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


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, fever 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 antisecratory 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 immuno-reactivity for AF peptide has been registered in the colonic mucosa (15). The endogenous stimulation of AF synthesis,
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® 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...
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
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-haptoglobinulin (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, haptoglobin and platelets) showed any differences.

Discussion

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

Acknowledgements

Financial support was provided by AS Factor AB, Nectin AB, The Swedish State under the LUA agreement (grant no. I 33913), Västra Götaland FoU-fond (grant no. KVG-20, I-33823), Inga-Lisa och Bror Björnssons Stiftelse, Adlerbertska Forskningsfonden and Magnus Bergvalls Foundation.

References
