Attachment I

Date:

(FACE SHEET)

ETHICAL REVIEW COMMITTEE, ICDDR,B.

Principal Investigator: Dr Nur Haque Alam

Application No. 2003-043

Title of Study: Efficacy of Salovum egg powder containing antisecretory factor (AF) in the treatment of severe cholera in adult

Trainee Investigator (if any): _____

Supporting Agency (if Non-ICDDR,B) _____

Project Status: _____

[X] New Study

[ ] Continuation with change

[ ] No change (do not fill out rest of the form)

Circle the appropriate answer to each of the following (If Not Applicable write NA)

1. Source of Population:
   - (a) Ill subjects — (Yes) No
   - (b) Non-ill subjects — Yes (No)
   - (c) Minor or persons under guardianship — Yes (No)

2. Does the Study Involve:
   - (a) Physical risk to the subjects — Yes (No)
   - (b) Social risk — Yes (No)
   - (c) Psychological risks to subjects — Yes (No)
   - (d) Discomfort to subjects — (Yes) No
   - (e) Invasion of privacy — Yes (No)
   - (f) Disclosure of information damaging to subject or others — Yes (No)

3. Does the Study Involve:
   - (a) Use of records (hospital, medical, death or other) — (Yes) No
   - (b) Use of fetal tissue or abortus — Yes (No)
   - (c) Use of organs or body fluids — (Yes) No

4. Are Subjects Clearly Informed About:
   - (a) Nature and purposes of the study — (Yes) No
   - (b) Procedures to be followed including alternatives used — (Yes) No
   - (c) Physical risk — (Yes) No
   - (d) Sensitive questions — (Yes) No NA
   - (e) Benefits to be derived — (Yes) No
   - (f) Right to refuse to participate or to withdraw from study — (Yes) No
   - (g) Confidential handling of data — (Yes) No
   - (h) Compensation &/or treatment where there are risks or privacy is involved in any particular procedure — (Yes) No

5. Will Signed Consent Form be Required:
   - (a) From subjects — (Yes) No
   - (b) From parents or guardian (if subjects are minor) — Yes (No)

6. Will precautions be taken to protect anonymity of subjects — (Yes) No

7. Check documents being submitted herewith to Committee:
   - ____ Umbrella proposal - Initially submit an with overview (all other requirements will be submitted with individual studies
   - ✓ Protocol (Required)
   - ✓ Abstract Summary (Required)
   - ✓ Statement given or read to subjects on nature of study, risks, types of questions to be asked, and right to refuse to participate or withdraw) (Required
   - ✓ Informed consent form for subjects
   - NA Informed consent form for parent or guardian
   - ✓ Procedure for maintaining confidentiality
   - NA Questionnaire or interview schedule*

   * If the final instrument is not completed prior to review, the following information should be included in the abstract summary
   1. A description of the areas to be covered in the questionnaire or interview which could be considered either sensitive or which would constitute an invasion of privacy
   2. Example of the type of specific questions to be asked in the sensitive areas
   3. An indication as to when the questionnaire will be presented to the Comr ittee for review

We agree to obtain approval of the Ethical Review Committee for any changes involving the rights and welfare of subjects before making such change.

Principal Investigator

Trainee

ICDDR,B: Centre for Health & Population Research **RRC APPLICATION FORM**

RESEARCH PROTOCOL

Protocol No. 2003-043

FOR OFFICE USE ONLY		
RRC Approval:	☐	☐Yes / ☐No Date:
ERC Approval:	☐Yes / ☐No	Date:
AEEC Approval:	☐Yes / ☐No	Date:

Project Title: Efficacy of Salovum egg powder containing Antisecretory Factor (AF) in the treatment of severe cholera in adult

Theme: *(Check all that apply)*

- ☐ Nutrition
- ☐ Emerging and Re-emerging Infectious Diseases
- ☐ Population Dynamics
- ☐ Reproductive Health
- ☐ Vaccine evaluation
- ☐ HIV/AIDS
- ☐ Environmental Health
- ☐ Health Services
- ☐ Child Health
- ☒ Clinical Case Management
- ☐ Social and Behavioural Sciences

Key words: Antisecretory factor; cholera

Relevance of the protocol:

Treatment of cholera is still rehydration fluid, antimicrotrial drug and usual food. Since cholera is a seratory diarrhoea, an antisecratory drug has long been desired by the physiciansand researchers. Several antisecretory drug has been evaluated in clinical trial, none of the drugs has yet been found beneficial for the treatment of cholera. Salovum egg powder containing antisecratory factor if found to be effective, it might be a prospective antidiarrhoeal drug in the treatment of cholera and related diseases.

Programmes

- ☐ Child Health Programme
- ☐ Nutrition Programme
- ☒ Programme on Infectious Diseases & Vaccine Science
- ☐ Poverty and Health Programme
- ☐ Health and Family Planning Systems Programme
- ☐ Population Programme
- ☐ Reproductive Health Programme
- ☐ HIV/AIDS Programme

Principal Investigator: N H Alam **Division:** CSD

Phone: 8811751-60 Ext: 2332

Address: Scientist, CSD, ICDDRB **Email:** nhalam@icddrb.org

Co-Principal Investigator(s):

Co-Investigator(s): H Ashraf, R Meier, S Sattar, M Oleson, J Troup, MA Salam

Revised on:15th April 2003

Student Investigator/Intern:

Collaborating Institute(s):Gastroenterology Dept, Liestal, University of Basel, Switzerland; Novartis Consumer Health, Novartis, Bern, Switzerland

Population: Inclusion of special groups *(Check all that apply)*:

Gender
- [x] Male
- [ ] Females

Age
- [ ] 0 – 5 years
- [ ] 5 – 9 years
- [x] 10 – 19 years (15 to 55 years)
- [ ] 20 – 64 years
- [ ] 65 +

- [ ] Pregnant Women
- [ ] Fetuses
- [ ] Prisoners
- [ ] Destitutes
- [ ] Service providers
- [ ] Cognitively Impaired
- [ ] CSW
- [ ] Others (specify _____)
- [ ] Animal

Project / study Site *(Check all the apply)*:
- [x] Dhaka Hospital
- [ ] Matlab Hospital
- [ ] Matlab DSS area
- [ ] Matlab non-DSS area
- [ ] Mirzapur
- [ ] Dhaka Community
- [ ] Chakaria
- [ ] Abhoynagar
- [ ] Mirsarai
- [ ] Patyia
- [ ] Other areas in Bangladesh _____
- [ ] Outside Bangladesh
  name of country: _____
- [ ] Multi centre trial
  (Name other countries involved)
  _____

Type of Study *(Check all that apply)*:
- [ ] Case Control study
- [ ] Community based trial / intervention
- [ ] Program Project (Umbrella)
- [ ] Secondary Data Analysis
- [x] Clinical Trial (Hospital/Clinic)
- [ ] Family follow-up study
- [ ] Cross sectional survey
- [ ] Longitudinal Study (cohort or follow-up)
- [ ] Record Review
- [ ] Prophylactic trial
- [ ] Surveillance / monitoring
- [ ] Others

Targeted Population *(Check all that apply)*:
- [x] No ethnic selection (Bangladeshi)
- [ ] Bangalee
- [ ] Tribal groups
- [ ] Expatriates
- [ ] Immigrants
- [ ] Refugee

Consent Process *(Check all that apply)*:
- [x] Written
- [ ] Oral
- [ ] None
- [x] Bengali language
- [ ] English language

Proposed Sample size:

Total sample size: 40 ☐

Sub-group _____ ☐ _____ ☐

☐ _____ ☐

Determination of Risk: Does the Research Involve *(Check all that apply)*:

- ☐ Human exposure to radioactive agents?
- ☐ Fetal tissue or abortus?
- ☐ Investigational new device?
  (specify_____)
- ☐ Existing data available from Co-investigator
- ☐ Human exposure to infectious agents?
- ☐ Investigational new drug
- ☐ Existing data available via public archives/source
- ☐ Pathological or diagnostic clinical specimen only
- ☐ Observation of public behaviour
- ☒ New treatment regime

Yes/No

☒ ☐ Is the information recorded in such a manner that subjects can be identified from information provided directly or through identifiers linked to the subjects?

☐ ☒ Does the research deal with sensitive aspects of the subject's behaviour; sexual behaviour, alcohol use or illegal conduct such as drug use?

Could the information recorded about the individual if it became known outside of the research:

☐ ☒ a. place the subject at risk of criminal or civil liability?

☐ ☒ b. damage the subject's financial standing, reputation or employability; social rejection, lead to stigma, divorce etc.

Do you consider this research *(Check one)*:

- ☐ greater than minimal risk
- ☐ only part of the diagnostic test
- ☒ no more than minimal risk

Minimal Risk is "a risk where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physical, psychological examinations or tests. For example, the risk of drawing a small amount of blood from a healthy individual for research purposes is no greater than the risk of doing so as a part of routine physical examination".

Yes/No

☐ ☐ Is the proposal funded?

If yes, sponsor Name: _____

Yes/No

☒ ☐ Is the proposal being submitted for funding ?

If yes, name of funding agency: (1) ___Novartis, Bern Switzerland_____

(2) _____

Do any of the participating investigators and/or their immediate families have an equity relationship (e.g. stockholder) with the sponsor of the project or manufacturer and/or owner of the test product or device to be studied or serve as a consultant to any of the above?

IF YES, submit a written statement of disclosure to the Director.

Dates of Proposed Period of Support	**Cost Requested for the Budget Period ($)**
(Day, Month, Year - DD/MM/YY)	*a. Ist Year* *2nd Year* *3rd Year* *Other years*
Beginning date____February 1, 2004_____	_____ _____ _____ _____
End date_November 31, 2004_____	*b. Direct Cost :*__29797_____ *Total Cost :* 37,246_____

Approval of the Project by the Division Director of the Applicant

The above-mentioned project has been discussed and reviewed at the Division level as well by the external reviewers. The protocol has been revised according to the reviewer's comments and is approved.

DR. MA Salam
Name of the Associate Director

Signature

19/11/03
Date of Approval

Certification by the Principal Investigator

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

Signature of PI _____

Date: 19-11-03

Name of Contact Person (if applicable)

_____

Table of Contents

Check here if appendix is included

Principal Investigator: Last, first, middle _____

PROJECT SUMMARY: Describe in concise terms, the hypothesis, objectives, and the relevant background of the project. Describe concisely the experimental design and research methods for achieving the objectives. This description will serve as a succinct and precise and accurate description of the proposed research is required. This summary must be understandable and interpretable when removed from the main application. (**TYPE TEXT WITHIN THE SPACE PROVIDED**).

Principal Investigator Dr. Nur Haque Alam

Project Name Efficacy of Salovum egg powder containing Antisecretory Factor (AF) in the Treatment of Severe Cholera in Adult

Total Budget US $ 37,246 Beginning Date February 1, 2004 Ending Date November 30, 2004

Since cholera involves stimulation of secretory process, efforts to identify and test potential antisecretory agents in order to reduce the severity of diarrhoea and consequently reduced need for rehydration fluids seems logical. Until now, none of the antisecretory drugs has been recommended for the clinical management of cholera patients. Antisecretory factor is a naturally occurring protein produced in the brain as well as in other secretory organs including intestine with antisecretory properties demonstrated in animal and human models of secretory diarrhoea. The Salovum egg yolk powder contains antisecretory proteins in a much higher (500 times) concentrations than that found in normal hen eggs. This is achieved by feeding hens with specially processed cereals, capable of inducing antisecretory proteins in the yolk, from which an egg yolk powder is produced. The objective of this study is to examine the effect of salovum egg yolk powder containing antisecretory factor in the treatment of adult cholera This clinical trial is a pilot open randomized controlled study in 40 adult patients (20 in each group). Adult male cholera patients attending with a history of watery diarrhoea of less than 24 hours with signs of severe dehydration will be screened to participants in the study. During the observation period, all patients will be rehydrated with intravenous fluid (cholera saline) over 4 hours. Patients fulfilling the inclusion criteria will be randomized to receive either Salovum egg yolk powder along with ORS or ORS alone. All patients will receive a single dose (300mg) of doxycycline. In take of ORS, food and output of stool, urine, vomits will be measured every 6 hours. After completion of the study outcome variables such as stool output, ORS intake, duration of diarrhoea and number of patients receiving unscheduled IV therapy will be compared between the groups. If the Salovum egg powder is found to be beneficial, then a clinical trial with adequate sample size will be conducted to further establish its therapeutic use.

KEY PERSONNEL (List names of all investigators including PI and their respective specialties)

	Name	Professional Discipline/ Specialty	Role in the Project
1.	N.H Alam	Medicine / Gastroenterology	Pr. Investigator
2.	H. Ashraf	Medicine / Gastroenterology	Co Invetigator
3.	R. Meier	Medicine / Gastroenterology	"
4.	S. Sattar	Medicine	"
5.	M Olesen	Statistics	"
6.	J Troup	Nutrition	"
7.	MA Salam	Medicine	"

DESCRIPTION OF THE RESEARCH PROJECT

Hypothesis to be tested:

Concisely list in order, in the space provided, the hypothesis to be tested and the Specific Aims of the proposed study. Provide the scientific basis of the hypothesis, critically examining the observations leading to the formulation of the hypothesis.

Treatment of severe cholera with Salovum egg yolk powder rich in antisecretory factor (AF), as an adjunct to standard treatment, will significantly enhance recovery from diarrhoea and reduce severity of disease in adult cholera patients.

Specific Aims:

Describe the specific aims of the proposed study. State the specific parameters, biological functions/ rates/ processes that will be assessed by specific methods **(TYPE WITHIN LIMITS).**

Study objectives

Primary Objective

Assess the effects of AF on the duration of diarrhoea and the volume of abnormal stool in severely purging adults with cholera.

Secondary Objectives

The secondary objectives of this study are to:

1. Compare intake of intravenous fluid and ORS between the 2 groups
2. Compare the proportion of patients requiring "unscheduled intravenous fluid therapy"
3. Assess the safety, acceptability of and compliance to the intervention.

Background of the Project including Preliminary Observations

Describe the relevant background of the proposed study. Discuss the previous related works on the subject by citing specific references. Describe logically how the present hypothesis is supported by the relevant background observations including any preliminary results that may be available. Critically analyze available knowledge in the field of the proposed study and discuss the questions and gaps in the knowledge that need to be fulfilled to achieve the proposed goals. Provide scientific validity of the hypothesis on the basis of background information. If there is no sufficient information on the subject, indicate the need to develop new knowledge. Also include the **significance and rationale** of the proposed work by specifically discussing how these accomplishments will bring benefit to human health in relation to biomedical, social, and environmental perspectives. **(DO NOT EXCEED 5 PAGES, USE CONTINUATION SHEETS).**

Background

Cholera is an infectious disease caused by enteric bacterial pathogen *V. cholerae*. The disease can often be very severe characterized by frequent passage of voluminous watery stools, and vomiting leading to severe dehydration, and if not efficiently treated might result in death rates as high as 50-80% [1]. The primary cause of death in cholera is hypovolemic shock due to severe dehydration. The mechanism of fluid loss in cholera involves the production of heat labile cholera toxin (CT) produced by the bacteria, which stimulates cellular adenylate cyclase in small and large intestinal mucosa and increases the intraepithelial concentration of cyclic adenosine monophosphate (cAMP) resulting in electrolyte and water secretion [2-5]. In addition to direct effect of CT on intestinal mucosa, recent observations suggest that enteric nervous system and release of neurotransmitters is also involved in intestinal water and electrolyte secretion in cholera [6-9]. Prevention of dehydration, and rehydration using appropriate oral or intravenous fluids, as appropriate, and the use of an effective antimicrobial agent along with continued feeding are important in the management of cholera [10, 11]. Since cholera involves stimulation of secretory process, efforts to identify and test potential antisecretory agents in order to reduce the severity of diarrhea and consequently reduced need of rehydration fluids seems logical. Indeed, several drugs have been demonstrated to possess antisecretory effect in experimental animal models [12-15] and some of them have been assessed in cholera patients [13-19]. Most of these agents have been assessed to have no or minimal effect, and those assessed to reduce stool volume are not suitable for use due to their adverse effects [20-24]. Until now, none of the antisecretory drugs has been recommended for the clinical management of cholera patients.

Endogenous factors

As possible antisecretory agents, some of the endogenous factors have recently drawn attention of the researchers.

Enkephlinase inhibitors

The enkephalins are endogenous opiate substances, first discovered in 1975, play an important physiological role by acting as neurotransmitters, most notably along the digestive tract where they elicit intestinal antisecretory activity without affecting intestinal transit time or motility [22]. After release, they are rapidly inactivated by enkephalinase, also present throughout the gastrointestinal tract. Racecadotril (an enkephalinase inhibitor) is a dipeptide with a single amide bond developed from research into structure-activity relationship in the enkephalinase molecule. The active metabolite of racecadotril interacts specifically with the active site of enkephalinase to produce potent blockade of the enzyme [23]. Racecadotril reinforces the physiological activity of endogenous enkephalins and shows intestinal antisecretory activity without affecting intestinal transit [22]. The antisecretory mechanism involves activation of the opiod receptors reading to reduced secretion of electrolytes and water through a reduction in intracellular cAMP [24]. These antisecretory and antidiarrhoeal effect have been demonstrated in dogs with CT-induced secretion [25] and in rats and man after administration of CT [26, 27]. Some clinical studies have demonstrated a better antisecretory efficacy of racecadotril in acute diarrhoea (other than cholera) relative to placebo and loperamide [28, 30]. However, a recent large clinical study has failed to demonstrate any beneficial effect of racecadotril in cholera patients[31], although it was found to be effective in acute non-cholera diarrhoea [32]

Antisectory Factor

Anti-secretory factor (AF) is a naturally occurring protein, thought to be the primary regulator of fluid secretion with an ED_{50} of 1-5 pmol in an antisecretory animal model (33). The protein is produced in the brain as well as in secretory organs such as the gallbladder, the lungs, the kidneys and the intestine. AF appears to be stored in cytoplasmic vesicles, and the mode of action is thought to be autocrine (33,35).

Although the native protein is 382 amino acids, antisecretory activity is retained in an eight amino acid (8-aa) N-terminal fragment. The protein is accumulated in the gut and pituitary gland after challenge with enterotoxins such as CT, heat labile toxin of *Escherichia coli*, and the *Clostridium difficile* toxin A (33-37). AF is also secreted in the blood, bile and breast milk in response to intestinal enterotoxin challenge (38) and the content in Sows' milk is probably crucial for protection against neonatal diarhoea in suckling piglets (39). It has also been found that the carbohydrates and amino acids of specially processed cereals are also able to induce secretion of AF or AF-like proteins (40), which might be useful for therapeutic applications. Preclinical animal studies have shown that the AF can have both prophylactic and therapeutic applications in the treatment of various diarrhoeas (33- 42). The peptide has effects on intestinal permeability as well as chloride secretion, which may be fundamental to its mechanism of action(43). These animal studies also demonstrated AF to possess strong anti-inflammatory properties (44).

A strong correlation between endogenous levels of AF in blood and the severity of diarrhoea has been demonstrated in both animals and humans (33,38). Likewise, AF has been found to be absent in the intestine of patients with active inflammatory bowel disease (IBD), but has been found to return towards normal levels during the period of remissions(33). Consistent with this observation, a recent double-blind placebo-controlled study with Lantmännens' functional food demonstrated that IBD patients receiving AF-inducing food had higher levels of endogenous AF associated with significantly reduced disease scores (43).

The Salovum egg yolk powder contains antisecretory like proteins in a much higher (500 times) concentration than found in normal hen eggs. This is achieved by feeding hens with specially processed cereals, capable of inducing production of anitsecretory-like proteins in the yolk, from which an egg yolk powder is produced (44). A number of studies have evaluated the effect of Salovum egg yolk powder in the treatment of inflammatory bowel disease and observed encouraging results in terms of reduction in the stool frequency and inflammatory blood parameters (44, 45,46). In view of its apparent central role in the regulation of pathological fluid secretion, AF could be therapeutically useful in numerous indications(e.g., secretory diarroea, inflammatory bowel dusease etc.) In gastrointestinal (GI) disorders, AF may have an use in the treatment of diarrhoeal disease of various etiologies(e.g secretory and inflammatory diarrhoea) .Furthermore, results from animal studies, as well as the above-mentioned clinical trials in humans, suggests that there are more indications that the product could be useful.

Safety of Salovum egg powder

Salovum, is a freeze dried egg yolk powder rich in antisecretory factors and some vitamins (Vit A, B12, Biotin and vitamin E) and minerals (Phosphorus, Selenium and fluoride). It has been classified as a food for special medical purposes by the Swedish National Food Administration (Paper attached with the procol). There are no limitations or warnings for use from the authority, and there is no side effects reported so far. These products are sold in health food stores/pharmacy for people with gastrointestinal disorders in Sweden. This product has been used in patients with ulcerative colitis (46), in patients with Crohn's disease (47), in non infectious secretory diarrhoea(48) and in Meniere's disease (49). Clinical studies done so far with this product were not found to be associated with any adverse effects (50).

Hypothesis

In the light of the above background, we hypothesise that AF would reduce the duration and the severity of diarrhoea in adults with cholera.

However, due to past experiences showing no benefit of antisecretory agents that have been assessed to be useful in animal models, we would like to take a cautious approach and undertake a pilot study to investigate the effect of Salovum egg powder rich in antisecretory factor in the management of adults with cholera. Study patients will be selected from those who attend the Dhaka Hospital of ICDDR,B with a history of diarrhoea of less than 24 hours and signs of severe dehydration.

Research Design and Methods

Describe in detail the methods and procedures that will be used to accomplish the objectives and specific aims of the project. Discuss the alternative methods that are available and justify the use of the method proposed in the study. Justify the scientific

validity of the methodological approach (biomedical, social, or environmental) as an investigation tool to achieve the specific aims. Discuss the limitations and difficulties of the proposed procedures and sufficiently justify the use of them. Discuss the ethical issues related to biomedical and social research for employing special procedures, such as invasive procedures in sick children, use of isotopes or any other hazardous materials, or social questionnaires relating to individual privacy. Point out safety procedures to be observed for protection of individuals during any situations or materials that may be injurious to human health. The methodology section should be sufficiently descriptive to allow the reviewers to make valid and unambiguous assessment of the project. **(DO NOT EXCEED TEN PAGES, USE CONTINUATION SHEETS).**

Investigational plan

Overall study design

This will be a single-centre, prospective, open, controlled pilot study in 40 adult patients with severe cholera who would be randomized in equal numbers to receive either:

1. Anti-secretory Factor (AF) rich Salovum egg yolk powder in addition to standard treatment
2. or the standard treatment alone

Methods and Procedures

Sample size and power considerations

A total of 40 patients will be randomized in a ratio of 1:1. This is a pilot study to investigate the effect of AF in this serious diarrhea condition, thus no power calculation have been made.

Study site and study population

Study patients will be selected from among those attending the Dhaka Hospital of ICDDR,B with a history of watery diarrhoea of <24 hours and severe dehydration. Eligibility for participation in the study would be as follows:
Inclusion Criteria

Patients would be eligible for inclusion in the study if the following criteria are met:

- Age: 18 – 55 years
- Gender: Male (women would be excluded due to difficulties in separation of their urine from stools, particularly in those with altered mentation due to severe dehydration and shock).
- Duration of diarrhoea: 24 hours or less
- Clinical features of severe dehydration.(Dhaka Methods, appendix 2)
- Demonstration of *V. cholerae* in dark-field microscopy of a fresh stool specimen
- Written informed consent for participation in the study (for patients with temporary inability to provide consent due to their severe dehydration and obtunded mentation their major attendant would provide initial consent; however, the consent process would be reapplied once they are fully oriented and able to provide consent themselves)

Exclusion Criteria

Patients will not be enrolled in the study if any of the followings are present:

- Chronic diarrhoea including IBS
- Dysentery (presence of visible blood in stool)
- History of receiving antimicrobial or antidiarrhoeal drugs within one week prior to admission
- History of renal or hepatic dysfunction
- Failure to obtain informed consent
- Known allergies to eggs

Patient Assessment Schedule

Upon initial screening patients would be taken to the research ward of the Dhaka Hospital, weighed, and placed on a cholera cot. A nurse would record vital signs, and the investigators\ study medical officer would take medical history and perform a thorough physical examination including assessment of dehydration using modified WHO guidelines (Dhaka Method). All findings would be recorded in pre-designed forms. The patient enrolment in the study will be done from 6 AM to 2 PM in 7 days a week.

Initial Rehydration Phase

Patients will be rehydrated over a 4-hour period using intravenous fluid (cholera saline), and a stool specimen would be subjected for dark-field microscopy for identification of *V. cholerae*. Those identified to have cholera will be eligible for final study enrollment.

Patients will be closely observed in the Research Ward of the Dhaka Hospital, and the duration of trial would be 4 days. The assessment of the patients would be done in accordance with the following schedule:

Screening (Day 1, Hour: (- 4) to 0)

The following assessments will be done, and the findings will be recorded in the pre-tested Case Report Forms (CRFs).

- Determine eligibility for participation in the study using the pre-defined criteria (please see above)
- Obtain written informed consent from the patients or their guardian/attendant (in the event they are temporarily unable to provide the consent themselves due to obtundation, and in such cases the consent process would be reapplied to the patients as soon as they are fully oriented) by the investigator or his representative before any study-specific procedure/intervention is undertaken
- Record medical history including the duration of diarrhoea prior to study inclusion, the stool and vomiting frequency in the preceding 24 hours, stool characteristics, medication including the use of antimicrobials and/or antidiarrhoeal agent(s)
- Record significant past medical/surgical history, demography and concurrent illnesses, and concomitant medication(s)
- .Perform thorough physical examination including vital signs (oral/axillary temperature, respiratory rate, supine pulse rate and blood pressures) and body weight, assessment of dehydration using the modified WHO guidelines (Dhaka Method), and record findings in pre-designed forms.
- Correct dehydration using intravenous solution (Dhaka Solution: containing 133 mmol/L, 98 mmol/L, 48 mmol/L and 13 mmol/L of sodium, chloride, bicarbonate as acetate and potassium respectively) during the first 4 hours.
- Collect stool specimen for dark-field microscopy for demonstration of *V. cholerae*.

Final enrollment to study (Day 1, Hour: 0)

If *V. cholerae* is demonstrated in the dark-filed microscopy of the admission stool specimen, the followings would be done:

- Assessment of dehydration using the modified WHO guidelines.
- Assessment of eligibility for inclusion into the study.
- Assessment of stool output and rate of stool output during the observation period
- Assessment of the volume of intravenous rehydration used during the observation period
- Assessment of the physical findings as described above
- Collection of 5.0 ml of venous blood for laboratory assays (described below), and stool specimen for culture of enteric pathogens (described below)

Treatments

Rehydration and maintenance of hydration

Patients with severe dehydration on enrollment would be rapidly rehydrated using a poly-electrolyte intravenous solution. Maintence of hydration will be done by administering ORS solution (Na 75, K 20, Cl 65, citrate 10 glucose 75 mmol/L and osmolarity 245 mosmol/L) until resolution of diarrhoea with a minimum volume equal to the volume stool passed during the previous 6 hour period. However, the same intravenous solution used for initial rehydartion would be used if indicated.

Antimicrobial therapy for cholera

All patients will receive a single-dose therapy for cholera with 300 mg dose of doxycycline capsules.

Diets

All patients will receive the routine adult diets of the Dhaka Hospital three times a day:

Breakfast (7:00 a.m.) bread, sugar and banana
Lunch (12:30 p.m.) rice, lentil and fish/meat curry, and
Supper (7:00 p.m.) rice, lentil/vegetables and meat/fish curry.

Investigational therapy

Description of products

Antisecretory Factor (AF)-rich egg yolk powder

Manufacturer and supplier of the drug for the study: Novartis Consumer Health.

Randomization to study intervention and management during the study

Eligible patients will be randomized in equal numbers to one of the following :

a) Anti-secretory Factor(AF) in addition to standard treatment

or

b) the standard treatment alone

Route and Dosing regimen (TBD)

Salovum egg yolk powder 2 sachets (2 gram each i.e. total of 4 g) will be dissolved in 100 ml of the assigned ORS and fed orally every 2 hours during the first 24 hours, and 4 hourly until resolution of diarrhoea but upto a maximum of 72 hours.
(The dose decided is partially arbitrary and is based on previous study in inflammatory bowel disease, the dose used 4 g 4 hourly for 14 days. Since cholera is a severe disease, we have decided frequent dosing (2 hourly) at least for 24 hours).
Dispensing will be done by a nurse
Assignment to Study Intervention

A sequential patient number will be assigned to the patients enrolled in the study in accordance with the inclusion/exclusion criteria, and such numbers will have been randomly pre-assigned in a 1:1 ratio to either of the two groups- AF or no AF.

Concomitant therapy

With the exception of antimicrobial therapy for cholera (300 mg single-dose dixycycline) no concomitant medication is anticipated. However, all concomitant medications, if required would be recorded on the CRF at each assessment of the study patients..

Disallowed treatment(s)

The following substances and therapies are not permitted during the course of the study as they may confound the results of the study:

1. any antibiotics other than doxycycline
2. any antisecretory agent or antidiarrhoeal drugs

Interruption or discontinuation of treatment

Whether or not study is completed would be documented for each participant. If either the study intervention or observations are discontinued for any reason(s) that would be recorded. A patient might voluntarily withdraw from the study or the investigator might withdraw patients for the following reasons:

1. occurrence of adverse event(s)
2. undesired therapeutic effect (that interfere with with patient's normal health or any medication is needed for this undesired side effects)
3. protocol violation
4. subject withdrew consent
5. referral to another hospital for complications or other medical problems
6. lost to follow-up

The date and reason(s) for any premature discontinuation of the trial should be recorded and the final assessment made in accordance with the protocol instructions/guidelines (also to be provided in appropriate section of the CRF).

Patients withdrawing or discontinued from participation will not be replaced by a new patient. No data will be collected for the study purpose after withdrawal of the patients from the study, although treatment would continue if required.

During study evaluations: (0-24, 25-48, and 49-72 hours)

An investigator or the Research Medical Officer would assess patients several times on the day of admission, and at least once every morning. Patient will be closely observed in the Research Ward of the Dhaka Hospital by the nurses and other health workers, and the followings will be assessed and recorded:

- Volume of watery/liquid stools (6 hourly from the time of randomization)
- Recording of stool frequency (6 hourly from the time of randomization)
- Recording of vomiting frequency (6 hourly from the time of randomization)
- Recording of all diarrhea associated symptoms (each day)
- The amount of intravenous fluid(s), ORS, and water consumed (6 hourly from the time of randomization)
- Vital signs i.e. radial pulse rate, respiratory rate, oral/axillary temperature, and blood pressures every 6 hours
- Body weight (every 24 hours from the time of randomization)
- Requirement of unscheduled intravenous fluid therapy

Assessment at the end of the study period (On Day 4)

The last assessment of the patents would be done after completion of 72 hours (from the time of randomization) of the study. The final assessment might be done earlier for patients discontinued from the study due to any reason. Irrespective of the time of final assessment, all of the required evaluations must be performed :

Adverse Events will be addressed, concomitant medications will be checked.
Patients will be examined by the physician (full examination and vital signs) to evaluate the status of their disease. The End of study form will be completed.

Description of Procedures (Methods & Measurements)

Concomitant Medication

All concomitant medications including dietary supplements (by trade name) taken either at or within the last 6 weeks of study entry should be recorded at the first assessment. Records should include the indication, start date, dose and mode of administration. Any change of concomitant medication during the study including a change of dose should also be recorded. All concomitant medications/diets/supplements will be recorded throughout the study.

Vital signs

Radial pulse (heart rate in the event radial pulse in absent e.g. in severely dehydrated patents with hypovolaemic shock), respiratory rate and body temperature (oral or axillary), and the recumbent blood pressures (systolic and diastolic) would be obtained at initial enrollment before initiation of rehydration, after rehydration, and every 6 hours from the time of randomization to study intervention. Body weight will be measured using an electronic balance with a precision of 10 grams, at the time of enrollment to the study before initiation of rehydration, after rehydration, and daily from the time of randomization to the study intervention.

Physical Examination

A full physical examination will be performed at the time of enrollment to the study before initiation of rehydration, after rehydration, and daily from the time of randomization to the study completion.

Demographics

Information on date of birth/age, gender and occupation would be collected at study entry.

Stool collection

Cholera cot has been designed for the treatment and research on diarrhoeal diseases, This is a modified camp cot with a central hole in the canvas that leads through a plastic sheave to a plastic bucket underneath for collection of watery/liquid stools. All study patients would be placed on cholera cot and their stool volume would be measured for the rehydration period, and for every 6 hours from the time of randomization using an electronic balance with a precision of 1 gram.

Diarrhoea associated symptoms

- Associated symptoms include nausea, vomiting, abdominal pain or cramps, tenesmus and urgency of stools, and they would be recorded on a daily basis using a 4 point scale:

  0 Absent

  1 Mild

  2 Moderate

  3 Severe

Unscheduled intravenous therapy

After the initial rehydration with intravenous fluid, its requirement at any time during the study due to failure of maintenance of hydration using study ORS resulting from any reason including high purging rate (>10 ml/kg/hr) and frequent vomiting, would constitute "unscheduled intravenous therapy". The frequency of such requirements and the volume of intravenous fluid required on each of such occasions would be recorded and analyzed.

Criteria for unscheduled intravenous therapy:

1. Reappearance of signs of severe dehydration after initial intravenous rehydration or
2. Persistence of some dehydration for >6 hours with/without excessive vomiting.

Routine Laboratory Assessments

The following laboratory tests would be performed for each patient enrolled in the study:

Before randomization and at the end of treatment (on day 4):

Stool: Dark-field microscopy for demonstration of *V. cholerae.*

Culture for isolation and identification of *V. cholerae, Shigella, Salmonella* and *Campylobacter jejuni*

Blood: Haemoglobin and haematocrit, and total and differential white blood cell counts; serum specific gravity, BUN, creatinine, sodium, potassium, chloride and bicarbonate

EFFICACY AND SAFETY ASSESSMENT

Primary efficacy parameter

The primary efficacy parameter will be:

1. The duration of diarrhoea, in hours, from the time of randomization to the resolution of diarrhoea [this was mentioned under the hypothesis]

2. Total stool output (expressed as gram/kg of body weight), from the time of randomization (initiation of study intervention) to the resolution of diarrhoea, and for each 24-hour period of the study.

Secondary efficacy parameter

The followings would be the secondary efficacy parameters:

1. Proportion of patients requiring "unscheduled intravenous therapy' in the two intervention groups
2. Proportion of clinical success (and failures),
3. Proportion of success (and failure) of oral rehydration therapy
4. Frequency and amount of vomit.
5. Severe abdominal cramps

Definitions

- **Duration of diarrhoea**: The interval, in hours, from randomization to the last watery stool that is followed by either 2 consecutive normal stools or 12 hours of no stool.

- **Clinical success:** defined as resolution of diarrhoea (please see the above definition) and associated symptoms (vomiting, abdominal crapms, anorexia) within 72 hours on initiation of the study intervention.

- **Clinical failure**: continuation of diarrhoea and/or associated symptoms (please see definitions above) beyond 72 hours of initiation of the study intervention.

- **Success of oral therapy:** defined as no requirement of intravenous rehydration therapy at any time during the study after the initial intravenous rehydration (i.e. no requirement of "unscheduled intravenous therapy").

- **Failure of oral therapy:** defined as the reappearance of the signs and/or symptoms of dehydration, at any time during the study after initiation of intervention, despite the use of ORT, due to high rates of purging or frequent vomiting or both, requiring reinstitution of intravenous fluid therapy.

SAFETY ASSESSMENT

Safety will be assessed by the following parameters:

1. Safety assessments will consist of monitoring and recording of all **adverse events**, including **serious adverse events**.

2. In addition, blood parameters and vital signs will be monitored regularly, and a physical examination will be performed at the final visit.

Adverse Event Recording

For each adverse event, date of onset, severity, relation to study product, action taken and outcome are recorded. It would be the obligation of the investigator to assess the relationship between study product and the adverse event, and to fill up relevant part of the CRF as well as to inform the sponsor of the study as well as the Ethical Review Committee of ICDDR,B on the serious adverse event, within 24 hours of its occurrence.

o Adverse events

An adverse event is any undesirable sign, symptom or medical condition occurring after starting study product, whether or not they are related to the study product (or therapy). Study product includes the product under evaluation, and placebo given during any phase of the study.

Medical conditions/diseases present before starting study treatment are considered as adverse events ONLY if they worsen after starting study treatment . Adverse events (but not serious adverse events) occurring before starting study treatment but after signing the informed consent form are recorded on the Medical History/Current Medical Conditions CRF. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant or require therapy, and are recorded on the Adverse Events CRF under the signs, symptoms or diagnosis associated with them.

Information about all adverse events, whether volunteered by the patient, discovered by investigator during questioning, or through physical examination, laboratory test or other means, will be collected and recorded on the Adverse Event CRF. All events will be followed as appropriate.

As far as possible, each adverse event will also be described by:

1. its duration (onset date and ongoing/end dates)
2. the severity grade (mild, moderate, severe)
3. its relationship to the study product(suspected/not suspected)
4. the action(s) taken and, as relevant, the outcome

Severity of an adverse event is defined as a qualitative assessment of the degree of intensity of an adverse event, as determined by the investigator, or as reported to him/her by the patient. The assessment of severity is made irrespective of study product relationship or seriousness of the experience, and should be evaluated according to the following scale:

1 = mild: noticeable to the patient, did not require reduction or discontinuation of study product

2 = moderate: interfered with patient's daily activities, possibly required reduction of study product and/or additional therapy, but did not require discontinuation of the study product

3 = severe: was intolerable and necessitated reducing the dose of or discontinuing the study product and/or required additional therapy

Serious adverse events

Information about all serious adverse events will be collected and recorded on the Novartis Consumer Health Serious Adverse Event Report Form. All serious adverse events which are judged as possibly or probably linked to the study product must also be reported to NCH within 24 hours of learning of its occurrence. A serious adverse event is an undesirable sign, symptom or medical condition which:

1. is fatal or life-threatening
2. requires or prolongs hospitalization
3. results in persistent or significant disability/incapacity
4. constitutes a congenital anomaly or a birth defect
5. is medically significant, in that it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Events **not** considered to be serious adverse events are hospitalizations for the:

1. treatment, which was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under study and did not worsen
2. admission to a hospital or other institution for general care, not associated with any deterioration in condition
3. treatment on an emergency, outpatient basis for an event **not** fulfilling any of the definitions of serious given above and **not** resulting in hospital admission.

Any serious adverse event occurring after the patient is randomized or begins taking study medication and until 4 weeks after the patient has stopped the study product must be reported.

Serious adverse events occurring more than 4 weeks after study product discontinuation need only be reported if a relationship to the NCH study product (or therapy) is suspected.

Hematology

The results of CBC with differential leukocytes count will be evaluated and recorded.

Serum chemistry

Glucose, creatinine, AST (SGOT), ALT (SGPT), total bilirubin, albumin, sodium, chloride, alkaline phosphatase, and total protein will be evaluated and recorded.

Physical examination

Information obtained through physical examination must be recorded in the source documentation at the study site. The investigator (or research medical officer) would perform physical examination and record significant findings prior to the start of the study on relevant section (Medical History/Current Medical Conditions) of the Case Report Form (CRF). Significant findings that occur after the start of the study which meet the definition of an AE must be recorded on the Adverse Events Case Report Form.

PROTOCOL AMENDMENT OR OTHER CHANGES IN THE STUDY CONDUCT

Protocol amendments

Any changes to the protocol will be documented in the form of an amendment.

Other changes in study conduct

Changes in study conduct are not permitted. Any unforeseen changes in the study conduct will be recorded in the clinical study report.

Facilities Available

Describe the availability of physical facilities at the place where the study will be carried out. For clinical and laboratory-based studies, indicate the provision of hospital and other types of patient's care facilities and adequate laboratory support. Point out the laboratory facilities and major equipments that will be required for the study. For field studies, describe the field area including its size, population, and means of communications. **(TYPE WITHIN THE PROVIDED SPACE).**

The study will be done in the study ward of CRSC of ICDDR,B. About 100,000 patients with diarrhoea attend every year in hospital of the centre, of these the study patients will be selected according to the inclusion and exclusion criteria.

Data Analysis

Describe plans for data analysis. Indicate whether data will be analyzed by the investigators themselves or by other professionals. Specify what statistical soft wares packages will be used and if the study is blinded, when the code will be opened. For clinical trials, indicate if interim data analysis will be required to monitor further progress of the study. **(TYPE WITHIN THE PROVIDED SPACE).**

Study Schedule: Patient recruitment and data collection are expected to complete within 6 months and data analysis and report writing will take another 3 months.

DATA MANAGEMENT

Data collection

Designated investigator staff must enter the information required by the protocol onto the Case Report Forms (CRFs). Novartis assigned monitors will review the CRFs for completeness and accuracy, and instruct site personnel to make any required corrections or additions.

Original copies of the CRFs will be forwarded to the designated Data Management Contract Research Organisation (CRO) by the monitors, one copy being retained at the investigational site and one by the monitors. Once the CRFs are received by Data Management, their receipt will be tracked and acknowledged, and the original copy will be retained as the working copy. All CRFs will be reviewed for any serious adverse events.

Database management and quality control

1. Data items from the CRFs will be entered centrally into the study database by CRO Data Management staff using double data entry with verification and reconciliation. Subsequently, the entered data will be systematically validated by reviewing computerized edit checks, manual checks and data cleaning listings. Approved data corrections will be filtered by listing and updated and documented in the database.
2. Other errors, omissions or inconsistencies will be entered on Data Query Forms, which are returned to the field monitors and if necessary to the investigator at site for resolution. Monitor-generated queries received with the CRFs will be reviewed before generating queries, to avoid duplication. The signed and resolved original Data Query Forms will be sent to Data Management so any necessary amendments can be made to the database, while one copy is retained at the investigational site and one by the field monitors. All Data Query Form resolutions will be checked twice.
3. The CRF and Data Query Forms will be stored in an Archive room by center and patient order.
4. Quality control audits of all key safety and efficacy data in the database will be made after each patient is complete with no outstanding queries. All data for 15% of all patients will also undergo an audit. When all errors are corrected, the CRO's Clinical Research Quality Assurance group will conduct an independent audit according to the Audit Protocol.
5. When the database has been declared to be complete and accurate, the database will be locked.

Statistical methods

Data will be summarized with respect to demographic and baseline characteristics, efficacy observations and measurements, safety observations and measurements, and other assessments. Continuous variables will be summarized by calculating the mean, standard deviation, median, minimum and maximum values, quartiles if appropriate, and number of observations. Categorical variables will be summarized by absolute and relative frequencies.

All statistical tests used to compare the two treatment groups will be two-sided, with probability of Type I error = 0.05.

Background and demographic characteristics

Information collected at the Baseline visit will be summarized for the purpose of characterizing the patient populations. This information includes demographics, weight and height, medical history, and concomitant medications.

Categorical variables (e.g., sex, presence/absence of relevant medical history, any previous/current medication,) will be summarized by the number and percentage of patients with each relevant characteristic in each treatment group. In addition, listings of medical conditions and previous/current medications will be presented.

Continuous variables will be summarized by calculating the mean and standard deviation, and the median, minimum and maximum values in each treatment group. These variables include age, weight and height.

Homogeneity at baseline will be examined between treatment groups; if deemed necessary, variables on which the treatment groups differ will be accounted for in the efficacy analysis.

Study products

The hospital medical staff will be instructed to record the product usage.

Concomitant therapy

Standard tables will be provided for concomitant therapy. All concomitant medications will also be listed by treatment group and patient. However,. Concomitant therapy will be avoided unless it is essential.

Efficacy evaluation

Primary efficacy parameter

Stool output will be summarized by calculating the mean, 95% confidence limits on the mean, and the median, minimum and maximum values in each treatment group and. The two-sided t-test will be used to determine the statistical significance of any difference in volume between treatment groups. Duration of diarrhoea will be compared between groups using student's t-test

The distribution of the data will be assessed by the Shapiro-Wilk test. If the data are not found to be normally distributed ($p<0.05$), then the data will be ranked and the analyses will be performed on the ranked data.

Secondary efficacy parameters

The proportions of patients having therapeutic success/failure, and proportion of patients requiring "unscheduled intravenous fluid therapy will be compared between the 2 treatment groups, using the chi-square test or Fisher's exact test.

ORS intake will be compared by ANOVA and Students-t-test, and a non-parametric test (Mann-Whitney-U test) will be used where appropriate

Safety evaluation

- The assessment of safety will be based mainly on the frequency of adverse events.
- Adverse events will be summarized by presenting, for each treatment group, the number and percentage of patients having any adverse event, an adverse event in each body system, each individual adverse event, and an adverse event suspected to be related to study medication. Any other information collected (e.g., severity) will be listed as appropriate.
- The number and percentage of patients in each treatment group who experience a serious adverse event will also be presented.
- Vital signs, serum chemistry and hematology results, and changes and percentage changes in these parameters, will be summarised by calculating the mean, 95% confidence limits on the mean, and the median, minimum and maximum values.
- Any clinically significant observations will be listed by treatment group/ patient.
- The number and percentage of patients with abnormal findings on physical examination after treatment, which were not present at baseline, will be presented. Such abnormal findings will also be listed by treatment group and patient.

Interim analyses

No interim analyses are planned for this pilot study.

Sample size and power considerations

A total of 40 patients will be randomized in a ratio of 1:1. This is a pilot study to investigate the effect of AF in this serious diarrhea condition, thus no power calculation have been made. (The sample size decided is arbitrary. Since there is no efficacy data with this new product in human cholera , if this is found to efficacious, data generated in this study will help in calculation of sample size for full protocol).

Ethical Assurance for Protection of Human Rights

Describe in the space provided the justifications for conducting this research in human subjects. If the study needs observations on sick individuals, provide sufficient reasons for using them. Indicate how subject's rights are protected and if there is any benefit or risk to each subject of the study.

All patients eligible for this study will be asked for written informed consent. The study will be enrolled according to good clinical practice and the declaration of the Helsinki and relevant national guideline with the prior approval by the RRC and ERC

# of Animals

Describe in the space provided the type and species of animal that will be used in the study. Justify with reasons the use of particular animal species in the experiment and the compliance of the animal ethical guidelines for conducting the proposed procedures.

Not applicable.

Literature Cited

Identify all cited references to published literature in the text by number in parentheses. List all cited references sequentially as they appear in the text. For unpublished references, provide complete information in the text and do not include them in the list of Literature Cited. There is no page limit for this section, however exercise judgment in assessing the "standard" length.

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Principal Investigator: Last, first, middle _____

Dissemination and Use of Findings

Describe explicitly the plans for disseminating the accomplished results. Describe what type of publication is anticipated: working papers, internal (institutional) publication, international publications, international conferences and agencies, workshops etc. Mention if the project is linked to the Government of Bangladesh through a training programme.

The finding of the study will be disseminated through presenting the result in International and rational conferences and also through publication in Peer reviewed journals.

Collaborative Arrangements

Describe briefly if this study involves any scientific, administrative, fiscal, or programmatic arrangements with other national or international organizations or individuals. Indicate the nature and extent of collaboration and include a letter of agreement between the applicant or his/her organization and the collaborating organization. **(DO NOT EXCEED ONE PAGE)**

This will be a collaborative study between the University hospital, Liestal and Basel, Switzerland, Novartis Consumer Health, Bern, Switzerland and ICDDR,B.

Procedures and instructions

0.1 Special safety-related procedures

0.1.1 Instructions for rapid notification of serious adverse events

Reporting responsibility

Each serious adverse event must be reported by the investigator to the Primary Safety Responsible Person (PRSP) within 24 hours of learning of its occurrence, even if it is not felt to be treatment-related (see contact persons and numbers below).

Follow-up information about a previously reported serious adverse event must also be reported within 24 hours of the investigator receiving it. If the serious adverse event is not previously documented (new occurrence) and is thought to be related to the NCH study product, a Quality Assurance and Pharmacovigilance (QAP) associate may urgently require further information from the investigator for Health Authority reporting. NCH may need to issue an investigator notification, to inform all investigators involved in any study with the same product that this serious adverse event has been reported.

Reporting procedures

The investigator must complete the Serious Adverse Event Report Form in English, assess the relationship to study treatment and send the completed, signed form by fax within 24 hours to the PSRP. The PSRP, after ensuring that the form is accurately and fully completed, must then fax it to the Quality Assurance and Pharmacovigilance Department (see telefax number below) within 24 hours of receipt. The original copy of the Serious Adverse Event Form and the fax confirmation sheet must be kept with the Case Report Form documentation at the study site.

Follow-up information is sent to the same person to whom the original Serious Adverse Event Form was sent. A new serious adverse event form is sent, stating that this is a follow-up to the previously reported serious adverse event and giving the date of the original report. Each re-occurrence, complication or progression of the original event should be reported as a follow-up to that event. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not, and whether the patient continued or discontinued study participation. The form and fax confirmation sheet must be retained. Refer to the NCH SOPs as needed for instructions for completing the Serious Adverse Event Form.

Contact persons and numbers

The telephone and telefax numbers of the contact persons in the Clinical Research department and in the local NCH affiliate, are provided below.

SAE reporting will be ensured by:

Maryam Kadjar Olesen

Head of Clinical development and operations

Novartis Consumer Health SA

CH-1260 Nyon, Switzerland

Tel: +41 22 363 3567

Fax: +41 22 363 3013

e-mail: maryam.olesen@ch.novartis.com

0.1.2 Emergency procedure for unblinding

For blinded studies 1 (one) complete set of emergency code break cards is provided to the investigators. The investigator will receive a blinded code break card for each patient in a sealed envelope, with the details of product treatment. In an emergency only, e.g., whenever knowledge of the treatment assignment is deemed essential by the patient's physician/investigator for the patient's care, can the envelope be opened to determine the treatment given.

The envelopes are not to be opened for any reason, other than the emergency defined in the previous paragraph. When the investigator opens the envelope, (s)he must note the date, time and reason for removing it and retain this information with the Case Report Form documentation. (S)he must also immediately inform the NCH local monitor that the code has been broken, and withdraw the patient from the study.

In case the investigator cannot have access to the code break envelopes, a NCH representative from Quality Assurance and Pharmacovigilance (QAP), independent from the Clinical Research department, can provide him/her the treatment assigned to the patient encountering the emergency situation. A contact report will be immediately completed by the QAP representative and provided to the Clinical Project Leader. The QAP contact persons and numbers will be provided to the investigator at the beginning of the study.

0.1.3 Instructions for completing adverse event Case Report Forms

Each adverse event is to be reported on the Adverse Event Case Report Form provided.

0.2 Administrative procedures

0.2.1 Changes to the protocol

Any change or addition to this protocol requires a written protocol amendment that must be approved by NCH and the investigator before implementation. Amendments significantly affecting the safety of patients, the scope of the investigation or the scientific quality of the study, require additional approval by the IRB/IEC/REB of all centers, and, in some countries, by the regulatory authority. A copy of the written approval of the IRB/IEC/REB, which becomes part of the protocol, must be given to the NCH monitor. Examples of amendments requiring such approval are:

1. an increase in product dosage or duration of exposure of patients
2. a significant change in the study design (e.g., addition or deletion of a control group)
3. an increase in the number of invasive procedures to which patients are exposed
4. addition or deletion of a test procedure for safety monitoring.

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or by NCH in the interests of preserving the safety of all patients included in the study. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented by him/her for safety reasons NCH should be notified and the IRB/IEC/REB at the center should be informed within 10 working days.

Amendments affecting only administrative aspects of the study do not require formal protocol amendments or IRB/IEC/REB approval but the IRB/IEC/REB of each center must be kept informed of such administrative changes. Examples of administrative changes not requiring formal protocol amendments and IRB/IEC/REB approval that can be treated as administrative amendments include:

1. changes in the staff used to monitor studies (e.g., NCH staff versus a CRO)
2. minor changes in the packaging or labeling of study product.

0.2.2 Monitoring procedures

Before study initiation, at a site initiation visit or at an investigator's meeting, a NCH representative will review the protocol and Case Report Forms with the investigators and their staff. During the study the field monitor will visit the site regularly, to check the completeness of patient records, the accuracy of entries on the Case Report Forms, the adherence to the protocol and to Good Clinical Practice, the progress of enrollment, and also to ensure that study medication is being stored, dispensed and accounted for according to specifications. Key study personnel must be available to assist the field monitor during these visits.

The investigator must give the monitor access to relevant hospital or clinical records, to confirm their consistency with the Case Report Form entries. No information in these records about the identity of the patients will leave the study center. NCH monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of serious adverse events and the recording of primary efficacy and safety variables. Additional checks of the consistency of the source data with the Case Report Forms are performed according to the study-specific monitoring plan.

0.2.3 Recording of data and retention of documents

The investigator must complete the Case Report Forms provided, transmit the data as instructed by NCH at study initiation and must store copies of the Case Report Forms or the NCH computer that contains them with other study

documents (e.g., the protocol, the investigators' brochure and any protocol amendments) in a secure place. All entries to the Case Report Forms must be made as instructed by NCH at study initiation.

Data on patients collected on Case Report Forms during the study will be documented in an anonymous fashion and the patient will only be identified by the patient number, and by his/her initials if also required. If, as an exception, it is necessary for safety or regulatory reasons to identify the patient, both NCH and the investigator are bound to keep this information confidential.

The investigator must maintain source documents for each patient in the study, consisting of all demographic and medical information, including laboratory data, electrocardiograms, etc, and keep a copy of the signed informed consent form. All information on Case Report Forms must be traceable to these source documents in the patient's file. Data without a written or electronic record will be defined before study start and will be recorded directly on the Case Report Forms, which will be documented as being the source data.

Essential documents, as listed below, must be retained by the investigator for as long as needed to comply with national and international regulations (generally 2 years after discontinuing clinical development or after the last marketing approval). NCH will notify the investigator(s)/institution(s) when the study-related records are no longer required. The investigator agrees to adhere to the document retention procedures by signing the protocol. Essential documents include:

1. IRB/IEC/REB approvals for the study protocol and all amendments
2. all source documents and laboratory records
3. CRF copies (paper copies or electronic copies on a CDROM, depending on the study)
4. patients' informed consent forms (with study number and title of study)
5. FDA form 1572 (as required)
6. any other pertinent study document.

0.2.4 Auditing procedures

In addition to the routine monitoring procedures, a Quality Assurance and Pharmacovigilance Department exists within NCH. This unit conducts audits of clinical research activities in accordance with internal Standard Operating Procedures to evaluate compliance with the principles of Good Clinical Practice. A regulatory authority may also wish to conduct an inspection (during the study or even after its completion). If an inspection is requested by a regulatory authority, the investigator must inform Novartis immediately that this request has been made.

0.2.5 Handling of study product

All study products will be supplied to the principal investigator by NCH. Supplies must be kept in an appropriate, secure area (e.g., locked cabinet) and stored according to the conditions specified on the labels. The investigator must maintain an accurate record of the shipment and dispensing of study product in a product accountability ledger, a copy of which must be given to NCH at the end of the study. An accurate record of the date and amount of study product dispensed to each patient must be available for inspection at any time.

All product supplies are to be used only for this protocol and not for any other purpose. The investigator must not destroy any product labels, or any partly-used or unused supply. At the conclusion of the study, and, as appropriate during the course of the study, the investigator will return all used and unused product containers, labels and a copy of the completed product disposition form to the NCH monitor or to the NCH address provided in the investigator folder at each site.

0.2.6 Publication of results

Any formal presentation or publication of data from this study will be considered as a joint publication by the investigator(s) and appropriate NCH personnel. Authorship will be determined by mutual agreement. For multicenter studies it is mandatory that the first publication is based on data from all centers, analyzed as stipulated in the protocol by NCH statisticians, and not by the investigators. Investigators participating in multicenter studies agree not to present data gathered from one center or a small group of centers before the full publication, unless formally agreed to by all other investigators and NCH.

NCH must receive copies of any intended communication in advance of publication (at least 15 working days for an abstract or oral presentation and 45 working days for a journal submission). NCH will review the communications for accuracy (thus avoiding potential discrepancies with submissions to health authorities), verify that confidential information is not being inadvertently divulged and provide any relevant supplementary information.

The investigator may be required to sign the clinical study report, if it is to be used in a registration submission to the health authorities of some countries. For multicenter studies only the coordinating (principal) investigator nominated by NCH at the start of the study would provide any needed signature.

0.2.7 Disclosure and confidentiality

By signing the protocol, the investigator agrees to keep all information provided by NCH in strict confidence and to request similar confidentiality from his/her staff and the IRB/IEC/REB. Study documents provided by NCH (protocols, investigators' brochures, Case Report Forms and other material) will be stored appropriately to ensure their confidentiality. The information provided by NCH to the investigator may not be disclosed to others without direct written authorization from NCH, except to the extent necessary to obtain informed consent from patients who wish to participate in the study.

0.2.8 Discontinuation of study

NCH reserves the right to discontinue any study under the conditions specified in the clinical study agreement.

0.3 Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol and the principles of Good Clinical Practice, as described in NCH standard operating procedures and:

1. ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996.
2. Directive 91/507/EEC, The Rules Governing Medicinal Products in the European Community.
3. US 21 Code of Federal Regulations dealing with clinical studies (including parts 50 and 56 concerning informed consent and IRB regulations).
4. Declaration of Helsinki and amendments, concerning medical research in humans (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects). This includes the note of clarification on paragraph 29 accompanying the 2000 Edinburgh version of the Declaration of Helsinki.

The investigator agrees when signing the protocol to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

0.3.1 Institutional Review Board/Independent Ethics Committee

Before implementing this study, the protocol, the proposed informed consent form and other information to patients, must be reviewed by a properly constituted Institutional Review Board/Independent Ethics Committee/Research Ethics Board (IRB/IEC/REB). A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC/REB must be given to NCH before study initiation. The name and occupation of the chairman and the members of the IRB/IEC/REB must be supplied to NCH. Any amendments to the protocol, other than administrative ones, must be approved by this committee.

0.3.2 Informed consent

The investigator must explain to each patient (or legally authorized representative) the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits involved and any discomfort it may entail. Each patient must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the treating physician.

This informed consent should be given by means of a standard written statement, written in non-technical language. The patient should read and consider the statement before signing and dating it, and should be given a copy of the signed document. If the patient cannot read or sign the documents, oral presentation may be made or signature given by the patient's legally appointed representative, if witnessed by a person not involved in the study, mentioning that the patient could not read or sign the documents. No patient can enter the study before his/her informed consent has been obtained.

The informed consent form is considered to be part of the protocol, and must be submitted by the investigator with it for IRB/IEC/REB approval. NCH supplies a proposed informed consent form, which complies to regulatory requirements and is considered appropriate for the study. Any changes to the proposed consent form suggested by the investigator must be agreed to by NCH before submission to the IRB/IEC/REB, and a copy of the approved version must be provided to the NCH monitor after IRB/IEC/REB approval.

0.3.3 Declaration of Helsinki

The investigator must conduct the study in accordance with the principles of the Declaration of Helsinki. A copy of the latest amendment of the Declaration of Helsinki, that includes the note of clarification on paragraph 29 will be provided upon request, or can be found through the following link: www.wma.net/e/policy/17-c_e.html.

Biography of the Investigators

Give biographical data in the following table for key personnel including the Principal Investigator. Use a photocopy of this page for each investigator.

1 Name : Dr. Md. Nur Haque Alam

2 Present position : Scientist, Clinical Sciences Division

3 Educational background (last degree and diploma & training relevant to the present research proposal) : Doctor of Medicine (MD) 1993, Basel University, Switzerland

List of ongoing research protocols
(start and end dates; and percentage of time)

Efficacy of Benefiber added oral rehydration solution in the treatment adult cholera patients	2003-2005	Novartis Nutrition,Bern , Switzerland and University of Basel	$87000	% of time 25%
Introduction of new hypoosmolar ORS for routine use in the treatment of diarrhoeal diaseses	2002-2003	USAID	$106000	15%
Oral rehydration solution containing amylase resistant starch in severely malnourished children with watery diarrhoea due to *Vibrio cholerae*	2001—2003	Nestle Foundation	$159,000	20%

4.1. As Principal Investigator

Protocol Number	Starting date	End date	Percentage of time
2002-026	2002	2003	15
2002-034	2003	2005	25
2001-007	2001	2003	20

4.2. As Co-Principal Investigator

Protocol Number	Starting date	End date	Percentage of time

4.3. As Co-Investigator

Protocol Number	Starting date	Ending date	Percentage of time

5 Publications

Types of publications	Numbers
a) Original scientific papers in peer-review journals	10
b) Peer reviewed articles and book chapters	1
c) Papers in conference proceedings	17
c) Letters, editorials, annotations, and abstracts in peer-reviewed journals	3
c) Working papers	
b) Monographs	

Five recent publications including publications relevant to the present research protocol

1. **Alam NH.** Oral rehydration and hyponatraemia. Lancet (Letter) 1999;354:1733-4.

2. **Alam NH,** Meier R, Schneider H, Sarker SA, Bardhan PK, Mahalanabis D, Fuchs GJ, Gyr K. Partially hydrolysed guar gum supplemented oral rehydration solution in the treatment of acute diarrhoea in children JPGN 2000;21:503-7.

3. **Alam NH,** Ashraf H. Treatment of infectious diarrhoea in children . Pediatr Drugs 2003;5(3):151-165

4. **Alam NH**, Ashraf H, Khan WA, Karim M, Fuchs GJ. Racecadotril in the treatment ofadult cholera Gut 2003;52:1419-1423

5. Alam NH, Hamadani J, Dewan N, Fuchs GJ. Efficacy and safety of a modified oral rehydration solution (ReSoMaL) in the treatment of severely malnourished children with watery diarrhoea. J Pediatr. 2003 Nov;143(5):614-619.

Short Title: ***"Antisecretory factor in adult cholera"***
Funding Source: Novartis

Account Description	Effort %	Monthly rate	Man month	Amount in US$
Personnel				
Dr. NH Alam	30%	1,795	9	4,847
Dr. H Ashraf	10%	1,413	6	848
Dr. Shamima Sattar	10%	767	6	460
Dr. MA Salam	1%	11,412	6	685
Medical Officer (NOA)	50%	741	9	3,332
Research Asstt (2)	100%	328	9	5,904
Study Nurse (2)	50%	160	9	1,441
Health Worker (3)	50%	79	9	1,060
Total Personnel Cost				**18,577**
International Travel				3,500
Total travel				**3,500**
Supplies and Materials:				
Office Stationery				100
Drugs and ORS				260
Non-stock supplies				160
Total Supplies				**520**
Interdepartmental Services				
Pathological Tests				350
Microbiological tests				465
Biochemistry Tests				360
Patients in Study Ward				4,800
Transport				225
Total Interdepartmental Services				**6,200**
Capital Expenditure (Computer, accessories)				1,000
Total Capital				**1,000**
Total Direct Cost				**29,797**
Indirect Cost (25%)				**7,449**
TOTAL PROJECT COST (US$)				**37,246**

S. Hoi
19-Nov-2003

Principal Investigator: Last, first, middle _____

Budget Justifications

Please provide one page statement justifying the budgeted amount for each major item. Justify use of man power, major equipment, and laboratory services.

1. Personnel salaries are requested according to the percentage of work of each person involved in the study.
2. Charge of patients' study include the daily bed charge, clinical care, fluid, diet and drugs.
3. Laboratory costs are estimated as per ICDDR,B laboratory charge for each test.
4. Capital expenditure includes the costs of items considered to be essential to support the study such as computer accessories, file cabinets etc.
5. International travel is requested for dissemination of study findings and other study related matters.

Principal Investigator: Last, first, middle _____

Appendix I

International Centre for Diarrhoeal Disease Research, Bangladesh
Voluntary Consent Form

Title of the Research Project: Efficacy of Salovum egg powder containing antisecretory factor in the treatment of cholera in adults

Principal Investigator: Dr Md. Nur Haque Alam

Before recruiting into the study, the study subject must be informed about the objectives, procedures, and potential benefits and risks involved in the study. Details of all procedures must be provided including their risks, utility, duration, frequencies, and severity. All questions of the subject must be answered to his/ her satisfaction, indicating that the participation is purely voluntary. For children, consents must be obtained from their parents or legal guardians. The subject must indicate his/ her acceptance of participation by signing or thumb printing on this form.

We suspect that you are perhaps suffering from cholera, which is caused by infection of the human intestine by a germ called *Vibrio cholerae*. Patients with cholera may lose huge amounts of water and salts from the body in diarrhoeal stools and vomiting. If not appropriately treated, the amount of loss of body water and salts can be severe, which may lead to deaths of the patients. The main treatment of cholera is correction of the losses of water and salts using appropriate intravenous or oral saline. The usual diet should also be continued, and antibiotic treatment is useful in the treatment of cholera. Although very effective in the correction of fluid and salt losses, oral saline does not reduce the amount of stools. Scientists all over the world are trying to find ways to reduce the stool volume in cholera and other diarrhoeas. Salovum is an egg yolk powder rich in antisecretory factor and expected to reduce the diarrhoeal loss by its antiscretory properties.

We are conducting a research study at this hospital to examine the efficacy of Salovum egg powder in the treatment of adults with cholera. To test if our assumptions are correct, we would recruit 40 patients to be divided into two groups. One group of cholera patients will be treated with Salovum egg powder in addition to standard treatment (ORS, antibiotics and normal diet).The other group will get only the standard therapy. We will compare the volume of stool, the duration of diarrhoea in these two groups of patients. It would be possible to improve the current treatment of cholera if Salovum egg powder is found useful in our study. So far, no adverse effect has been reported with the use of this product.

Since you are suffering from cholera, we are inviting you to participate in this study and help us in our efforts to find better treatment of cholera. If you agree to participate in this study, the followings would be done:

1. We would admit you to a research ward of this hospital until you recover from diarrhoea

2. We will ask you some questions related to your illness and perform your thorough physical examination

3. Following the routine practice of this hospital, we would begin your treatment using an intravenous fluid to be given over 3-4 hours, and collect a sample of your stool for a quick test to determine if you are actually suffering from cholera. If you are not found to have cholera, we will not enroll you in this study and you would receive the standard treatment of this hospital in another ward.

4. If the test reveals cholera, we would finally enroll you in our study, and collect 5.0 ml (one teaspoonful) of blood from a vein on your arm. The blood would be used to perform tests that would help us assess your condition. At the end of the study , again 5.0 ml of blood from vein in the arm will be drawn for similar tests as at enrolment.

5. After completion of treatment with intravenous fluid, we would give you one of the two types of treatment: One group will receive Salovum egg powder in addition to standard therapy and the other group will receive the standard therapy alone. This allocation would be done through a process, called randomization, whereby you would have equal chance to receive either of these treatments.

6. We would enquire about how are you doing and also examine you at least once every day, and collect and measure your stool, urine and vomit separately for each 6-hourly period of your stay at the hospital.

of skin around the needle prick and a remote chance of infection, collection of this amount of blood would cause you no harm. We would use sterile, disposable syringe and needle for collection of blood and also take measures to prevent these problems. You will be benefited from participation in this study since we would more closely observe you in the research ward. You would also be able to help us in improving our knowledge about treatment of cholera and thus benefit the society.

8. Your participation in the study is entirely voluntary. You may refuse to participate in this study and also withdraw your consent at any time during the study, and in either case you would continue to receive the usual good care and treatment of this hospital.

9. All information collected from you and the results of your laboratory tests would be kept confidential, in a secured place, and none other than the investigators of this study, and the Ethical Committee of the ICDDR,B would have an access to that information. Your name or identity would not be used in the analysis of data or in publishing the results of this study.

10. You will be treated free of charge but no compensation will be given for participating in the study except free treatment available in the country for injury to your health that occurs as a result of participation in this study.

11. We would be happy to answer to your question about your illness and about our study now, and you would also be able to communicate with us at the following address in the event you want to have further information or you want to know the results of your laboratory tests.

Dr. N.H. Alam	2332
Dr. H Ashraf	2313
Dr. Shamima	2332/2311

If you agree to participate in the study, please sign or give your thumb impression at the space indicated below.

Signature/thumb impression of the patient	Signature of the Investigator/representative	Signature of witness
Date:	Date:	Date:

আন্তর্জাতিক উদরাময় গবেষনা কেন্দ্র, বাংলাদেশ

স্বেচ্ছায় সম্মতি পত্র

গবেষণার নাম ঃ কলেরা রোগে নিঃসরন বিরোধী বস্তু সমৃদ্ধ স্যালোভাম ডিমের কুসুমের পাউডারের কার্যকারীতা পরীক্ষা

গবেষকের নাম ঃ ডাঃ এন এইচ আলম

আমরা মনে করছি যে আপনি হয়ত কলেরায় ভুগছেন যা ভিবরিও কলেরি নামক এক জীবাণু মানুষের অন্ত্রে সংক্রমণের ফলে হয়ে থাকে। কলেরা রোগীদের পাতলা পায়খানা ও বমিতে শরীর থেকে বিপুল পরিমাণে পানি ও লবণের ক্ষয় হয়। যদি সঠিকভাবে চিকিৎসা করা না হয় তাহলে এই বিপুল পরিমান পানি ও লবণের ক্ষয় মারাত্মক আকার ধারণ করতে পারে এবং মৃত্যুও ঘটতে পারে। রক্তের শিরায় স্যালাইন বা খাবার স্যালাইন দিয়ে পানি ও লবণের ক্ষয় পূরণ করাই কলেরার প্রধান চিকিৎসা। খাওয়া দাওয়া নিয়মিত চালিয়ে যেতে হবে এবং এ্যানটিবায়োটিক চিকিৎসাও বেশ উপকারী। যদিও খাবার স্যালাইন পানি ও লবণের অভাব কমাতে খুব কার্যকর। কিন্তু এটা পায়খানার পরিমান কমাতে পারে না। সারা বিশ্বে বিজ্ঞানীরা কলেরার কারনে পাতলা পায়খানা এবং অন্যান্য ডাইরিয়া রোগের তীব্রতা কমানোর উপায় বের করতে চেষ্টা করছেন। আশা করা হচ্ছে নিঃসরণ বিরোধী প্রোটিনে পরিপূর্ণ স্যালোভাম নামক এক ডিমের কুসুমের গুঁড়ো ডাইরিয়া কমাতে কার্যকরী হবে।

আমরা এই হাসপাতালে একটি গবেষণা কার্যক্রম পরিচালনা করছি পূর্ণবয়স্কদের চিকিৎসায় স্যালোভাম কুসুমের গুঁড়োর কার্যকারিতা পরীক্ষা করার জন্য। আমাদের অনুমান ঠিক কি না তা পরীক্ষা করার জন্য ৪০ জন রোগী নেওয়া হবে যাদেরকে দুটি দলে ভাগ করা হবে। কলেরা আক্রান্ত রোগীর একটি দলকে স্যালোভাম কুসুমের গুঁড়োর সাথে প্রচলিত চিকিৎসা (ওরালস্যালাইন, এ্যানটিবায়োটিক ও স্বাভাবিক খাওয়া) দেওয়া হবে। আর অন্য দলটিকে শুধু প্রচলিত চিকিৎসা দেওয়া হবে। আমরা এ দুটি দলের মধ্যে তাদের পায়খানার পরিমাণ, ডাইরিয়ার মেয়াদকাল তুলনা করব। যদি স্যালোভাম ডিমের কুসুমের গুঁড়োর উপকারিতা পাওয়া যায় তাহলে এ চিকিৎসায় উন্নতি সাধন সম্ভব হবে। এ পর্যন্ত এ খাদ্য বস্তুটির কোন ক্ষতিকর প্রতিক্রিয়া প্রকাশিত হয়নি।

যেহেতু আপনি কলেরায় ভুগছেন, তাই আমরা আপনাকে এ গবেষণা কার্যক্রমে অংশগ্রহণ করতে আমন্ত্রণ জানাচ্ছি এবং আমাদেরকে ভাল চিকিৎসা বের করতে সাহায্য করতে। যদি আপনি এ গবেষণায় অংশগ্রহণ করতে রাজী হন, তাহলে নিম্নেবর্ণিত কাজগুলি করা হবে ঃ

১। আপনাকে একটি গবেষণা ওয়ার্ডে ভর্তি করা হবে যতক্ষণ পর্যন্ত না আপনি সুস্থ হন।

২। আমরা আপনাকে আপনার রোগসংক্রান্ত কিছু প্রশ্ন জিজ্ঞেস করব এবং শারীরিক কিছু পরীক্ষা সমাধা করব।

৩। এ হাসপাতালের নিয়মানুযায়ী আমরা ৩-৪ ঘন্টা ধরে আপনার রক্তের শিরায় স্যালাইন দিয়ে চিকিৎসা শুরু করব এবং আপনি সত্যিই কলেরায় আক্রান্ত কি না তা নির্ণয় করার জন্য পায়খানার একটি নমুনা নিব এবং একটি দ্রুত পরীক্ষা চালাব। আপনি যদি কলেরা আক্রান্ত না হন, তাহলে আপনাকে এ গবেষণায় অন্তর্ভুক্ত করা হবে না এবং অন্য ওয়ার্ডে আপনাকে প্রচলিত চিকিৎসা দেওয়া হবে।

৪। যদি পরীক্ষায় প্রমাণ হয় যে আপনি কলেরা আক্রান্ত তাহলে আপনাকে এ গবেষণা অন্তর্ভুক্ত করা হবে এবং পাঁচ মিঃ লিঃ (এক চা চামচ) রক্ত আপনার বাহুর শিরা থেকে সংগ্রহ করা হবে। আপনার অবস্থা নির্ণয় করার জন্য সেই রক্ত দিয়ে কিছু পরীক্ষা করা হবে। গবেষণার শেষে আবার বাহুর শিরা থেকে পাঁচ মিঃ লিঃ রক্ত বের করে পরীক্ষা করা হবে।

৫। রক্তের শিরায় স্যালাইন দিয়ে চিকিৎসা শেষ হলে আমরা আপনাকে দুটোর যে কোন একটি চিকিৎসা দিব : একটি দলকে স্যালোভাম কুসুমের গুঁড়ো সাথে প্রচলিত চিকিৎসা দেওয়া হবে এবং আরেকটি দলকে শুধু প্রচলিত চিকিৎসা দেওয়া হবে। র‍্যানডম পদ্ধতির মাধ্যমে রোগীরা কোন দলে যাবে সেটি নির্ধারণ করা হবে যেখানে সবারই সমান সুযোগ থাকবে যে কোন একটি দলে যাওয়ার।

৬। আপনার শরীরের অবস্থা কি হচ্ছে এ ব্যাপারে আমরা আপনার খোঁজ খবর নেব এবং প্রতিদিন কমপক্ষে একবার আপনাকে পরীক্ষা করব। হাসপাতালে থাকা অবস্থায় প্রতি ছয় ঘন্টা পর পর আপনার পায়খানা, প্রস্রাব ও বমি আলাদা আলাদা করে সংগ্রহ করে মাপা হবে।

৭। আশা করা হচ্ছে স্যালোভাম খাদ্যটি নিরাপদ এবং যে পরিমানে এটি গবেষণায় ব্যবহার করা হবে তা ক্ষতিকর নয়। রক্ত সংগ্রহের জন্য সূঁচ ফোঁড়ানোর কারনে অল্প ও সাময়িক ব্যথা, সূঁচ ফোঁড়ের চারদিকের ত্বকের রঙের হালকা পরিবর্তন এবং সংক্রমণ হওয়ার খুব মৃদু সম্ভাবনা ছাড়া এই রক্ত সংগ্রহ অন্য কোন ক্ষতি করবে না। আমরা জীবাণুমুক্ত এবং একবার ব্যবহারযোগ্য সূঁচ ও সিরিঞ্জ ব্যবহার করব এবং অসুবিধা দূর করার জন্য পদক্ষেপ নিব। যেহেতু রিসার্চ ওয়ার্ডে আপনাকে অনেক কাছ থেকে নজর রাখা হবে তাই এ গবেষণায় অংশগ্রহণ করে আপনি লাভবান হবেন। কলেরার চিকিৎসা সম্পর্কে আমাদের জ্ঞানের উন্নতি সাধন করতে সহায়তা করায় আপনি এ সমাজের উপকার সাধন করবেন।

৮। এ গবেষণায় আপনি পুরোপুরি স্বেচ্ছায় অংশগ্রহণ করবেন। আপনি যে কোন সময় এ গবেষণা থেকে বিরত থাকতে পারেন এবং আপনার সম্মতি প্রত্যাহার করতে পারেন এবং যে কোন অবস্থাতেই এ হাসপাতালে আপনার চিকিৎসা সেবা বহাল রাখা হবে।

৯। আপনার সবরকম তথ্য এবং ল্যাবরেটরী পরীক্ষা একটি নিরাপদ স্থানে গোপন রাখা হবে এবং গবেষণাকারীরা ও আই সি ডি ডি আর বির ইথিকাল কমিটি ছাড়া এ তথ্য অন্য কেই জানবে না। ডাটা অ্যানালাইসিস ও এই গবেষণার ফলাফল প্রকাশে আপনার নাম ও পরিচয় ব্যবহার করা হবে না।

১০। গবেষণায় অংশগ্রহণ করার জন্য বিনা পয়সায় চিকিৎসা ছাড়া অন্য কোন ক্ষতিপূরণ আপনাকে দেওয়া হবে না। তবে অবশ্যই গবেষণার কারণে স্বাস্থ্যের কোন ক্ষতি হলে বিনা পয়সায় দেশে প্রচলিত চিকিৎসা দেওয়া হবে।

১১। যে কোন সময় আপনার অসুখ ও আমাদের গবেষণা সম্পর্কে আপনার প্রশ্নের জবাব দিতে আমরা খুশি হব, এবং আরও তথ্য বা ল্যাবরেটরী পরীক্ষার ফলাফল যদি আপনি জানতে আগ্রহী হন তবে নিম্নেলিখিত ফোন নম্বরে যোগাযোগ করতে পারেন।

ডাঃ এন এইচ আলম ২৩৩২

ডাঃ এইচ আশরাফ ২৩১৩

ডাঃ শামিমা সাত্তার ২৩৩২/২৩১১

আপনি যদি এই গবেষণায় অংশগ্রহণ করতে চান তাহলে নিম্নে আপনার স্বাক্ষর অথবা টিপ সহি দিন।

গবেষকের স্বাক্ষর	স্বাক্ষীর স্বাক্ষর	অংশগ্রহনকারী রোগীর/ অভিভাবকের স্বাক্ষর/বৃদ্ধাঙ্গুঁলির ছাপ

Abstract Summary for Ethical Review Committee

Efficacy of Salovum egg powder containing antisecretory factor (AF) in the treatment of severe cholera in adult

Cholera is potentially a severe disease characterized by profuse watery diarrhoea leading to severe dehydration, circulatory collapse and death if not treated properly and timely. Since cholera involves stimulation of secretory process, efforts to identify and test potential antisecretory agents in order to reduce the severity of diarrhoea and consequently reduced need for hospital admission, rehydration fluids seem logical. Until now, none of the antisecretory agents has been recommended for the treatment of cholera. Antisecretory factor is a naturally occurring protein produced in the intestine with antisecretory properties demonstrated in animal and human studies including in several clinical trials. The salovum egg yolk powder contains antisecretory proteins in a much higher (500 times) concentration than that found in normal hen eggs. This is achieved by feeding hens with specially processed cereals, capable of inducing secretion of antisecretory proteins in the yolk from which an egg yolk powder is produced. Thus this product has the potential to reduce the severity of diarrhoea in patients with cholera. Therefore, we are proposing this study to assess the efficacy of salovum egg powder containing antisecretory factor in the treatment of cholera.

1. Adult cholera patients will be enrolled in the study.
2. There is no risk involved in this study except some discomfort during blood drawing time.
3. Not applicable.
4. The investigators will handle all information collected from the patients. Records will not be used for any other purposes except to fulfill the study objective.
5. Signed informed consent will be obtained from the patients after explaining the objectives, procedures, benefits and risks of the study.
6. Patients' information will be taken by interviewing as part of the routine management of diarrhoea in this hospital. No extra interview for this study will be required.
7. This study will not cause any risk to the patients, the patients will be benefited as part of receiving routine treatment of this hospital. The study results if found effective will help in improving the patient management.
8. The study will require only the hospital records (patients' history, intake and output record, laboratory results which will be done for routine management of the patients; no extra investigations will be performed for this study).

Annexure II: Assessment of Dehydration (Dhaka Method)

Assess	Condition*	Normal	Irritable/Less Active*	Lethargic/Comatose*
	Eyes	Normal	Sunken	
	Mucosa	Normal	Dry	
	Thirst*	Normal	Thirsty*	Unable to Drink*
	Skin turgor*	Normal	Reduced*	
	Radial Pulse*	Normal		Uncountable or Absent*
Diagnose		**No Sign of Dehydration**	If at least 2 signs including one (*) sign is present, diagnose **Some Dehydration**	If some dehydration plus one of these (*) signs are present, diagnose **Severe Dehydration**

Response to Reviewer # 1

Q.1. This is a new product with relatively little published information on humans. The authors have carefully planned to monitor adverse effects. Therefore, one of the primary objectives of the study should be "to evaluate the safety of the product".

Ans. The study material although is a new product has been classified as food for special medical purposes. No serious adverse events have been reported so far. Therefore, evaluation of safety parameters has been considered under secondary objective.

Q.2. Page 7- Study Interventions - Is it possible to request the manufacturer of AF to manufacture a placebo powder? This will greatly strengthen the study findings.

Ans. We have checked with the manufacturer of AF factor for preparation of a placebo. At this moment, they are unable to prepare a placebo. But for the full protocol they can make it later on.

Q.3. Page 7 - Route & Dosing - What is the rationale for monitoring subjects for only 72 hours? Is this based on previous data?

Ans. Yes, based on previous studies, we have decided for monitoring the subjects for period of 72 hrs.(page 9)

Q. 4. Page 8, Line 7 - Undesired side effect - The investigators need to define this further. For example, one would not discontinue treatment if a few patients complained of headache. However, if any patient experienced seizures or severe anaphylactic reaction, they will probably want to discontinue the intervention.

Ans. As suggested the `undesired side-effect' for discontinuation of study has been defined as 'that interfere with patients normal health or any medication is needed for this undesired side-effects' - this has been incorporated in the protocol (page 10).

Q.5. Page 10, line 8 - Define ``High purging rate".

Ans. `High purging rate' has been defined as stool output >10ml/kg/hr - has been incorporated in the protocol(page 12).

Q.6. Page 11 - Secondary efficacy parameters - Severe abdominal cramps - duration should be recorded.

Ans. Although severe abdominal cramps is uncommon in cholera, as suggested we have included severe abdominal cramps and its duration in the secondary efficacy parameters (page 13).

Q.7. Page 12 - Adverse events - I found the second paragraph confusing. How can you have an adverse event before entering the study? I would suggest that individuals who have signs and symptoms that could be considered to be serious adverse event (if they were in the study), be excluded from enrollment.

Ans. 'Adverse events' before starting the study intervention, here we mean any associated symptoms (eg. headache, fever, abdominal cramps etc.) other than diarrhoea and vomiting in cholera patients. Patients with serious adverse events will not be considered for inclusion in the study.

Response to Reviewer # 2

Q.1. A biography of Charles Larson was included in the package; there was nothing for Dr. Alam.

Ans. We are sorry for the mistake, now the CV of the PI has been included in the protocol.

Q.2. A major reference is omitted: Salazar et al, NEJM, study of racecadotril in children with acute diarrhea. (I don't have the exact ref with me.)

Ans. As suggested by the reviewer, the mentioned paper has been cited in the protocol (Ref. No. 32).

Q.3. Even though this is an open study, there should be some reason for the numbers chosen.

Ans. This is a new product and there is no efficacy data in the treatment of cholera with this product. So, we have decided for a pilot study and sample size determination is an arbitrary. If we find any reduction of stool output, a full protocol will be developed with adequate sample size.

Q.4. Is it wise to exclude women from this study? I would think that possible side effects would also show up in women. Certainly the duration of diarrhea could be measured, as well as fluid requirements and need for extra ivs. All aims could be studied except for the volume of stool, and this could probably be done adequately enough in this type of pilot study.

Ans. As the sample size is small and primary outcome variable is stool output, and there is a doubt of separation of stool output in female patient, so excluding women in this study is logical. For full protocol we can include women also.

Q.5. Exclusion criteria; receiving antimicrobials for how long before admission?

Ans. Exclusion criteria; 'Receiving antimicrobial for within 1 week prior to admission' - incorporated in the protocol (page 7).

Q.6. Page 6; the composition of iv fluids is given, but not that of ORS.

Ans. Composition of ORS has been incorporated (page last 8 line).

Q.7. Probably the most important: How was the dosage established? It seems somewhat arbitrary, and no rationale is given for using these doses at these intervals. This needs to be further defined.

Ans. Yes, the dosage decided partially arbitrary and it is based on previous study (The dose used 4grams, 4 times daily for 14 days in inflammatory bowel disease)
Since cholera is a severe disease we have decided frequent dosing for at least first 24 hrs.

Q.8. Stools should be cultured for ETEC as well as the other enteric pathogens listed. ETEC can give a clinical picture almost identical to cholera.

Ans. We agree that ETEC can mimic cholera. We will include only cholera after examining dark field microscopy. Since this is a pilot study and tests for ETEC is time consuming and

Reviewer 1

Title: Efficacy of Antisecretory Factor (AF) in the Treatment of Severe Cholera in Adult

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of project	x		
Adequacy of project design	x		
Suitability of methodology	x		
Feasibility within time period	x		
Appropriateness of budget	x		
Potential value of field of knowledge	x		

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification (MINOR)

- on technical grounds ☑

- on level of financial support ☐

I do not support the application ☐

Date: 10/30/03

Reviewer 1

Detailed Comments

Please briefly provide your opinions of this proposal, giving special attention to the originality and feasibility of the project, its potential for providing new knowledge and the justification of financial support sought; include suggestions for modifications (scientific or financial) where you feel they are justified. (Use additional pages if necessary)

Title: Efficacy of Antisecretory Factor (AF) in the Treatment of Severe Cholera in Adult

PI: Dr NH Alam

Reviewer:

1. This is a new product with relatively little published information on humans. The authors have carefully planned to monitor adverse effects. Therefore, one of the primary objectives of the study should be "to evaluate the safety of the product".
2. Page 7 – Study Interventions- Is it possible to request the manufacturer of AF to manufacture a placebo powder? This will greatly strengthen the study findings.
3. Page 7 – Route & Dosing- What is the rationale for monitoring subjects for only 72 hours? Is this based on previous data?
4. Page 8, Line 7 – Undesired side effect – The investigators need to define this further. For example, one would not discontinue treatment if a few patients complained of headache. However, if any patient experienced seizures or severe anaphylactic reaction, they will probably want to discontinue the intervention.
5. Page 10, line 8 – Define "High purging rate".
6. Page 11 – Secondary efficacy parameters – Severe abdominal cramps - duration should be recorded.
7. Page 12 – Adverse events – I found the second paragraph confusing. How can you have an adverse event before entering the study? I would suggest that individuals who have signs and symptoms that could be considered to be serious adverse even: (if they were in the study), be excluded from enrollment.

1 Reviewer 2. Dr. W.H. Alam

Title: Efficacy of Antisecretory Factor (AF) in the Treatment of Severe Cholera in Adult

Summary of Referee's Opinions: Please see the following table to evaluate the various aspects of the proposal by checking the appropriate boxes. Your detailed comments are sought on a separate, attached page.

	Rank Score		
	High	Medium	Low
Quality of project		✓	
Adequacy of project design		✓	
Suitability of methodology	✓		
Feasibility within time period	✓		
Appropriateness of budget	✓		
Potential value of field of knowledge	✓		

see my notes

CONCLUSIONS

I support the application:

a) without qualification ☐

b) with qualification

- on technical grounds ☒
- on level of financial support ☐

I do not support the application ☐

Name of Referee: R. Bradley Sack MD

Signature: R. Bradley Sack MD Date: Oct 28 03

Position: Professor

Institution: Johns Hopkins Bloomberg School of Public Health

Address: (full postal address)

Reviewer. 2

Dr. N. H. Alam

October 28, 2003

Efficacy of Antisecretory Factor (AF) in the Treatment of Severe Cholera in Adults,
PI: Dr. NH Alam

The proposal is an intriguing one and sounds like it is worth pursuing. I have a number of questions however, that I think need to be addressed.

1.A biography of Charles Larson was included in the package; there was nothing for Dr. Alam.

2. A major reference is omitted: Salazar et al, NEJM, study of racecadotril in children with acute diarrhea. (I don't have the exact ref with me.)
3. Even though this is an open study, there should be some reason for the numbers chosen.
4. Is it wise to exclude women from this study? I would think that possible side effects would also show up in women. Certainly the duration of diarrhea could be measured, as well as fluid requirements and need for extra iv's. All aims could be studied except for the volume of stool, and this could probably be done adequately enough in this type of pilot study.
5. Exclusion criteria; receiving antimicrobials for how long before admission?
6. Page 6; the composition of iv fluids is given, but not that of ORS.
7. Probably the most important: How was the dosage established? It seems somewhat arbitrary, and no rationale is given for using these doses at these intervals. This needs to be further defined.
8. Stools should be cultured for ETEC as well as the other enteric pathogens listed. ETEC can give a clinical picture almost identical to cholera.

The protocol is a standard one used at ICDDR,B and should answer the question being asked. But we need the rationale for the dose.

Principal Investigator: Last, first, middle _____

Check List

After completing the protocol, please check that the following selected items have been included.

1. Face Sheet Included [√]
2. Approval of the Division Director on Face Sheet [√]
3. Certification and Signature of PI on Face Sheet, #9 and #10 [√]
4. Table on Contents [√]
5. Project Summary [√]
6. Literature Cited [√]
7. Biography of Investigators [√]
8. Ethical Assurance [√]
9. Consent Forms [√]
10. Detailed Budget [√]

English translation Salovum notification according to FSMP regs

11.10.2001

Livsedelsverket
Box 622 751 26 Uppsala

Notification of product according to SLVFA 2000:15

The product Salovum egg powder with a high content of anti-secretory proteins is hereby notified according to SLVFS:15.

Information about labelling is shown on the attached packaging and non-complying minerals and vitamins are shown on the attachment.

With best wishes

.....

Attachment to notification for Foods for Special Medical Purposes
Salvoum egg powder with high content of anti-secretory proteins

Salovum egg protein with a high content of anti-secretory proteins exceeds the maximum values in SLVFS 2000:15 attachment 3 tables 3 and 4 for vitamin A, vitamin B12, biotin, vitamin E and the minerals phosphorus, selenium and fluoride.

The product is based on eg yolk which causes this non-compliance.

Stockholm 11.10.2001

Approval of Salovum in Sweden (added by Alison)

National Food Administration

26.11.2001

Notification of sale of Food for Special Medical Purposes

The National Food Administration have received the notification from AF Factor AB of the sale of the product Salovum as a Food for Special Medical Purposes with the following area for use:

- For dietary treatment of complaints of increased intestinal secretions

The notification c0omplies with The National Food Administration's regulations (SLVFS 2000:15) on Food for Special Medical Purposes.

This confirmation does not mean that the National Food Administration have approved the labelling of the product. For more information on the requirements for labelling see relevant regulations

.....

AS-FAKTOR AB

2001-10-11

Livsmedelsverket
Box 622
751 26 UPPSALA

Anmälan av produkt enligt SLVFS 2000:15

Härmed anmäles Salovum äggulepulver med hög halt antisekretoriska proteiner enligt SLVFS:15.

Uppgifter om märkning framgår av bifogat förpackning samt avvikande mineraler och vitaminer enligt bilaga.

Med vänliga hälsningar

AS-Faktor AB

Lars Sjöstrand/

Carola Lindholm

AS-FAKTOR AB
Box 30192, S-104 25 Stockholm
Besöksadress: S:t Göransgatan 160A
Org.nr: 556530-9720
Tel: +46-8 657 42 70
Fax: +46 8 618 69 32

AS-Faktor AB

Bilaga till anmälan för livsmedel för speciella medicinska ändamål
Salovum äggulepulver med hög halt antisekretoriska proteiner

Salovum äggulepulver med hög halt antisekretoriska proteiner överskrider maxvärdena i SLVFS 2000:15 bilaga 3 tabell 3och 4 för vitamin A, Vitamin B12, Biotin , vitamin E samt mineralerna fosfor, selen och fluor.

Produkten är baserad på äggula vilket medför denna avvikelse.

Stockholm 2001-10-11

Carola Lindholm

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AS-Faktor AB

CONFIDENTIAL

Product Review
Salovum®, SPC - Enteral
Summary

The AF protein and technology is a significant commercial opportunity for the following reasons:

- With potent anti-secretory and anti-inflammatory properties, AF appears to be a fundamental regulator of pathologic fluid secretion, with potential for multiple therapeutic applications

- The suggested indications serve large markets in the areas of IBD, IBS, MB Ménière and inflammatory disease .

- The intellectual property position around AF is regarded as strong

- AF has the promise of high commercial value

- Salovum is a egg yolk powder from hens, which have been fed with a patented feed so the content of anti-secretory proteins in the egg yolk is approximately 500-750 times higher than in "normal" egg yolk.

  SPC-Enteral is a product produced with a high content of SPC (special processed cereals), designed for enteral feeding.
  SPC-Enteral "trigger" the body to produce more protein AF.

- Contrary to most larger proteins, human and animal data show that AF is readily absorbed and has high bioavailability.

- A 100% evolutionary conservation at the amino acid level across all mammalian species examined to date, suggest an important physiological role for AF

- The clinical value of high endogenous AF levels has been demonstrated in livestock as well as humans.

The Business Opportunity

This proprietary technology evolves around the antisecretory (AF) protein, a fundamental regulator of pathological fluid secretion with potent antisecretory and anti-inflammatory properties. As well known a pathological fluid secretion is associated with a large number of diseases, therefore we can see a range of potential therapeutic indications, particularly in the large gastrointestinal, Mb Ménière and inflammatory markets which can be treated with Salovum ® and SPC-Enteral as "food" for special medical purposes.

The path to market is expected to be short since the products are ready for production at a large scale and are approved by the Swedish Food Agency according to EU directive 1999/21/EC (Food for special medical purposes i.e medical food).

It should be noted that we already have commercialised several aspects of this technology, including animal feed and human functional food products (MagiForm®) capable of stimulating endogenous AF levels.

Background

In the mid 1980's Sweden banned the addition of antibiotics to animal feed. As a result, Lantmännen, a farmers co-operative, began investigating alternative ways of controlling diarrhoea disease in piglets. Research by Drs. Stefan Lange and Ivar Lönnroth, assistant professors at the University of Gothenburg, eventually discovered that a single naturally occurring protein is extremely potent in regulating intestinal fluid secretion. They termed this protein "antisecretory factor" (AF).

It was eventually discovered that the protein's potent regulation of pathological fluid secretion is not restricted to the intestine, but rather appears to be involved in fluid regulation in a variety of secretary organs. Today, Lantmännen sell animal feed, which is hydrothermally processed and capable of increasing endogenous AF levels. Similarly, it has recently developed a line of functional food, which include such items as muesli, rusks, bread and pasta, also manufactured from hydrothermally processed grains. These products are sold in health food stores/pharmacy and are intended for people with GI disorders.

Although it is apparent that the protein itself has significant potential as a human therapeutic, AS-Faktor AB a wholly owned subsidiary of Lantmännen, is seeking to out-license the rights for the egg yolk powder and SPC-Enteral for clinical nutritional use in human. Exclusive rights to the technology are held by AS-Faktor.

The AF protein - Proof-of-Principle

AF is a naturally occurring primary regulator of fluid secretion with an ED50 of 1-5 pmol in an antisecretory animal model, arguably the most potent regulatory peptide known to man. The protein is produced in the brain, as well as in secretory organs such as the gallbladder, the lungs, the kidneys and the intestine. AF appears to be stored in cytoplasmic vesicles, and the mode of action is thought to be autocrine.
Although the native protein is 382 amino acids, antisecretory activity is retained in an eight amino acid (8-aa) N-terminal fragment. Preclinical animal studies have shown that the 8-aa peptide can be used prophylactically and therapeutically in the treatment of various forms of diarrhoea. The peptide has effects on intestinal permeability as well as chloride secretion, which may be fundamental to the mechanism of action. These animal studies also demonstrated that AF has strong anti-inflammatory properties.

The synthesised peptid is developed by Biofactor Therapheutic AB a company where AS-Faktor are the major owner together with a venture capital group.

Clinical Experience

Trials with egg yolk powder in humans have shown that it is very effective both for secretion and inflammation. The egg yolk is used as a acute treatment and in combination with SPC-Enteral for a longer period.
We have so far completed clinical trials with

* carcinoids
* short bowels
* acute colitis
* Mb Ménière

They will or have been published during this year.

We have on going studies in Mb Chron, Ménière, pediatric sector and have started pilot trials in the inflammatory sector e.g. RA.

A strong correlation between endogenous blood levels of AF and the severity of diarrhoea has been demonstrated in both animals and humans. Likewise, patients with active inflammatory bowel disease have no AF in the intestine, but during periods of remission, AF levels return to a more normal level. Consistent with this observation, a recent double-blind placebo-controlled study with Lantmännens' functional food demonstrated that IBD patients receiving AF-inducing food had higher levels of endogenous AF and significantly reduced disease scores.

Intellectual Property Position

The egg yolk powder and the SPC-Enteral products have a strong patent protection. The egg yolk have recently got a Swedish patent and applications have been filed in appr 70 countries (PCT/SE99//02340).
The SPC-products have patent granted in several countries and the process is on going (PCT/SE97/01918).

Potential Clinical Indications

In view of its apparent central role in the regulation of pathological fluid secretion, AF could be therapeutically useful in numerous indications. In GI disease, AF may be used to treat diarrhoea disease of various etiologies (AIDS-related, IBS-related, carcinoid etc). Furthermore, results from animal studies, as well as the above-mentioned clinical trials in humans, suggests that there are more indications that could benefit from treatment with those products.

The Potential Market

All of the indications mentioned above lead us to believe that the market is large. The AF egg yolk powder and SPC-Enternal products have the possibility to play an important role in the dietary treatment therapy of those indications in the future.

The Path Forward

The scientists connected to AS-Faktor has conducted extensive quality research at the academic level over the last years. AS Faktor AB have together with the scientists developed a range of products with unique results. The products are easy to use for the patients, and for more than 5 years there have been no side effects reported.

AS Faktor would prefer one exclusive partner to market the products for the clinical nutritional use.

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ORIGINAL ARTICLE

Taylor&Francis
healthsciences

Effect of Antisecretory Factor in Ulcerative Colitis on Histological and Laborative Outcome: a Short Period Clinical Trial

A. Eriksson, M. Shafazand, E. Jennische & S. Lange
Depts. of Internal Medicine and Clinical Bacteriology, Sahlgren's University Hospital, Göteborg, Sweden; Dept. of Anatomy and Cell Biology, University of Göteborg, Sweden

Eriksson A, Shafazand M, Jennische E, Lange S. Effect of antisecretory factor in ulcerative colitis on histological and laborative outcome: a short period clinical trial. Scand J Gastroenterol 2003;38:1045–1049.

Background: The antisecretory factor (AF) is a 41 kD endogenously produced protein capable of mediating protection against diarrhoea diseases and intestinal inflammation. High concentrations of AF-like proteins are present in egg yolk, and AF can consequently be administrated in the form of egg yolk drinks. In this study, performed in patients suffering from acute onset of ulcerative colitis (UC), we evaluate the influence of orally administrated AF on the histological and clinical laboratory outcome. **Methods:** A total of 20 patients fulfilled this prospective, double-blind and randomized protocol. The intake of AF was used as an additive treatment to conventional UC medication. Patient registrations were extended to two outward visits, performed 2–4 and 8–12 weeks after hospital discharge. **Results:** During AF treatment, a reduction in the histological severity from mucosal biopsies received from the mid-rectum was found. In addition, a lowering in the inflammatory blood parameters ESR, CRP and orosomucoid was demonstrated. **Conclusion:** In the AF-treated group a late and significant lowering of various inflammatory parameters combined with a histological recovery was demonstrated. These findings suggest that administration of AF mediates a long-lasting anti-inflammatory effect in cases of acute UC.

Key words: Antisecretory factor; functional food; immunohistochemistry; inflammation; ulcerative colitis

Anders Eriksson, Dept. of Internal Medicine, Gastroenterology Unit, Sahlgren's University Hospital/ Östra, SE-416 85 Göteborg, Sweden (fax. +46 31 259254, e-mail. anders.s.eriksson@vgregion.se)

Ulcerative colitis (UC) is characterized by a chronic mucosal inflammation and epithelial dysfunction involving the rectum and to various extents the colon. The symptoms consist of diarrhoea, bloody stools, abdominal pain, fewer and decreased well being. Acute ulcerative colitis can be a life-threatening condition and requires extensive symptomatic therapy. At present, no curative therapy is available. Consequently, even death due to UC, cannot be fully prevented (1). Patients refractory to the conventional acute treatment will finally be obliged to undergo colectomy. So far, neither aetiology nor pathogenesis has been specifically defined. Despite pharmacological treatment of patients suffering from UC, prospective studies show that about 50% relapse within 12 months (2, 3). Today, valid evidence of the dietary component involvement in the pathogenesis of UC is still missing. High intake of mono- and polyunsaturated fatty acids and of vitamin B6 has been proposed as being involved in the pathogenesis of UC (4). Some reports also indicate that balanced supplementary diets may be beneficial for the outcome in UC (5–8), whereas, others do not (9).

The antisecretory factor (AF) (10), present in most of the mammalian organs (11), is a 41-kD protein originally found to inhibit experimental intestinal hypersecretion (12). The protein has been cloned (13) and the active, i.e. antisecretory, site of the protein has been located (14). Stimulation of endogenous AF synthesis has been registered in man (15) as well as in animals (16). The endogenous synthesis of the AF protein may be linked to exposure of intestinal pathogens, since it has been demonstrated that high AF levels are present in breast-milk from Pakistani mothers, whereas, considerably lower levels have been detected in milk from lactating Swedish women (17). Experimentally, stimulation of endogenous AF synthesis can be achieved after intestinal challenge with bacterial toxins (12), after peroral intake of sugar and amino acids (16) or after intake of specially processed cereals (SPC) (15). High concentrations of AF-like proteins have also been demonstrated in egg yolk (18). Antisecretory factor has been found to decrease experimental intestinal inflammation (19), but also inflammation in patients suffering from inflammatory bowel disease (IBD) (15). During the inflammatory state of IBD, decreased immunoreactivity for AF peptide has been registered in the colonic mucosa (15). The endogenous stimulation of AF synthesis,

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Table I. Numerical clarification of the patients included and excluded/dropouts

	No. of included patients Egg yolk treatment $n = 16$	Placebo treatment $n = 19$
Dropouts	$n = 1$	$n = 3$
Mb Crohn	$n = 1$	$n = 1$
Dead		$n = 1$ (ischaemic heart disease)
Acute colectomy	$n = 4$	$n = 4$
Completed	$n = 10$	$n = 10$

achieved by intake of special processed cereals (SPC), has been found to increase the clinical performance of patients suffering from short bowel syndrome (20) and also from Crohn disease (21).

AF therapy can therefore be achieved either actively by intake orally of SPC (i.e. stimulation of endogenous AF synthesis) or by intake of AF protein in the form of AF containing egg yolk drinks (10, 21, 22).

The present study was undertaken in patients suffering from severe exacerbation of UC. The study design was prospective and double blind. The working hypothesis was to evaluate whether AF therapy can improve the laboratory and clinical outcome in a short period of time.

Methods

Patients

The ethics committee at Göteborg University approved the study protocol. A prospective and double-blind technique was used and all patients were included after admittance to the ward because of a severe exacerbation of extensive UC (colonoscopy verified). Patients below 18 or above 70 years, pregnancy, hypersensitivity to egg, and urgent need of colectomy or expected inability to follow the protocol were excluded. The patients were randomized for comparison of AF versus placebo treatment in parallel to the standardized medical treatment (see below).

Thirty-five subjects (men $n = 20$, women $n = 15$) were included in consent to the study protocol (Table I) and 20 fulfilled the requirements of evaluation.

Treatment protocol

The standardized, medical treatment comprised intravenous betamethasone (0.06 mg/kg b.w. administered twice daily), local enema of prednisolone (0.25 mg/mL, 125 mL) given twice daily for 2 weeks and thereafter only at night for a further 2 weeks), sulfasalazin orally (14 mg/kg b.w. three to four times daily) and total parenteral nutrition (Kabimix basal[09] with addition of water- and fat-soluble vitamins and trace elements, 2560 mL/day). The use of parenteral nutrition lasted until the stools were macroscopically free of blood and numbered <5 per day. At this point in time, the parenteral betamethasone was changed to per oral prednisolone (0.6 mg/kg) followed by a scheduled decrease of the daily dose. If there were no side effects, sulfasalazin was used continuously for at least 3 months. All patients were clinically examined, including evaluation of blood samples, at 2 follow-up visits (3–5 and 12–13 weeks) after inclusion.

All patients have a run-in period of 48 h with standardized treatment before beginning intake of AF egg yolk drinks (2 g, 4 times daily for 14 days) or placebo treatment.

Antisecretory factor

In this study, spray-dried egg powder was used (AS Faktor AB, Stockholm, Sweden). The antisecretory activity of the egg yolk was tested in the rat ligated ileal loop assay, as described in detail previously (18). The antisecretory activity displayed by the control egg yolk was found to be non-significant (i.e. <0.5 AF units tested in a 1:10 dilution), while the AF egg yolk presented significant AF values, i.e. between 1.0 and 1.5 AF units, when tested in a dilution of 10^3. The egg yolk drinks were prepared by dissolution of 2 g egg powder in 10 mL of orange juice before intake.

Histology

Rectal mucosal biopsies from the mid-posterior portion of the rectum were taken at admittance and at the first outward visit. The biopsies were immersed in 4% paraformaldehyde, frozen in liquid nitrogen and later cryosectioned (6 μm). For histological examination, the sections were stained with haematoxylin (htx)/eosin and Periodic acid schiff (PAS)/htx. Further sections were used for immunohistochemical staining of inflammatory markers. Monoclonal antibodies against ICAM-1 (CD54, normally expressed on endothelial cells in small blood vessels), VCAM-1 (CD106, expressed on endothelial cells and inflammatory cells) and human neutrophil defensins (a marker of neutrophil cells). All antibodies were received from Novocastra Laboratories, Manchester, UK. The PAP procedure was used for visualization of the immunoreactions.

The sections were evaluated blindly by two examiners. By comparing the first and second biopsies from each patient, the effect of the treatment was ranked as no effect, moderate effect or good effect. The ranking was based on the status of the surface and crypt epithelium, the relative number of PAS-positive goblet cells, the relative number of inflammatory

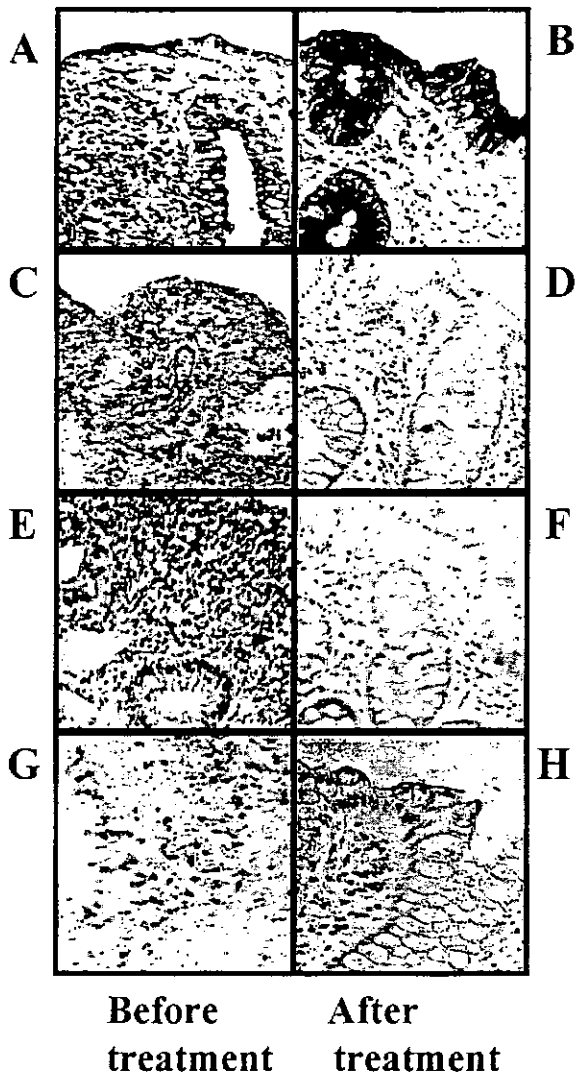

Fig. 1. Example of rectal biopsies from an AF-treated patient at admission and outward visit 1. Left panel: at admission. Right panel: at outward visit 1 (2–4 weeks). A, B. PAS stained 6-μm sections. C, D. Immunohistochemical staining (PAP/DAB) for ICAM-1 sections. ICAM-1 expression brown, nuclei counterstaining with haematoxylin (htx)/eosin. E. F. Immunohistochemical staining (PAP/DAB) for VCAM-1 sections. VCAM-1 expression brown, nuclei counterstaining with htx. G, H. Immunohistochemical staining (PAP/DAB) for defensin sections. Defensin expression brown, nuclei counterstaining with htx.

cells and the relative intensity of the staining for ICAM and VCAM. Furthermore, the relative number of neutrophils, using the staining for defensins, and the relative number of eosinophils, using htx/eosin staining, was ranked semiquantitatively as none, few or many.

Stool and blood samples

The daily weight of feces was measured beginning at midnight on the day of admittance. Blood samples for routine analyses were taken at regular intervals.

Statistical analyses

All data are presented as median throughout the study, except stool weight, which is presented as mean. All statistical analyses were performed using the Wilcoxon signed-rank test. A level of $P < 0.05$ or less was considered significant.

Results

No statistical differences between the patient groups were found concerning age or sex distribution or the length of hospitalization time. At the time of inclusion, both patient groups demonstrated the same values for blood sample parameters along with an equal histology outcome of rectal biopsies. Furthermore, the frequency of colectomy was equal in both groups. Statistical comparisons between the groups were impossible 9 days after inclusion because the number of patients dropped below the limits of statistical analysis.

Since some of the biopsies were too small to allow reliable evaluation, paired analyses could be made on biopsies from 7 patients in the placebo group and 10 in the AF group. On comparing the total effect of the treatments on mucosa histology, the Mann-Whitney test showed a trend towards a better effect in the AF group ($P = 0.129$) (Fig. 1). However, when comparing the change in relative number of neutrophilic and eosinophilic granulocytes between the first and second biopsies there was a significantly larger decrease in the relative number of these cell types in the AF group than in the placebo group ($P < 0.039$ for granulocytes, $P < 0.025$ for eosinophils), indicating an effect of the treatment on the inflammatory reaction.

The mean weight/stool was equal between the AF-treated and placebo-treated groups at all times (Fig. 2). Although not evaluated in this study, we noticed that stool weight/24 h was considerably higher in the groups of patients who went to

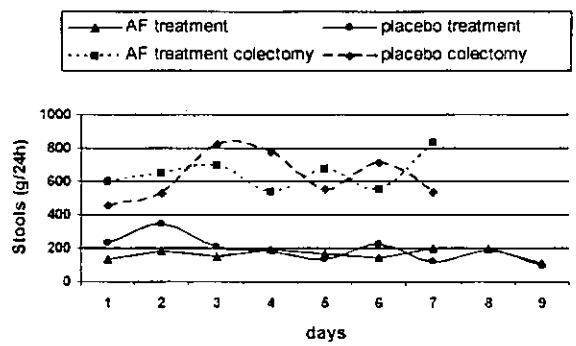

Fig. 2. Median weight/stool registered from inclusion to day 9 (▲ = AF treatment; ■ = AF treatment colectomy; ● = placebo treatment; ◆ = placebo colectomy).

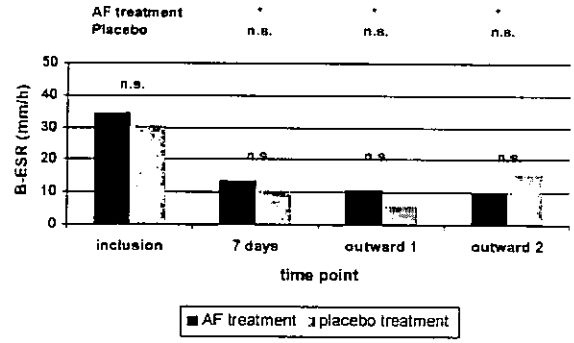

Fig. 3. B-ESR (mm/h) during hospital stay and at two outward visits in AF-treated versus placebo-treated patients (n.s. = non-significant; $^*P < 0.05$).

colectomy (exclusion criteria, not evaluated in this study) during the first 7 days. No differences in S-ESR and S-CRP between the groups were registered throughout the study (Figs. 3 and 4). In the AF-treated group, however, from the 7th day and onward, ESR was significantly reduced compared to the inclusion value (Fig. 3). This finding was also evident at both outward visits. S-CRP significantly decreased in both groups during the hospitalization time, but tended to increase in the group of placebo-treated patients at outward visits (Fig. 4). The group of AF-treated patients showed decreased levels of S-haptoglobulin (n.s.) and S-Orosomucoid ($P < 0.05$) in comparison to the placebo group at the first follow-up visit (not tested at second outward visit). Serum albumin was equal and slightly increased (not significant) in both groups. No other blood samples (haemoglobin, leucocyte counts, haptotglobin and platelets) showed any differences.

Discussion

Patients suffering from acute severe UC require systemic and local medical treatment in combination with parenteral or

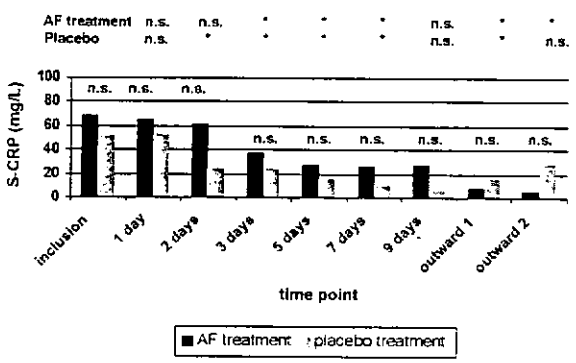

Fig. 4. S-CRP (mg/L) during hospital stay and at two outward visits in AF-treated versus placebo-treated patients (n.s. = non-significant; $^*P < 0.05$).

enteral nutrition. Today, because of the severe outcome of the progressive state of UC, variable forms of immunosuppressive or immunomodulating regimes are also under evaluation. However, our knowledge of the natural history of UC indicates that further approaches are required in handling this group of patients. Thus, in the present study patients with acute attacks of UC received AF or placebo treatment as a supplement to the conventional pharmacological and nutritional therapy usually performed. The supplementary AF treatment was given in order to investigate a possible influence on the histological biopsies along with the routine laboratory parameters, but also to evaluate a possible effect on the clinical outcome. Previous studies of patients with chronic IBD (15), endocrine diarrhoea (22) and diarrhoea due to intestinal resections (20) have shown variable, but significant effects on the number of bowel movements after active AF induction (i.e. oral intake of SPC), but also after passive AF therapy (i.e. intake of egg yolk drinks). In patients suffering from IBD, the results of AF treatment with SPC indicated a positive effect on the extent of inflammation in the rectal biopsies (15).

In the present study, a significant effect of AF is clearly demonstrated in the mucosal biopsies from the routine histology as well as in the immunohistochemical staining. In a previous study we have shown that an increase in AF activity can be demonstrated in plasma after intake of AF-rich egg powder, indicating that AF is still active after passage of the upper gastrointestinal tract. The observed reduction of mucosal inflammation in the biopsies strongly indicates that the orally administrated AF protein mediates a significant anti-inflammatory effect when registered in the intestinal mucosa.

Concerning patients with acute exacerbation of UC in the present study, further AF treatment for 2 weeks influenced neither the frequency of colectomy nor the hospitalization time. Furthermore, the weight of stool samples did not differ between the two patient groups. This finding indicates that the secretory component of acute UC is not affected by the AF containing egg yolk drinks at the tested dose. However, the secretory UC component might be reduced by AF treatment in higher doses, or by induction of increased endogenous AF synthesis via oral intake of SPC.

In the group of AF-treated patients, positive effects were noticed in some inflammatory blood sample parameters by demonstrating persistent low levels of B-ESR and S-CRP during hospital stay and at both outward visits. Both of these parameters tend to increase in the group of placebo-treated patients. These findings might be correlated to the histological improvements demonstrated by the mucosal biopsies in the AF-treated group.

The exact biological mechanisms for the effects of AF action are not known (10, 23). However, in clinical practice AF appears to have a dual effect by counteracting diarrhoea as well as the inflammatory reaction in the intestinal mucosa (15, 19, 21–23). Diarrhoea displays a wide and variable aetiology,

and AF therapy appears to be most effective in diarrhoea conditions where the secretory component is prominent, e.g. diarrhoea due to the endocrine tumours (22). In acute UC, the diarrhoea is probably secondary to the inflammatory process, tentatively as a result of the destructed mucosal barrier. Consequently, the antisecretory effect of AF in the UC disease should not be expected until the inflammation subsides. This hypothesis is supported by the present biopsy analysis, where the large-bowel mucosa is partially restored, as a result of the AF therapy. Consequently, it is therefore likely that the anti-inflammatory effect of AF precedes the antisecretory effect. A prolonged AF treatment might therefore affect other parameters related to the inflammatory response.

In conclusion, administration of AF as a supplement to conventional pharmacological and nutritional therapy demonstrated less inflammation in the rectal biopsies of the AF-treated patients than in the placebo controls. Furthermore, the inflammatory variables in blood samples were persistently low and almost normalized at the second outward visit in the AF group, whereas these variables demonstrated higher values in the placebo group. Thus, AF tentatively has a long-lasting and anti-inflammatory effect on intestinal mucosal in the acute phase of UC.

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LETTER TO THE EDITOR

Antisecretory Factor: A Clinical Innovation in Ménière's Disease?

PER HANNER[1], EVA JENNISCHE[2] and STEFAN LANGE[3]

From the Departments of [1]Audiology, [2]Anatomy and Cell Biology and [3]Clinical Bacteriology, Sahlgrenska University Hospital, Göteborg University, Göteborg, Sweden

Hanner P, Jennische E, Lange S. *Antisecretory factor: a clinical innovation in Ménière's disease?* Acta Otolaryngol 2003; 123: 779–780.

A 67-year old woman with severe Ménière's disease (MD) was referred to our ENT Department in 2001. Clinical examination revealed a normal otoneurological status, except for a right-beating nystagmus after headshake, indicating a vestibular disorder. The audiogram demonstrated sensorineural hearing loss affecting all frequencies in the right ear. The patient had initially experienced rotatory vertigo, hearing loss and tinnitus in her right ear in 1968 when aged 34 years. These attacks lasted between 8 h and 3 days and were frequently accompanied by severe nausea, vomiting and diarrhoea. No form of conventional medical treatment, either alone or in different combinations, was found to counteract the severity of the disease.

As we had previously achieved beneficial clinical outcomes for MD using a diet of specially processed cereals (SPC), a potent stimulator of antisecretory factor (AF), the patient was given an SPC diet for 2 months (1). However, the SPC therapy affected neither the frequency nor the duration of her MD attacks, and her AF plasma activity level remained low. Therefore, a passive form of AF therapy, i.e. AF in the form of egg yolk drinks, prepared from eggs with a verified high content of AF (Salovum®; BioDoc AB, Stockholm, Sweden), was offered to the patient five times daily. Each egg drink consisted of 2 g of freeze-dried egg yolk. On Day 18, the patient experienced total and complete remission. According to the American Academy of Otolaryngology—Head and Neck Surgery guidelines for MD, the patient's status changed on the functional scale from 6 to 1 (2). After 6 months of health on a constant regimen of AF-egg drinks, the patient reduced the dosage and experienced an immediate relapse in terms of severe rotatory vertigo attacks. A return to the original dosage promptly produced a clinical improvement, and the patient was subsequently free of vertigo attacks, without the need for other forms of medical treatment. However, the right-sided hearing loss did not respond to the AF-egg therapy.

AFs are endogenous proteins with the capacity to counteract experimental hypersecretion in the small intestines of the rat and pig. AF has been cloned and sequenced (1). Endogenous stimulation of AF occurs in response to peroral intake of bacterial toxins, sugars, amino acids and SPC. Stimulation of AF synthesis has proven effective in reducing not only intestinal hypersecretion, but also intestinal inflammation, in humans and animals (1). However, some patients do not respond to SPC intake, and we suggest that this type of "non-responder" should be offered passive AF administration in the form of AF-egg therapy.

Regulatory dysfunction in the cells that control transport and synthesis of endolymph in the inner ear may cause what is pathophysiologically classified as endolymphatic hydrops (3, 4). Using immunohistochemistry, we found irregular AF immunoreactivity in the epithelial cells lining the endolymphatic space of the human cochlea, in addition to distinct staining of the neuronal cells of the spiral ganglion. This localization suggests an important role for endogenous AF activity in endolymphatic production, circulation and inner ear pressure. Furthermore, these morphological results suggest a regulatory role of endogenous AF in the development and/or clinical outcome of MD. The clinical handling of our MD patient represents a new type of treatment according to the definitions outlined in the previous *Lancet* debate (5). Although proof of concept requires a randomized controlled study, we suggest that those MD patients who are classified as "non-responders" to SPC intake may benefit from AF induction in the form of AF-egg drinks. Moreover, the development of the AF-egg drink treatment should result in both clinical efficacy and economic benefits.

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Address for correspondence:
Per Hanner, MD
Department of Audiology
Sahlgrenska University Hospital
SE-413 45 Göteborg
Sweden
E-mail: per.hanner@orlss.gu.se

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ORIGINAL ARTICLE

Antisecretory factor counteracts secretory diarrhoea of endocrine origin

A. LAURENIUS,* B. WÄNGBERG,† S. LANGE,‡ E. JENNISCHE,§ B. K. LUNDGREN,* I. BOSAEUS*

*Department of Clinical Nutrition, Göteborg University, Göteborg, Sweden, † Department of Surgery, Göteborg University, Göteborg, Sweden, ‡ Department of Clinical Bacteriology, Sahlgrenska University Hospital, Göteborg University, Göteborg, Sweden, and §Department of Anatomy and Cell Biology, Göteborg University, Göteborg, Sweden (Correspondence to: IB, Department of Clinical Nutrition, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden)

Abstract—Background: Dietary induction of antisecretory factor (AF) can reduce diarrhoea in patients with inflammatory bowel disease. Patients with neuroendocrine tumours may suffer from diarrhoea with a prominent secretory component. We studied if AF-therapy could affect this type of diarrhoea.

Methods: Six patients with the midgut carcinoid syndrome and two with metastasizing medullary thyroid carcinoma (MTC) participated. Effects of intake of AF, in the form of AF-rich egg powder (AF-egg), and induction of endogenous AF-activity by intake of specially processed cereals (SPCs) were studied. In an initial open part of the study all patients received AF-egg for 4 weeks, followed by a double-blind crossover period with SPC and control cereals (CCs) for 6 weeks each. Daily number of bowel movements at the end of each treatment period was registered.

Results: Treatment with AF-egg resulted in a decrease of bowel movements in seven patients ($P<0.01$). Registrations of bowel movements from both SPC and CC diet periods were obtained from five patients. The daily number of bowel movements was lower during the SPC-period compared to the period with CC ($P<0.05$). All patients had low levels of AF-activity in serum at baseline. During treatment with AF-egg, the mean level increased slightly. AF-activity was higher ($P<0.05$) after SPC compared to the CC diet.

Conclusions: In a group of patients with endocrine diarrhoea, AF-activity could be induced, and AF-therapy reduced the number of bowel movements.

Key words: antisecretory factor; diarrhoea; functional foods; metastatic medullary thyroid carcinoma; midgut carcinoid syndrome

Introduction

Antisecretory factor (AF) is a 41 kDa protein, originally characterised as a pituitary substance, suppressing experimental diarrhoea (1, 2). Endogenous AF-activity can be induced in humans and animals by a diet with specially processed cereals (SPCs) (3–5).

Previous studies have shown that SPC can reduce symptoms in patients with inflammatory bowel disease (5). In a pilot study on patients with reduced small bowel length after surgical resections AF-induction by SPC diet was significantly correlated to the length of remaining small intestine (6, 7). In patients with moderate intestinal resections, the SPC diet resulted in an increase in AF-activity and a decrease in the number of daily bowel movements. However, in patients with the most extensive resections there was no significant AF-induction and no effect on the number of bowel movements. Thus, clinical results so far indicate that SPC may be of value in the treatment of certain cases of diarrhoea. It is therefore of interest to identify which conditions are responsive to this therapy.

Patients with neuroendocrine tumours may suffer from diarrhoea (8, 9). The diarrhoea can be severe and therapy-resistant, persisting even in optimally treated patients. Since this type of diarrhoea is a significant clinical problem, and since it is one of the few known conditions with chronic secretory diarrhoea in adult humans we found it of interest to study the effect of AF-therapy in this group of patients.

The aim was two-fold: firstly, to investigate if AF-activity could be induced in patients with endocrine diarrhoea; secondly, if increased AF-activity could be correlated to reduced diarrhoea.

Two modalities of AF-treatment were studied: (1) In a double-blind crossover study on a small number of patients with advanced midgut carcinoid tumours or medullary thyroid carcinoma (MTC) the effects of SPC was compared to that of placebo cereals. (2) Egg yolk can contain high levels of AF (10) and with specific breeding conditions eggs with a high content of AF can be produced. Egg drinks were prepared from egg yolk with high AF content, and the effect on diarrhoea of

this alimentary treatment with preformed AF was also investigated.

Material and methods

Patients

At Sahlgrenska University Hospital, about 50 patients with midgut carcinoid tumours and about 30 patients with MTC are treated. Fifteen of these complained of diarrhoea in spite of treatment and were invited to participate in the study. Exclusion criteria were an operation within the previous 2 months, ongoing treatment with antibiotics or intolerance to egg. Eleven patients were included in the study. Of these, three patients, two women with midgut carcinoid tumours and one woman with MTC, could not follow the protocol and left the study during the second week of the first diet period.

Eight patients completed the whole study or parts of the study. Six patients (P1–P6), five women and one man, had the midgut carcinoid syndrome and two, both men (P7 and P8), metastasizing MTC. Mean age was 57 ± 14 years. Table 1 shows the clinical data and pharmacological treatment of the patients.

All subjects gave informed consent, and the Human Ethics Committee of Göteborg University approved the study design.

Protocol

Two modes of AF-therapy were studied. Firstly, intake of AF-enriched egg yolk and secondly, intake of SPC aiming at inducing endogenous AF-activity.

The study lasted 17 weeks. It consisted of an initial open part in which all patients received AF-egg drinks, followed by a double-blind, crossover part with SPC or control cereals (CCs), respectively.

During the first week, baseline registrations of frequency of bowel movements and plasma levels of AF were made. All patients were then treated with AF-egg drinks for 4 weeks. At the beginning of the fifth week, the patients were randomised to SPC or CC. The two kinds of cereals were consumed for 6 weeks each.

The AF-egg drinks and the cereals were given as an additional treatment and the usual medication of the patients was kept unchanged. The egg drinks consisted of 2 g freeze-dried egg yolk with a high content of AF, solubilised in 0.25% acetic acid. The antisecretory activity of the egg yolk was tested in the rat ligated ileal loop assay as described in detail previously (11). The freeze-dried egg yolk used in the present study had high AF-activity (i.e. per 2 g, between 1.0 and 1.5 AF-units, when tested in a dilution of 10^3).

The initial dose was 2 g egg yolk daily, which was successively increased during 10 days to the final dose of 2 g four times daily. All patients received the same amount of egg yolk, irrespective of body weight.

SPCs, and CCs were produced by BioDoc AB (Stockholm, Sweden) and analysed as previously described (5). SPCs or CCs were given in a final dose of 1 g/kg b.w. divided in four daily doses. The dose was gradually increased during 2 weeks and the final dose was given during 4 weeks. The daily volume of cereals consumed was about 1 dl.

Compliance was monitored by telephone contact every second week.

Registration of bowel movements

The patients registered their bowel movements for 1 week during the following four periods: at the start of the study, during AF-egg treatment and during the last week of each cereal treatment period. The number of daily bowel movements as well as the subjective evaluation of the size and consistency of the faeces was registered.

Analyses of AF-activity

Analyses of AF-activity in plasma were performed as previously described (11). Blood samples were drawn before and after each treatment period. Previous studies on man and animal indicates that AF-values of more than 0.5 AF-units are correlated with a reduction of diarrhoeal disease (3–5).

Statistics

Data are presented as mean ± standard deviation (SD). Means were compared using Student's *t*-test. All calculations were made in SPSS version 10.0 (SPSS, Chicago, IL, USA).

Table 1 Patient data

Diagnosis	Duration (years)	Age, sex	Intestinal resection (cm)	Therapy*	BMI
P1 carcinoid syndrome	15	59, F	Small intestine: 150	O, C	19.3
P2 carcinoid syndrome	7	65, F	Small intestine: 175 Colon: 10	O, C, L	17.5
P3 carcinoid syndrome	5	72, M	Small intestine: 100 Colon: 15	O, C, P	20.4
P4 carcinoid syndrome	1	76, F	Small intestine: 80	O, L, P	23.3
P5 carcinoid syndrome	2	43, F	Small intestine: 20 Colon: 10	O	35.0
P6 carcinoid syndrome	5	47, F	Small intestine: 120 Colon: 50 %	O, C	15.2
P7 MTC	4	57, M	—	—	26.2
P8 MTC	4	37, M	—	—	24.6

*O = octreotide, C = codeine, L = loperamide, P = pancreatic enzymes.

Results

Bowel movements

The mean daily number of bowel movements before and during the various diet periods is shown in Table 2.

Effects of passive AF-treatment

Complete registrations were obtained from seven patients. There was a significant decrease in the number of bowel movements during the treatment period ($P<0.01$) (Tables 2 and 3). The treatment with AF-egg also resulted in an increase in the relative number of formed stools from 14% at the start of the study to 41% during the last week of egg treatment.

Effects of AF-inducing treatment

Complete registrations from both SPC and CC diet periods were obtained from five patients. In all these patients, there was a lower number of daily bowel movements during the SPC diet period than during the CC diet. The group difference between SPC and CC was significant ($P<0.05$) (Table 3). P4 and P7 were randomised to SPC the first period, P1, P2 and P8 to CC. The relative number of formed stools was similar during both treatment varieties, i.e. around 30%.

AF-analyses

Due to technical problems, a complete analysis of AF-activity in all samples could not be made. All patients had very low or undetectable levels of AF-activity in serum at the beginning of the study (Table 4). During treatment with AF-egg, analysed in six patients, there was a slight, but statistically significant increase in AF-activity (Table 5).

In five patients, AF-activity was measured during SPC-treatment. Of these, four patients reached AF-levels above 0.5 AF-units, i.e. the blood level which in previous studies has been correlated to positive effects on diarrhoea. In one patient, no AF-induction could be registered. There was a significant difference ($P<0.05$) in AF-activity between the two cereal diets in the three patients where blood samples were analysed for both periods (Table 5).

Table 2 Mean daily bowel movements during the diet periods

Patient	Baseline	AF-egg	CC	SPC
1	2.6	0.9	2.3	0.9
2	6.1	4.9	5.3	4.1
3	8.0	5.4	—	6.1
4	4.7	3.7	6.7	3.4
5	5.9	4.6	—	—
6	9.4	8.1	—	—
7	—	2.6	3.6	3.0
8	2.3	2.1	2.3	1.7

Table 3 Frequency of bowel movements during the diet periods compared by paired *t*-test

Test period	Mean ± SD	*n*	*P*
Baseline	5.6 ± 2.6	7	<0.01
AF-egg	4.2 ± 2.4	7	
CC	4.0 ± 1.9	5	<0.05
SPC	2.6 ± 1.3	5	

Table 4 AF-activity (AF-units) during the diet periods

Patient	Baseline	AF-egg	CC	SPC
1	0	0.3	0.2	—
2	0.1	0.3	—	0.8
3	0	0.3	—	0.2
4	0	0.3	0.2	0.7
5	0	—	—	—
6	0	0.1	—	—
7	0	—	0.1	1.0
8	0.1	0.2	0	1.0

Table 5 Mean AF-activity in plasma, during the diet periods compared by paired *t*-test.

Test period	Mean ± SD	*n*	*P*
Baseline	0.02 ± 0.05	6	<0.01
AF-egg	0.25 ± 0.08	6	
CC	0.12 ± 0.10	3	<0.05
SPC	0.74 ± 0.33	3	

Discussion

In the present study, the effect of AF in a clinical model of secretory diarrhoea was investigated. Obviously, diarrhoea induced by advanced neuroendocrine tumours may have several underlying causes, e.g. hormonal overproduction, malabsorption due to intestinal resections and bile acid malabsorption. However, the secretory component is prominent and the condition was therefore considered a suitable model for a trial with AF-therapy. Patients with endocrine diarrhoea are relatively rare and the number of patients available to us was very limited. The study therefore has the character of a *concept* study.

The studied patients were clinically severely ill. They received extensive pharmacological treatment and the AF-therapy was given as a supplement. In spite of the fact that the patients were optimally medicated, a significant positive effect of the AF-therapy was registered in several patients. The clinical effect of the supplementary AF-therapy varied between individuals. The number of daily bowel movements was used to assess treatment effects. Since the study was made on outpatient basis, registration of faeces volume or weight could not be performed for practical reasons and the patients own registrations were used. We believe that this allows a reliable estimation of intra-individual effects, although comparisons between individuals are more difficult to make.

AF-activity was measured with a bioassay (11). Field studies in animals have shown that an AF-level of 0.5 U is correlated to a decrease in diarrhoea (3–5) and an AF-level above 0.5 was therefore considered significant.

Two modes of AF-therapy were tested in the present study. First, intake of AF-rich egg powder, i.e. passive AF-therapy, then, intake of SPC, inducing endogenous AF-activity, i.e. active AF-therapy. All patients received AF-egg and the treatment resulted in a decrease in the number of bowel movements in all patients. However, this part of the study was open, and therefore these results should be confirmed in a controlled study. Treatment with AF-egg was included in the present study since a number of patients with diarrhoea due to intestinal resections had responded favourably to this treatment (unpublished data). Treatment with AF-egg was tolerated by all patients. The therapy with cereals required intake of a comparatively large volume of cereals and not all of the seriously ill patients were able to complete this part of the study. Thus, complete registrations of daily bowel movements and AF-activity were available for only three patients. All these patients had a significant AF-induction after SPC and this was accompanied by a reduced number of daily bowel movements in these patients.

A previous study has shown that a small intestinal length of at least 100 cm is necessary to induce AF-activity by dietary means (6). All patients with midgut carcinoid tumours in the present study had been subjected to intestinal resections but information about the length of remaining intestine was not available. However, the residual intestine was obviously in most cases sufficient for AF-induction. The two patients with MTC, with intact small intestine, reached the highest AF-levels after SPC. One patient, P3, failed to induce AF-activity. This could be due to insufficient compliance or to inability of AF-synthesis in this individual for unknown reasons. However, this patient responded favourably to the treatment with AF-egg, indicating that the effector system for AF was functioning.

Passive intake of AF had positive effects on the number of bowel movements, indicating that AF was still active after passing the upper gastro-intestinal tract. This is somewhat surprising, but it is possible that AF is protected from degradation by other substances in the egg yolk or that AF is activated by proteases in the upper gastro-intestinal tract. We have shown earlier that a short, eight-amino acid long peptide is sufficient for antisecretory effect (12). The retained biological effect of AF after passing the intestine is further supported by the observation that a small, but statistically significant, increase in AF-activity in blood could be measured after intake of AF-eggs.

The mechanism of action for AF is not known but the results of treatment with AF-eggs indicate that AF can have a local effect in the intestine, interacting with receptors and/or binding proteins in the mucosa. This could explain why positive effects on diarrhoea after treatment with AF-egg was seen at considerably lower systemic levels of AF than the 0.5 AF-units required for significant effects when endogenous AF-activity is induced by SPC.

Also, the link between ingestion of SPC and induction of AF-activity is at present unknown. However, since a certain length of small intestine appears to be required for AF-induction, a direct interaction between components in the SPC and the intestinal wall could be a crucial event. The hydrothermal processing of the cereals is likely to expose epitopes which are not exposed in CCs and which are possible ligands for binding sites in the intestinal mucosa.

In conclusion, in patients with endocrine diarrhoea AF-therapy reduced the number of bowel movements in most patients. Treatment with AF-egg was well tolerated and may be the most useful form of AF-therapy in patients with difficulties to ingest large amounts of cereals. Further studies are necessary to verify the clinical usefulness of AF-therapy in endocrine secretory diarrhoea.

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Antisecretory factor-induced regression of Crohn´s disease in a weak responder to conventional pharmacological treatment - a case report study

Anders Eriksson M.D., Ph.D.[1], Morteza Shafazand M.D. [1]*, Eva Jennische M.D., Ph.D. [2]†, Ivar Lönnroth Ph.D. [3], Stefan Lange M.D., Ph.D[4].

[1]Department of Medicine, Gastroenterology Unit, Östra Hospital, University of Göteborg. [2]Department of Anatomy and Cell Biology, University of Göteborg. [3]Department of Medical Microbiology and Immunology; University of Göteborg [4]Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden.

Correspondence to: Dr. Anders Eriksson, Department of Medicine, Gastroenterology Unit, Sahlgren´s University Hospital/Östra Hospital, Smörslottsgatan 16, SE-416 85 Göteborg, Sweden

Tel (Int): +4631-343 4000, Fax: (int) +4631-259254

E-mail: anders.s.eriksson@vgregion.se

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ABSTRACT

Severe forms of Crohn's disease, with patients undergoing repeated surgical intervention, are known to be associated with high morbidity. We report a case of a 38-year-old patient suffering from progressive severe, pharmacologically resistant Crohn's colitis. The patient was given passive treatment with antisecretory factor in the form of egg yolk drinks, followed by active endogenous induction of antisecretory factor, through intake of hydrothermally processed cereals. The clinical, endoscopic, biochemical and histological outcome was rapidly improved.

KEY WORDS

Crohn´s disease, inflammatory bowel disease, cereals, antisecretory factor

INTRODUCTION

Our case study is of a 38-year-old man, resistant to conventional pharmacological treatment, with long-standing diagnosis of Crohn's disease. During the past year, the patient's condition had severely worsened, with exaggerated weight loss, increasing abdominal pain, and more than 15 bloody stools per 24 hours.

Antisecretory factor (AF) is a 41 kDal protein evident in numerous human tissues (1). The protein is well represented in the gastrointestinal tract (2). Even if the exact mechanisms are not described at present, the physiological action of AF in the gastrointestinal tract is to regulate the transmembrane fluid, combined with a decrease of the inflammatory reaction in the mucosal tissue (4–5). A reduced recurrence of clinical signs from colonic inflammatory diseases has been observed after food-induced stimulation of the endogenous synthesis of AF (2). Vast clinical trials in pigs of different sex and ages, fed with HPCs, have invariably demonstrated a highly significant decrease of the incidence of diarrhea, correlated to increased endogenous synthesis of AF (6–7). In pigs however, not only has endogenous AF stimulation proven beneficial for the cure of diarrheal diseases, but passively absorbed AF via milk in suckling piglets has also been seen to decrease the incidence of diarrhea (8). High concentration of AF in egg yolk is achieved by feeding hens with hydrothermally processed cereals (HPCs) (3), which makes it suitable for drink administration.

The management and possible importance of this novel approach to treating Crohn's disease are discussed.

CASE

A 38-year-old man with a 20-year history of Crohn's disease, who had previously undergone colonic surgery (1985 and 1986) followed by an ileocecal resection (1988), attended our hospital in February 2001 due to a severe deterioration of his condition. The previous surgical interventions had resulted in a remaining colon length of about 50 cm. Over the past few years, he had been treated with corticosteroids, sulfalazine/5-ASA, metronidazole, immunosuppression, and imfliximab. For about the past year, the patient had suffered from abdominal pain, progressive weight loss (25% of his normal weight), and bloody stools (>15/d). The laboratory tests confirmed severe disease activity and colonoscopy revealed devitalized mucosa including the entire remaining large intestine and terminal neo-ileum. On the ward, initially the patient received total parenteral nutrition and high doses of intravenous betamethasone with no clinical or laborative improvements.

At this time, the patient had a Crohn's Disease Activity Index (CDAI) of >600 (Table). Since intensive medical treatment failed and the colonoscopy finding had been severe, colectomy was suggested as a reasonable established alternative. After consideration, the patient asked for alternative medical treatment. He was offered additional treatment with AF in order to achieve a reduction in intestinal hypersecretion and inflammatory activity. The patient was given a diet based on egg yolk drinks (i.e. passive AF administration) in combination with HPCs (i.e. active AF-inducing) as a complement to the traditional ongoing medication. The freeze-dried egg yolk with verified high AF activity (2 g dissolved in 6 ml orange juice) was given four times daily for 14 days. On day 4, finely ground HPCs 0.5 g/kg body weight (b.w.)/d were given in addition. The doses of unground HPCs were initiated on day 10 and slowly increased to 1 g/kg b.w./d. The introduction of HPCs was performed parallel with

termination of the egg yolk drinks and the finely ground HPCs. Normal feeding was introduced on day 10. A rapid and significant decrease of average daily stools, from >15 to 2–4 (Figure 1), in conjunction with a recovery of the patient's general and gastrointestinal well-being, was recorded in response to the treatment (Figure 2). In 16 weeks, a complete recovery of patents weight loss and normalization of the patient's CDAI (<150; Table) were registered. Today, the patient continues with HPC medication and a sparing use of codeine tablets. The histological outcome of the colon biopsies demonstrates a remarkable and almost complete mucosal healing (Figure 3).

DISCUSSION

Antisecretory factor is a 43 kDa endogenous protein, capable of inhibiting intestinal secretion and inflammation. Oral intake of a diet based on HPCs increases endogenous AF synthesis in mammalians. The exact antisecretory and anti-inflammatory action of AF is not known, but different mechanisms have been discussed (8). Our patient showed significant clinical improvement after 3 days of egg yolk administration, parallel to an increase in plasma concentration of AF. Twenty days after admittance, the patient's clinical condition was stable, allowing him to leave the hospital. At 35 weeks after introduction of HPC therapy, an acceptable clinical intestinal recovery had been achieved.

The patient's poor clinical condition, combined with his long-standing history of disease, made a spontaneous remission highly unlikely *sine medication*. In conclusion, the positive clinical response in our patient suggests a beneficial effect of AF therapy in severe Crohn's disease. This treatment may be considered as a complement to conventional medical treatment in patients with non-responding disease in situations where surgery is unsuitable.

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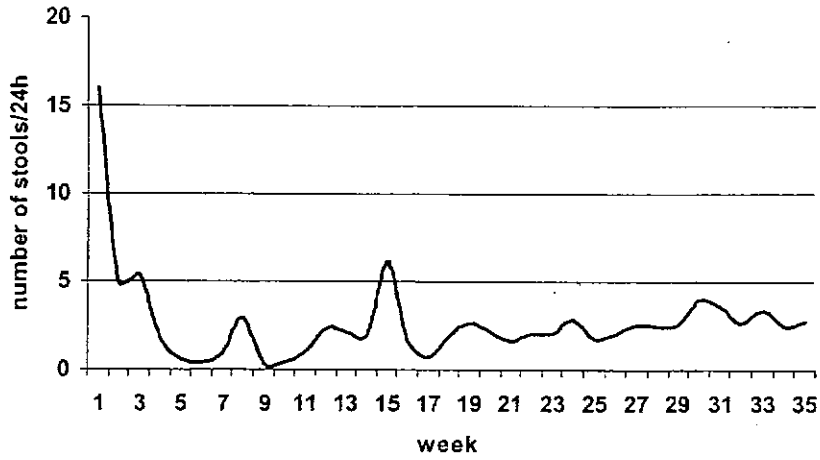
Table

week	AF units	CDAI
0	0.1	623
1	0	
2	0.6	
5	0.9	
16		147

Eriksson, figure 1



Eriksson, figure 2

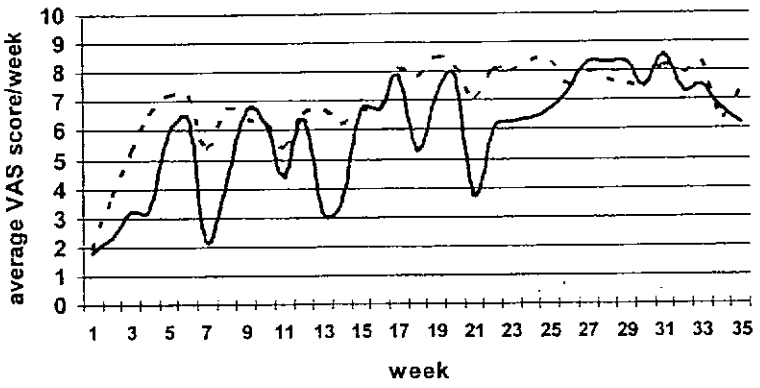
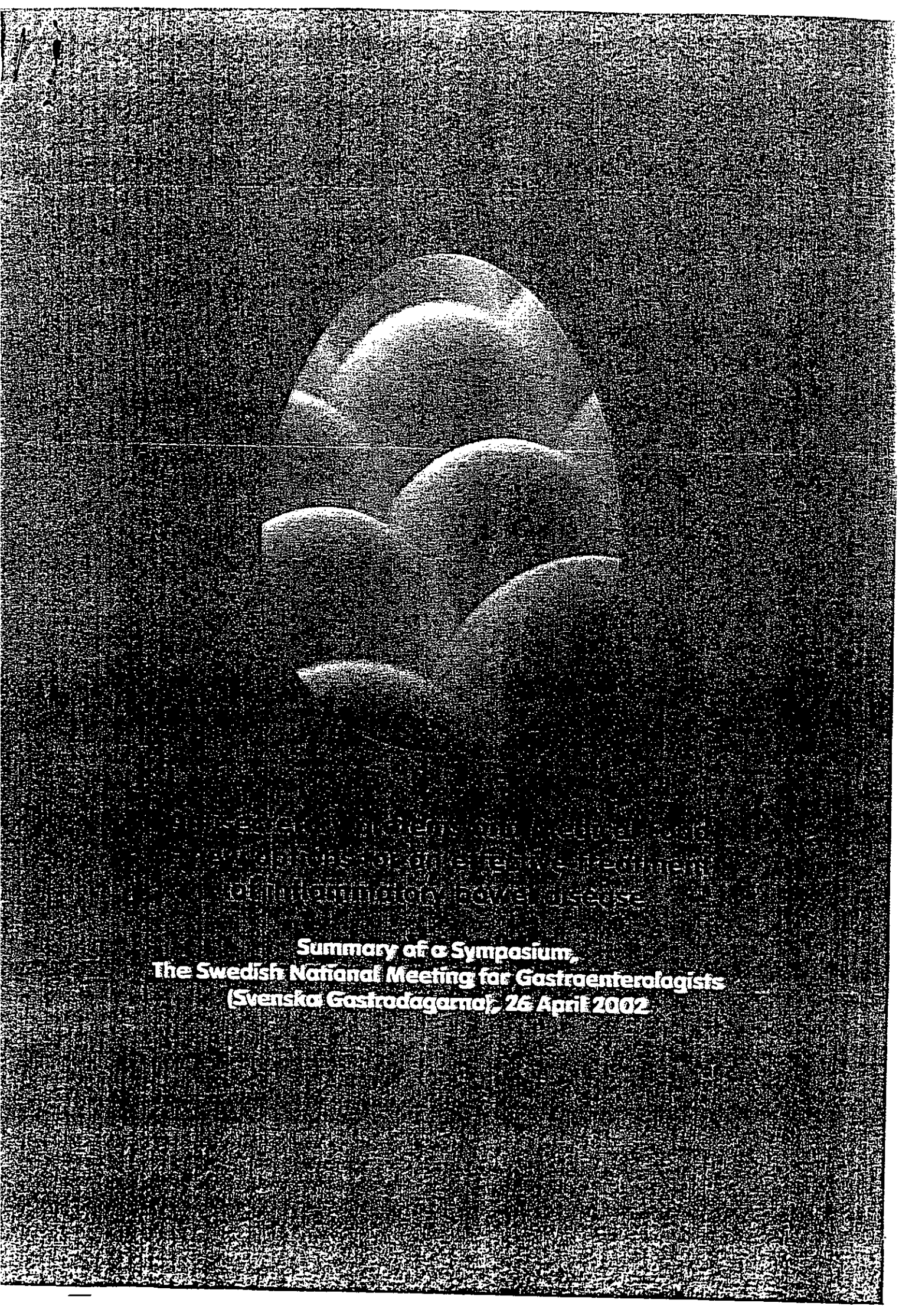


Table legend:

Table I. Antisecretory factor units (plasma) and Crohn's Disease Activity Index in plasma samples, 0–16 weeks.



Summary of a Symposium, The Swedish National Meeting for Gastroenterologists (Svenska Gastrodagarna), 26 April 2002

Protein AF: Synthesis and biological effects

Stefan Lange, M.D., PhD, The Institute of Clinical Bacteriology, University of Gothenburg, Sweden

The Antisecretory Factor (AF) is an endogenously produced peptide that modulates or regulates the salt and water balance in different membranes of the body. We have demonstrated that the AF peptide in different experimental systems, mainly in rat, inhibits cholera induced secretion. The antisecretory peptide also affects other types of secretions produced by other types of bacteria. All tissues in e.g. pig produce AF, such as in the intestines, lung, nose, gall bladder and the balance organs of the ear. The Swedish Farmers Cooperative, Lantmännen, contacted us in 1986 when the use of antibiotics in pig feed was banned in Sweden. As a result, the prevalence of diarrhoeas increased. We therefore developed a feed for small pigs that would trigger the production of AF. This treatment proved to be effective and the decrease in diarrhoea was correlated to the AF-production. Even the mortality was lowered. Thanks to these positive results in animals we discussed with Stellan Björck, M.D., PhD, and Prof. Ingvar Bosaeus how we should organize the first studies in patients. In the following studies in patients with short intestines, we were able to see that the bowel movements were affected when the concentration of AF was increased. An increased AF was only achieved in patients with short intestines exceeding a length of one meter.

The Antisecretory Factor – the development of a model and initial studies

The Antisecretory Factor (AF) is an endogenously produced peptide that modulates or regulates the salt and water balance in different membranes of the body. It is a regulative system that I worked on during my time as a PhD candidate. At first we developed a model to measure the hypersecretion or the pathological secretion in the intestines. In this model the rat is opened, a 10–15 cm long segment of the small intestine is sectioned and a bacterial toxin is injected, in this case cholera toxin. The segment is put back into the abdomen, the rat is sutured and then we had to await the time required. Maximal secretion occured after 60 minutes to 7 hours, depending on which toxin that has been used. It is difficult to choose another mode of measuring intestinal secretion, gut motility and different types of intestinal reactivity to bacterial toxins etc than conducting in vivo trials, often in non-sedated animals. In other words we have a dose-response, where we have a reactivity in the small intestine depending on the amount of the secretion releasing agent in question that we have been using.

In our studies we have been using cholera toxin. Using this toxin means that the intestinal integrity is unaffected, nothing affecting the morphology. There is only a pathological salt-water balance that can cause a loss of very large quantities of salt and water during a short period of time. There has been a case report of a patient who weighed 84 kg. He had, during a 24 hour period, registered a loss of 46 litres due to a cholera attack during his service in Vietnam. Consequently there is a very fast and dynamical flux change that occurs in these membranes when they are affected pathologically.

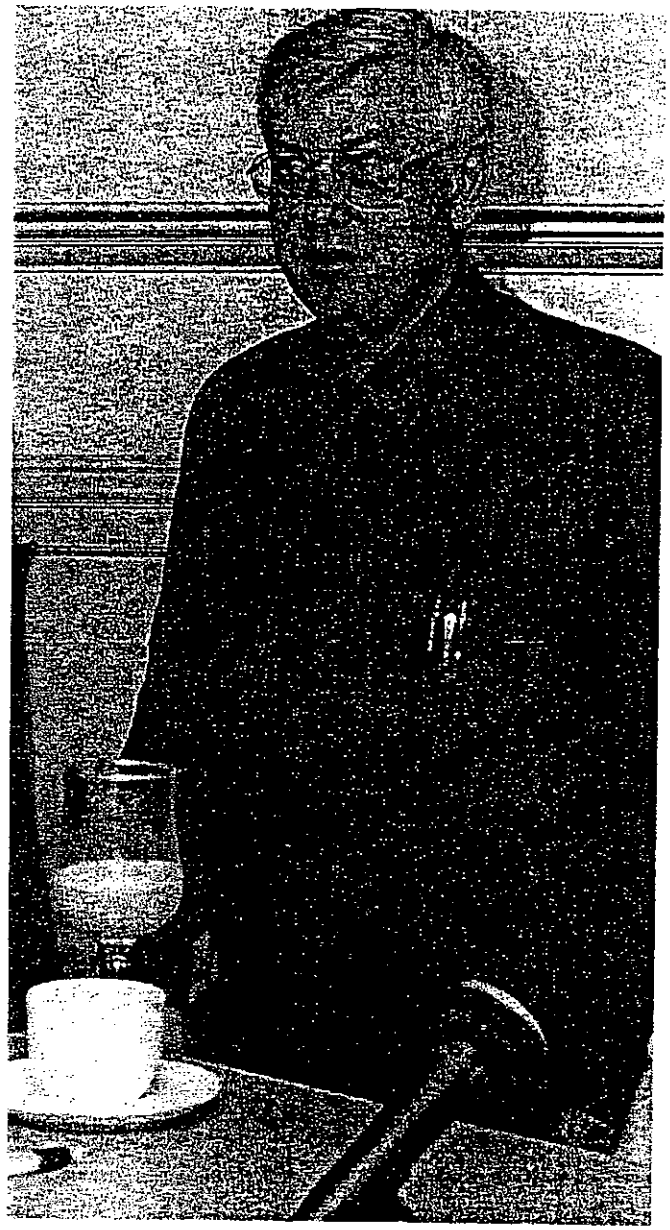
"Hereafter we showed that the peptide, in different experimental systems, affected the cholera toxin and inhibited secretion. It could, in other words, most certainly affect a very general system triggering secretion."

These results simply shows that the more toxin we use, the more water we have in the loops. A shift of the curve to the right shows less receptivity to the disease in question. The experienced clinician would say that this is nothing new. Specialists in infectious diseases and gastroenterologists know, for some 4000

years, that those who survive cholera or some other violent diarrhoeal infection are resistant 2–4 years afterwards. These people were forced to take care of sick or dead people since it was known that they would not get infected again. For the experimentalist, however, the above mentioned results imply several new aspects since we can now start to show interest in examining these resistance inducing mechanisms in the body, in this case in rat, and later on in pig.

In order to approach this problem we used a proven form of biological system. We immunised a large group of rats who were totally immune to cholera and transferred different types of extract from the immunised group to a virginal group that was very sensitive to cholera. We could then see which extracts that could influence the reactivity in the virginal rats, in other words a transfer from one system, insensitive, to a sensitive one; a very simple type of biology.

"The pigs responded effectively to this feed and in the initial trials we could see that the post weaning diarrhoea, PWD, decreased quickly from 50–60% to 10–20%. This reduction in diarrhoea was correlated to the AF-production."



My collaborator, Ivar Lönnroth, M.D., PhD, did all chemical preparations of the tissues. We transferred almost everything, from cells to different tissues, from the muscles to the CNS. We came to the conclusion that the pituitary gland is a good tissue to work with since it contains a large dose of what we wanted to research, namely a substance that is capable of bringing about an antisecretory or an inhibitory reactivity in the receptor animal.

We extracted a number of pituitary glands and separated the mixture after molecular weight. Then we selected the different fractions in order to test them in the receptor animal. The whole reactivity was collected in a span of approximately 60 kilodalton, as we knew that it was a protein and was to be found within this span. We refined the system and eventually discovered that it was a peptide. We sequenced it and sent these sequences to Gene Bank. To our great joy, they affirmed that what we had discovered was a totally new peptide that had not been described before.

A totally new peptide

Hereafter we showed that the peptide, in different experimental systems, especially in rat, affected the cholera toxin and inhibited secretion. It affected the Campylobacter toxin and Clostridium difficile toxin A, derived from two bacteria, and Okada acid, which is the toxin from sea mussels that is very effective in causing diarrhoea. It could, in other words, most certainly affect a very general system triggering secretion. The reason for testing all these toxins is that they provoke secretion biologically in totally different ways and which have not yet been described in detail. The antisecretory peptide however, affected all types of secretions. It affects something basic and phyllogenetically very old in the cell, namely a patho-physiological mechanism which probably comes before some cyclic nucleotides, before all types of chemical reactivity in the cell that have been described to this date as regards diarrhoea.

We were also able to split around 90% of the peptide and retain a small active fragment of 8–20

amino acids that still functions as an antisecretory unit. It is possible to perform this with almost any peptide that is biologically regulative in mammals. It also follows different types of reactivity for petides, we have a bell-shaped curve, we can get a maximum and a window in which the concentration operates.

The ban on antibiotics – negative and positive consequences

Now back to reality. We were contacted by The Swedish Farmers Supply and Crop Marketing Cooperative, Lantmännen, in the beginning of 1986 as the use of antibiotics was prohibited in pig feed. The diarrhoeas in the pig population started to increase. The production units are large and modern and closely monitored by skilled "biologists". If an infection occurs in this system, large economical values are at stake. The concentration of mammals is dense from a biological point of view. We co-operated with Kjell Martinsson, professor in pig health, and Leif Göransson, Doctor of agriculture, at the Lantmännen Pig Research Farm, Sveriges SvinCenter, in Svalöv. The only thing we knew about pigs at that point was that Ivar Lönnroth and I together with Erik Skadhauge at Fredriksberg's College of Agriculture had done exactly the same as in rats, namely a dose-response study with AF in pigs. Hence, we knew that they were sensitive to Antisecretory Factor and that it was produced endogenously. Looking back historically, how diarrhoeic diseases have been treated, we also knew that sugar and amino acids had been used. That is all. In cases of very severe hypersecretion, a mild or very sedating membrane stabilizing substance; ethanol or opiates, was added depending on the culture. I therefore tried the sugar alcohol sorbitol on the rat. I immunized the rat orally, provoked a secretion with 5,5% sorbitol and it proved much more efficient than cholera toxin in inducing a production of Antisecretory Factor. Together with especially Leif Göransson we produced a pig feed where we had manipulated the amounts of sugar and amino acids to obtain an optimal concentration of Antisecretory Factor. The pigs responded effectively to this feed and in the initial trials we could see that the post weaning diarrhoea, PWD, decreased quickly from 50–60% to 10–20%. This reduction in diarrhoea was correlated to the AF-production, i.e. the AF-levels were significantly higher in treated animals. We are now able to say that the problem with diarrhoea in the pig population has improved.

"We were our own first patients. We received pig feed from Svalöv from which we baked rolls and made muesli. Middle-aged doctors, PhD candidates and other co-workers all had a significant and sustainable increase in AF."

We could also note a growth increase, a daily weight gain, which is encouraging as we are now starting studies in children. If the pigs have healthy intestines due to a high production of AF they will gain weight significantly more than the controls who have a clinical or sub-clinical diarrhoea. In some children we have also seen this; not in a double-blind, controlled study, but in anecdotal cases we have noted this when the diarrhoeas have been reduced. The growth increase in these children with a reduced flow can be changed in a short time. Marie Krantz, M.D., PhD, at the Sahlgrenska Hospital and Prof. Yigael Finkel at the Karolinska Hospital are in charge of these paediatric investigations.

Also the mortality in the pig population was reduced due to their improved intestinal health. All tissues in pigs produce AF. With immunohistochemistry we can show that it is found in the intestines, in lymphocyte cells that morphologists are in the process of mapping. It is also located in the lung, shown using both in situ hybridisation and immunohistochemistry. AF is also found in the pig's nose, gall bladder and the balance organs of the ear.

The first studies in patients

Thanks to the positive results in pigs, we discussed with Stellan Björck, M.D., PhD, and Prof. Ingvar Bosaeus how we should organize the first study in patients. We were our own first patients. We received pig feed from Svalöv from which we baked rolls and made muesli. The manufacturing process is complicated. After having eaten the pig feed for up to four weeks, middle-aged doctors, PhD candidates and other co-workers all had a significant and sustainable increase in AF. Thereafter Ingvar Bosaeus wanted to test this on patients with short intestines, this being his speciality, and as these patients normally have high secretions. The first attempts showed no correlation whatsoever. But when we analysed the system more in detail we got a correlation in some patients. The bowel movements decreased when the AF-concentration increased as a result of the treatment with these cereals. We could not understand this fully since we also had some nonsense results. We discussed with Stellan and Ingvar how to interpret this. The lenght of the intestines probably have to exceed approximately 1 meter, which possibly is correlated to a certain ability to respond to the cereals with a resulting increase in AF and a reduced secretion. We are now continuing the measurements on Prof. Bosaeus's patients.

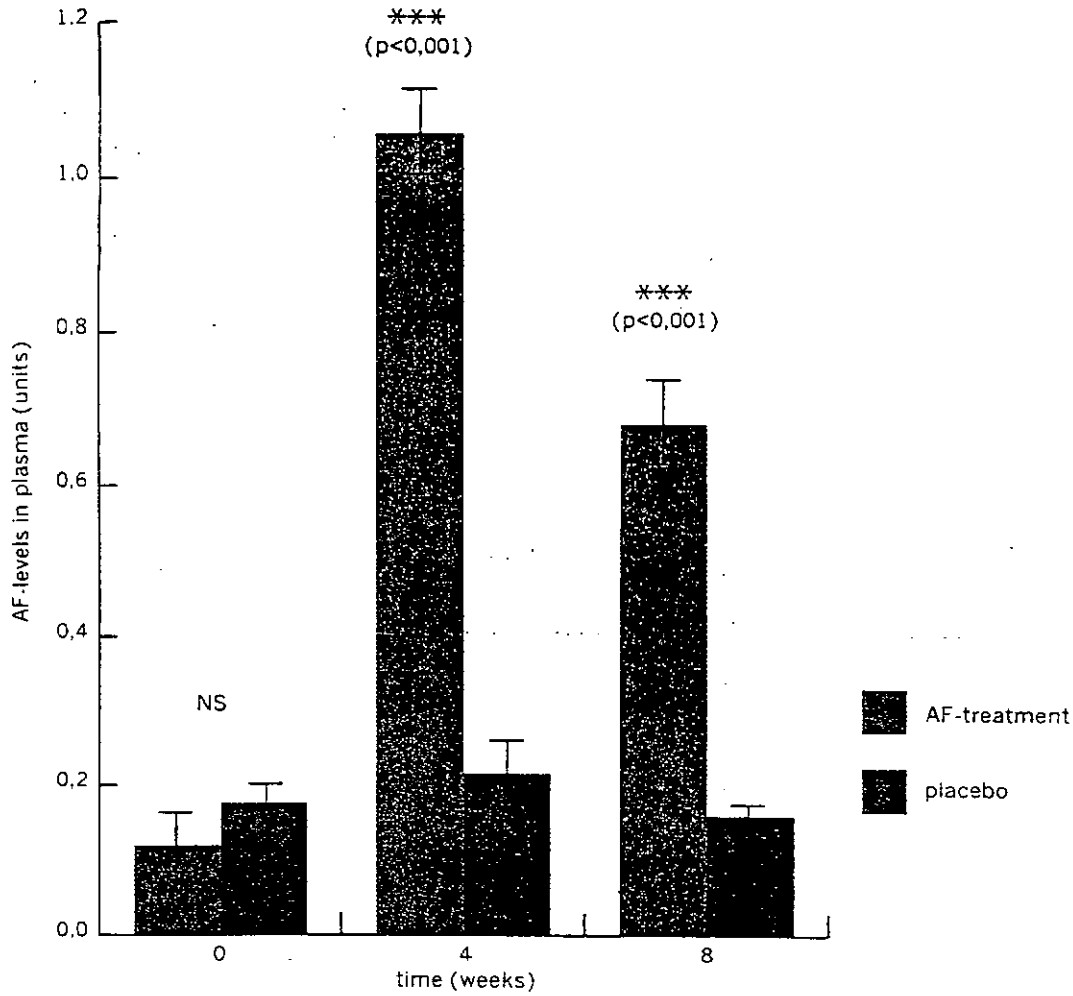


Figure 1. Patients treated with specially processed cereals (SPC) had significantly higher AF-levels in plasma than the placebo group. SPC-treated patients also had a significantly better intestinal function.

a drastic accumulation of AF is cytoplasmatically dissolved in the epithelial cells of the intestine. When looking at biopsies, it is clearly visible for the eye that something has happened from the start until the end of the trial.

Different prerequisites in different parts of the intestine

What are the prerequisites when we have to deal with patients whose intestines have been removed for one reason or another. If we start with the jejunum we know that it absorbs nearly all fat, carbohydrates and proteins from a meal. The proximal jejunum also absorbs iron, calcium and magnesium. The ileum has the same pattern of absorption as the jejunum, why the jejunum can be removed and its function can be taken over by the ileum. In the ileum there are two specific transport systems, partly for the biliary salts and partly for B12. The ileum also has a considerably higher adaptive ability than jejunum, so if one should be affected by something it should preferably be in the jejunal tract. In the ileocaecal region we know that it works as a unit regulating the gastric emptying and transit through the small intestine. We have also an important brake mechanism in the distal ileum which is called the ileal brake. When we are forced to remove the ileocaecal region we know that we accelerate gastric emptying, see a faster passage through the small intestine and get a proliferation of colon bacteria in the distal ileum. The colon is most important for the water absorption and the electrolytic absorption and the colon has a large extra and reserve capacity for absorption. It has been shown experimentally that the right part of the colon, caecum, is able to absorb up to 5 litres of liquid daily.

"My colleagues and I have now initiated treatment with AF to more patients undergoing operation in the alimentary canal and where we know by experience that the result will be an insufficient intestinal function."

After a partial ileocolic resection, the diarrhoeal volume is dependant on how much of the colon that has been removed and not so much of the ileal length.

Specially processed cereals – a treatment alternative for certain surgical patients

Stellan Björck, M.D., PhD, The Surgical Department, Sahlgrenska University Hospital, Mölndal, Sweden

We have conducted a randomised double-blind study in patients with ulcerative colitis and Crohn's disease. In the treatment group who received the specially processed cereals (SPC) as a supplement to their ordinary food, the amount of AF in plasma is drastically increased, and the increased levels are sustained, whereas nothing happens in the placebo group. The patients recorded their intestinal function in a diary and patients on the SPC treatment had a significantly better intestinal function. Intestinal biopsies also show that these patients will have a pronounced accumulation of AF in the epithelial cells of the intestines. These positive results have implied that we now initiate the treatment with AF to more patients who undergo operation in the alimentary canal and where we know by experience that the result will lead to an insufficient intestinal function. We start the treatment very early, sometimes already the first day, by giving eggs with a high concentration of Antisecretory Factor, AF (Salovum) dissolved in ordinary juice. This is a way of giving very high amounts of AF directly to the intestines without having the body producing it first. Most of the time we can then stop giving the egg powder and change to a maintenance dose of cereals. We believe the mode of action to be that the net absorption is increased by inhibition of the secretion. Moreover, there is no doubt that AF has very pronounced anti-inflammatory effects.

Positive results in ulcerative colitis and Crohn's disease

The first study that we performed was mainly in patients with ulcerative colitis but also with Crohn's disease. They all had an insufficient intestinal function and consequently visited the bathroom often. They were however not in a difficult, active phase of their disease, but they had a chronic insufficient intestinal function. We randomised patients to a specially processed diet or a placebo diet that they were instructed to eat every day during 1 month. The logistics worked very well and patients were eager to enroll in the study. 26 patients were given the specially processed diet and 24 patients received the placebo diet. Normally people have no measureable levels of AF, but upon receiving a specially processed diet like this in addition to regular food, the level of AF in plasma increases drastically, whereas nothing happens in the placebo group. Two months later these levels are still sustained in the AF-treated patients (figure 1). The patients kept diaries of their intestinal function and also stated their final result. The improvement in intestinal function is clearly significant in the group who had received the specially processed diet and who furthermore had high AF-levels in their blood. We also observed a large number of other factors, e.g. rectal biopsies. Among them, the only

"Normally people have no measureable levels of AF, but upon receiving a specially processed diet like this in addition to regular food, the level of AF in plasma increases drastically, whereas nothing happens in the placebo group."

significant change was found in the group with the most pronounced inflammatory changes of the mucosa. In this group as in the active group we had significant changes as regards the histology. We looked at various blood factors, such as CRP, cholesterol, triglycerides, HDL-cholesterol, but no significant changes were seen. After eating this diet

If we remove the colon, approximately 100 cm of jejunoileum is required not to develop a short bowel syndrome. In order to trigger AF-production in the body approximately one metre of small intestine is required.

Every time we have to remove parts of the intestine the intestinal adaptation takes place. This is a process that takes a certain number of months. Normally it takes three months to restore most of the function. This happens through extension and dilation of the remaining intestinal part. The villus height is also increased in the mucosa and with this the number of enterocytes, who will be high and cylindric,

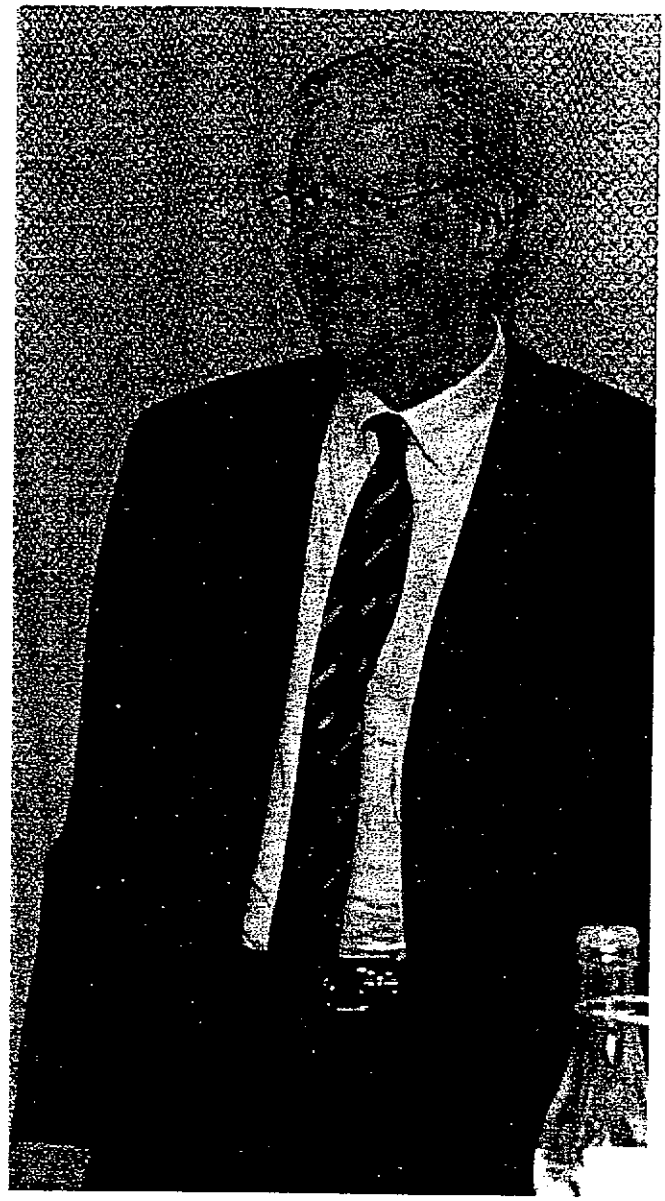
"We believe the mode of action to be a net absorption increased by inhibition of the secretion."

is also elevated. It is necessary to have food in the gut endothelium to trigger the system, and that the gall, pancreatic juice and also gastrointestinal hormones are present.

Treatment suggestion and mode of action

My colleagues and I at Sahlgrenska Hospital/Mölndal have now initiated treatment with AF to all patients undergoing operation in the alimentary canal and where we know by experience that the result will be an insufficient intestinal function. We start the treatment very early, sometimes already the first day, by giving eggs with a high concentration of Anti-secretory Factor, AF (Salovum) dissolved in ordinary juice, given orally or by probe. The mechanism is that if you give specially processed cereals to hens, they will lay eggs whose yolk contains very high amounts of AF. This will then be a way of giving AF directly to the intestines without having the body producing it first. However, we want the patient's body to start an endogenous AF-production as soon as possible and we initiate that by giving the patients the specially processed cereals orally. It takes about 10 days until we have maximal AF-levels with a stimulation of the endogenous production. Most often we can then stop giving the egg powder and change to a maintenance dose of cereals.

We believe the mode of action to be a net absorption increased by inhibition of the secretion. We know that there must be a balance between them. When there is a loss of less than one metre of distal small intestine we know that the patients are affected by secretion in the colon caused by biliary salts. It is probable that we inhibit that secretion with the high level of AF. When there is a loss of more than one



metre this will result in fatty acids in the colon that will start its own secretion. We believe that AF also has an inhibitory effect on this. Moreover, there is no doubt that AF has very pronounced anti-inflammatory effects.

Question: The MagiForm product range is recommended to more or less all patients suffering from gastroenterological problems. What happens to those not having an increased secretion?

Answer: The individual who has a normal intestinal function can nearly have a feeling of constipation if he or she eats the amount of cereals that we recommend in order to trigger the system maximally. The IBS patients have a very much disturbed motility as a cause and my experience is that many of them may worsen, the abdomen hurt more, they experince more flatulence. The patients suitable for this treatment are those who have diarrhoea. IBS with diarrhoea is an ideal patient.

Antisecretory proteins – a new therapy in IBD

Stefan Lange, M.D., PhD, The Institute of Clinical Bacteriology, University of Gothenburg, Sweden

Our double-blind study with eggs with a high concentration of Antisecretory Factor, AF (Salovum) in patients with severe relapses of ulcerative colitis shows that we had an effect mainly on the inflammatory parameters compared to the patients that were given the placebo egg powder. These differences are now being investigated.

Ulcerative colitis

I will present the result from Anders Eriksson's, M.D., PhD, and our study on the AF egg powder (Salovum) in patients with ulcerative colitis with severe relapses. The patients are on total parenteral nutrition, TPN. 22 patients participated in this study, 10 of them received eggs with a high concentration of AF, 12 patients were given a placebo egg powder.

More on the mode of action

Considering to the mode of action, we discussed which set of patients to choose. We evaluated that AF egg powder (Salovum) had a dramatic effect in Crohn's disease. We are now conducting very direct investigations on the peptide mechanism on different cellular and reactivity patterns in vitro and we intend to transpose them to in vivo. We can say that we have good trial assays to shed light upon the enteric nervous system, the brain-gut axis. We know that it affects the ion channels and has an anti-inflammatory effect. We are now focusing on which type of inhibition that it has and if it affects the vascular permeability.

The result of our double-blind study on ulcerative colitis came surprisingly late as regards the effect on the disease. We are now investigating the differences between the treatment groups, i.e. in the patients who received the egg powder with a high concentration of AF compared to the patients on the placebo egg powder.

"We are now conducting very direct investigations on the peptide mechanism. We know that it affects the ion channels and has an anti-inflammatory effect."

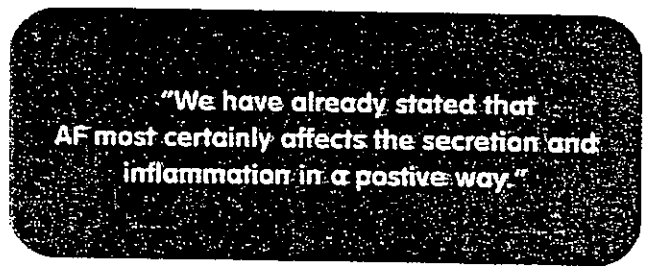
Treatment with specially processed cereals (SPC) in severe Mb Crohn

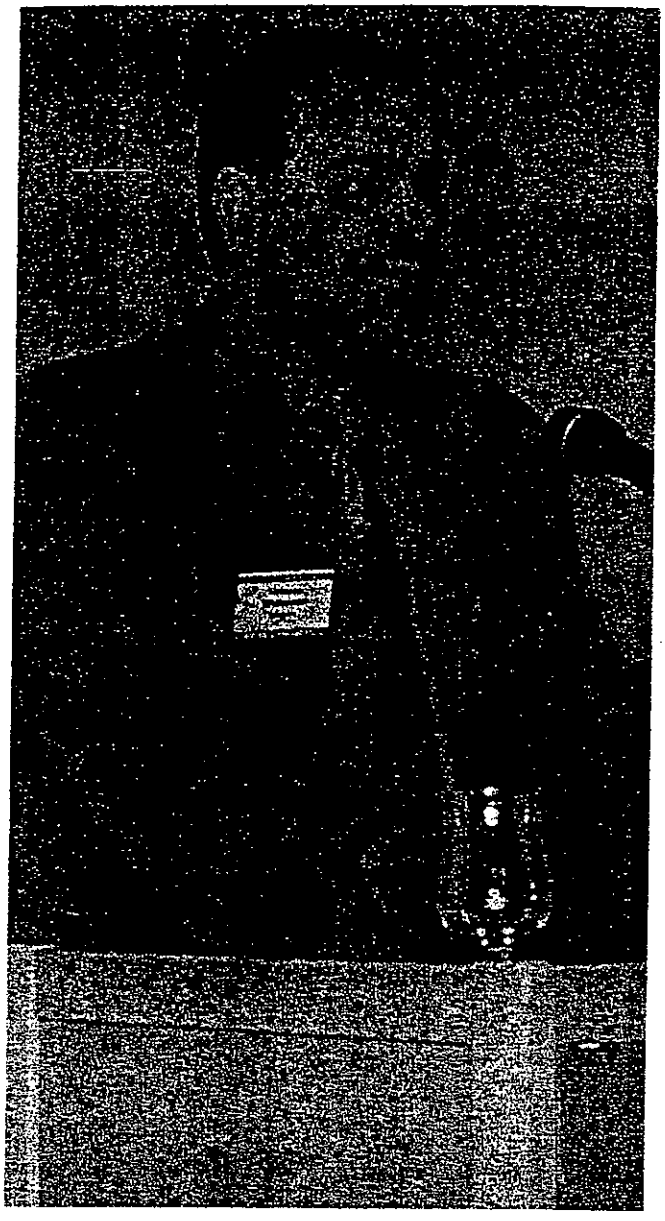
Morteza Shafazand, M.D., Department of Medicine, the Section of Gastroenterology, Sahlgrenska University Hospital/Östra Hospital, Sweden

We had tried all treatment possible, including infliximab, cortison, 5-ASA and TPN, in a patient with a 20-year past history of a severe Morbus Crohn. He got worse and relapsed with up to 15 bloodstained diarrhoeas daily and he experienced fever and stomach pain. His state deteriorated. Only surgery remained as a last alternative, but he refused, as he did not want to lose more intestine. He kept asking us if there was something else he could try, so we told him about Antisecretory Factor and he definitely wanted to try it. We initiated the treatment with eggs with a high concentration of Antisecretory Factor, AF (Salovum), which resulted in a reduction of his diarrhoea already after two days. After 12 days he had no diarrhoea at all. His fever disappeared, as well as the abdominal pain, and he felt a lot better. We noted an effect on the laboratory parameters and the inflammatory parameters were improved as well. We removed the TPN and he could be discharged with a total length of hospital stay of three weeks. Bearing in mind his severe condition and all other facts, including the failure of conventional treatment, we believe that it is AF that has had effect in his case and that it is an effective additional treatment.

Treatment with Salovum and SPC in severe Crohn's disease

I will tell you about a case report of a patient suffering from Crohn's disease where we have used this new treatment alternative. The case is about a 38-year old man with a 20-year past history of severe Morbus Crohn. He underwent two surgical operations, 1984 and 1986, with resection of parts of the colon, and about 60 cm of his large intestine remained. In 1988 his distal ileum was affected and he was operated. He had also been having problems with perianal fistulas. His disease had clearly progressed the last year before we initiated this treatment. He had an activity more or less all the time and we had to give him quite high doses of cortisone. He lost 25% of his habitual weight. Unfortunately he did not tolerate immunosuppresive treatment with Imurel and Puri-nethol. He experienced side effects from the pancreas and the liver, and general arthralgia. When we suggested alternative immunosuppresive treatment he declined since he and his wife had a strong desire to become parents. The treatment with immunomodulation, infliximab, gave no positive results on his intestine. His state worsened, he got a relapse with up to 15 bloodstained diarrhoeas daily, and he experienced fever and stomach pains. We quickly arranged a colonoscopy which was extremely painful for him. The remaining part of the colon's tissue was heavily inflamed, almost necrotic. We could not even get through with the anastomosis, being so narrow due to the swelling. Having a close collaboration with the colorectal surgeons at the Östra Hospital, we called on Dr Öresland. He saw no other way than removing the colon followed by a permanent stoma in his stomach. We initiated the treatment in the conventional way. The patient received high doses of cortisone rectally, 5-ASA, total parenteral nutrition, antibiotics, with the result that he only deteriorated. Only surgery remained as an alternative but the patient did not want to lose more of the colon. He kept asking us if there was nothing else to be done. At this





stage we informed him about the AF and he insisted on testing it.

We have already stated that AF most certainly affects the secretion and inflammation in a postive way. It has also resulted in a clinical improvement. Today AF exists in two forms, a spray dried egg powder, Salovum, that can be administered in a passive way or by stimulation of the patient's endogenous production of AF with specially processed cereals (SPC). We started giving the patient the powder form, with an overlapping treatment from day 5 when we gave him specially processed cereals. The only reason for giving him fine pressed cereals at first was that we were afraid that ordinary SPC would get stuck in his anastomosis. From day 8 he received regular specially processed cereals. Up to this day he is on this diet. At an early stage, already after 48 hours, we noted a re-duction in diarrhoeas. After 12 days they had stopped completely. He had loose defaecation a couple of times but no blood. His fever and abdominal pain disappeared and he felt much better. We saw the effect on the laboratory parameters and the inflammatory parameters improved as well. We removed the TPN and he could be discharged after three weeks length of hospital stay.

Figure 2 illustrates the effect of this treatment on his frequencies of diarrhoeas during different weeks; a fast reduction of his intestinal secretion and a sustained effect the remaining weeks.

On a VAS scale the patient registered how he felt about his intestinal function and general condition and we could see a clear improvement. Three months after initiation of the treatment he had regained his 25% weight reduction and was back to his normal weight. He had personally stopped to take all the medication that he had received over the years. We

"Three months after initiation of the treatment he had regained his 25% weight reduction and was back to his normal weight. He had personally stopped to take all the medication that he had received over the years."

performed a colonoscopy on him and this time there was no pain involved and it was the same colonoscopist who performed the procedure. There was no problem to get through the anastomosis and the small intestine looked fine. The colon was almost totally recovered, there was a slight tendency of oedema and a slight redness. Even the CDAI was lower, 150 compared to over 600 when he was admitted.

What remains today is that the patient still suffers from intermittent fever tops and arthralgia of unknown nature. We have taken systemic samples but have not found anything. He has been referred to a rheumatologist. He also has intermittent abdominal pain but we can not with certainty relate this to his intestine. The colonoscopy revealed that no obstruction existed, nothing that could explain his discomfort.

AF-powder and specially processed cereals is an effective additional treatment

Which conclusion can be drawn from the actual case? Bearing in mind his severe condition, the progression the last year and the acute relapse on top of this, where all customary treatment failed, followed by the rapid effect of the AF powder and the specially processed cereals which reversed his severe medical state, we can not interpret it in any other way than that AF has had effect in his case. Most certainly AF is an effective additional treatment.

We believe that it is wise to try this new treatment

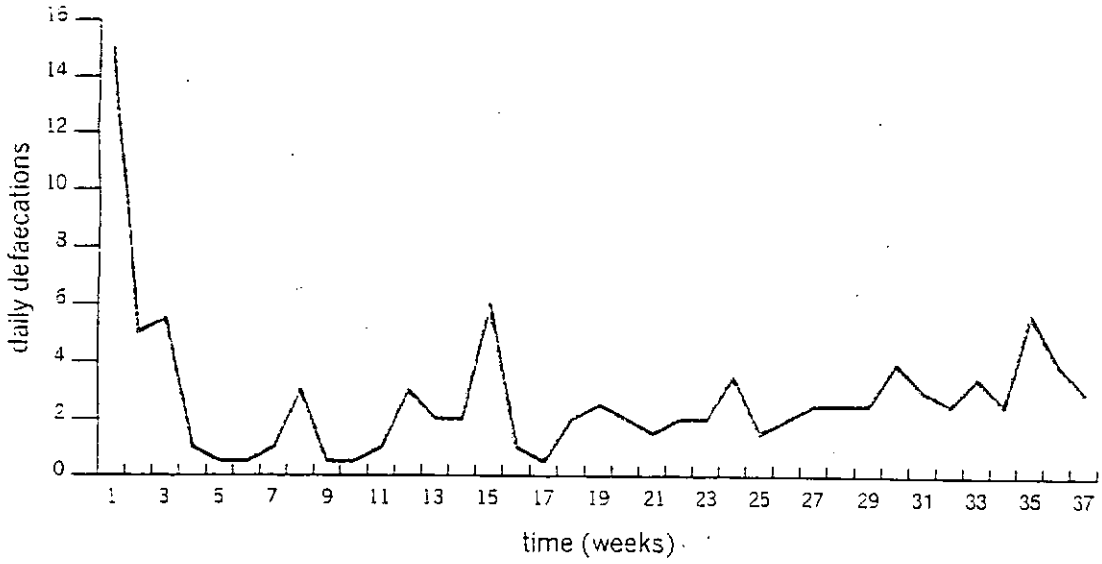


Figure 2. Initiation of treatment with Salovum and SPC dramatically decreased the diarrhoeic frequency from day 2. After 12 days the diarrhoeas had stopped completely and the good effect has been sustained.

on patients who, in one way or another, are refractory to customary treatment.

Question from the chairman: Can it be that the bacterial flora is changed in some way so there is a secondary effect not related to Antisecretory Factor?

Answer: No, we do not believe that. I can tell you that we had a couple of identical cases at the Östra Hospital with the same clinical symptoms and where ordinary treatment failed. We treated them with the same AF products and they are feeling very well.

Question from the chairman: When hearing these patient case stories one nearly gets the feeling that the anti-inflammatory effect is primary and the reduced secretion is secondary. But looking at the Factor per se one believes that antisecretion is primary.

Answer: There is obviously an effect on both parameters. His intestinal secretion was heavily reduced and we also noted an effect on the laboratory parameters and the inflammation, but which of them being most important is difficult to say with certainty. We know that it affects both parameters.

Question from the chairman: Have you tried to remove these specially processed cereals from this patient? Have you not dared to do it?

Answer: The patient declined. He does not want us to remove the AF products. The same applies for the other patients. On the other hand, he receives no other medication.

Question: The two other cases at the Östra Hospital that you were talking about, do you continue with the immunosuppressive treatment?

Answer: Yes, but one of them has stopped all his medication as far as I know.

"The rapid effect of the AF powder and the specially processed cereals which reversed his severe medical state, we can not interpret it in any other way than that AF has had effect in this patient's case. Most certainly AF is an effective additional treatment."

Question: When you initiate AF treatment, do you continue with ordinary treatment and add this?

Answer: In this dramatic case the patient received ordinary intensive treatment and then we added the AF products. It was the same in the other two cases. But it is noteworthy that we started with ordinary treatment, but without effect.

Question from the chairman: We now hope that you will continue with controlled randomised clinical studies.

Answer: In addition to the already conducted studies, more studies will be initiated.

Protein AF: Synthesis and biological effects
Stefan Lange, M.D., PhD, The Institute of Clinical Bacteriology,
University of Gothenburg, Sweden

Specially processed cereals – a treatment alternative
for certain surgical patients
Stellan Björck, M.D., PhD, The Surgical Department,
Sahlgrenska University Hospital, Mölndal, Sweden

Treatment with specially processed cereals (SPC)
in severe Mb Crohn
Morteza Shafazand, M.D., Department of Medicine,
the Section of Gastroenterology,
Sahlgrenska University Hospital/Östra Hospital, Sweden

Chairman: Claes-Henrik Florén, M.D., PhD,
Department of Medicine, University Hospital,
Lund, Sweden

AS-FAKTOR AB
Box 30192, SE-104 25 Stockholm. Phone +46 8 657 42 70. info@as-faktor.se

To: The Chairman
ERC, ICDDR,B
Dhaka.

31.12.2003

From: Prof. J. Ashraful Haq
Department of Microbiology, BIRDEM

Ref.: Comments on the protocol entitled "Efficacy of Salovum egg powder containing antisecretory factor (AF) in the treatment of severe cholera in adult "

The above protocol intends to investigate the effects of Salovum, a freez dried egg yolk powder, and rich in antisecretory factor on the duration of diarrhoea and volume of stool in cholera. It will also assess the safety, acceptability and compliance of the egg yolk powder. It is not clear from the protocol who the manufacturer of Salovum is. I assume that it is manufactured by either Novartis Consumer Health, Switzerland or BioDoc AB, Stockholm, Sweden. It should be clearly mentioned in the protocol.

It will be an open randomized clinical trial consisting of 40 adult male volunteers with severe cholera. The age range will be 15 to 55 years. All patients will be admitted in ICDDR,B Dhaka hospital. The participants will be equally distributed into two groups. Both groups will receive standard treatment for cholera. But the test group will be given 4 g of AF rich egg yolk powder (Salovum) every 2 hours in first 24 hours and thereafter 4 hourly up to 72 hrs. The dose has been arbitrarily selected based on the previous studies on IBD where dose used was 4 g 4 hourly for 14 days.

PI has mentioned that the product is sold in the stores/pharmacy in Sweden and is classified as a food for special medical purpose by the Swedish National Food Administration. There is no limitation for its use by the authority and according to the PI there is no side effects reported so far. However, documents in English language regarding its use in human should be furnished from the manufacturer and Swedish National Food Administration authority.

It may also be mentioned here that PI has not mentioned the concentration of AF in the egg yolk preparation. It is important to know the concentration for optimization and standardization of the dose. It may be mentioned here that the control group will not receive any placebo. It will be good if normal egg yolk preparation of same amount is given as placebo to the control group. Egg yolk contains many ingredients, which may have effect on the secretory status of the intestine.

Stools will be collected and tested for *V. cholerae* and other enteropathogens. A total of 10 ml venous blood will be collected of which 5 ml at the time of enrolment and 5 ml at the end of the resolution of diarrhoea (that is at 72 hrs). Though 10 ml amount is mentioned in the consent form, in the methodology section it is written that only 5 ml blood will be taken at the time of enrollment.

In page 15, it has been mentioned that any serious adverse events occurring in subjects receiving the egg product must be reported. Also, PI has mentioned that any adverse events that may occur even after 4 weeks must be reported if relationship to the product is suspected. But how these patients would be followed up to 4 weeks and later has not been elaborated in the protocol.

The Bengali consent form is not in conformity with the English version. Also, there are scopes for improving the language of the Bangla consent form.

In the face sheet, the item 2(a and (c) may be marked 'yes' instead of 'no'.

Recommendations

Overall, the protocol is well written. The protocol may be approved subject to the incorporation of the above observations.