APMIS 111: 985–8, 2003
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APMIS
ISSN 0903-4641

Food-induced antisecretory factor activity is correlated with small bowel length in patients with intestinal resections

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Lange S, Bosaeus I, Jennische E, Johansson E, Lundgren BK, Lönnroth I. Food-induced antisecretory factor activity is correlated with small bowel length in patients with intestinal resections. APMIS 2003;111:985–8.

Specially processed cereals (SPC) can increase antisecretory factor (AF) activity in humans with an intact intestine. The aim of the present study was to investigate whether AF synthesis could be induced in patients who had been subjected to intestinal resections. Eight patients with varying extents of intestinal resections due to Crohn's disease and six healthy controls participated. All subjects received 54 g SPC daily for 2 weeks. Plasma AF activity was determined before, during and after the treatment period. Baseline diet and medications were kept unchanged. The patients registered the daily number of bowel movements. The SPC diet increased AF activity in all controls. In the patients there was a significant correlation between the length of the remaining small intestine and AF induction ($r=0.94$, $p<0.01$) and only those patients with a remaining small intestine of about 3 m reached AF values comparable to those in healthy subjects. It is concluded that small bowel length is related to the ability of humans to induce AF activity by dietary means.

Key words: Antisecretory factor; Crohn's disease; diarrhoea; functional food; short bowel.

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Antisecretory factor (AF) is a 41 kDa protein originally isolated due to its capacity to counteract experimental diarrhoea (1, 2). Increased AF activity can be induced in animals (3, 4) and humans (5) by bacterial toxins or by dietary means. A diet supplemented with specially processed cereals (SPC) increases plasma AF activity and can improve the clinical outcome in patients suffering from inflammatory bowel disease (5) or from endocrine diarrhoea (6).

The source of the AF activity detected in plasma after SPC intake is not known. Previous studies have shown that several tissues are capable of AF synthesis; among these are the epithelial lining of the small and large intestine and lymphocytes in the lamina propria (5, 7). Tentatively, the combined length of the gastrointestinal tract could be one factor of importance for food-induced AF activity.

The aim of the present study was to investigate whether AF synthesis could be induced by SPC in patients with gastrointestinal resections and whether the obtained AF activity could be correlated with the clinical outcome. The effect of the SPC diet on AF activity in a small group of healthy subjects was also studied.

MATERIAL AND METHODS

Subjects and methods

Eight patients, three females and five males (45±9

Received June 10, 2003.
Accepted August 4, 2003.

years old, mean±SD), were asked to participate in the study. All patients had undergone intestinal resections due to Crohn's disease and were attending an outpatient clinic. All were in a stable condition. They had chronic diarrhoea, but there were no signs of active intestinal inflammation; all had been regularly followed for at least 4 years. Clinical information was available on intestinal resections and remaining small intestinal length, measured at their latest operation according to Nordgren et al. (8). The intestinal status of the patients is shown in Table 1.

The six healthy controls, two females and four males (49±5 years old), were enrolled from hospital and laboratory staff. None of the controls had a history of gastrointestinal disease, and none was taking any medication during the course of the study. All subjects gave their informed consent, and the Human Ethics Committee of Göteborg University approved the study design.

After baseline examination, patients and controls were supplied with 54 g of SPC per day, to be ingested in at least three divided doses along with regular meals during a 14-day treatment period. All subjects were instructed that the intake of cereals should be considered as a food supplement, and they were asked not to change their ordinary diet or medication during the study period.

Specially processed cereals (SPC) were produced by BioDoc AB (Stockholm, Sweden) and analysed as previously described (5).

Determination of AF activity

Blood samples were collected by venipuncture immediately before the start of the study and after 2 weeks of SPC diet in all patients and controls. In all controls and in four of the patients, blood samples were also drawn 4 weeks after termination of the SPC diet.

AF was purified from plasma by means of affinity chromatography as previously described (1). In brief, after passage of the plasma through a small agarose column (Sepharose 6B, Pharmacia LKB Biotechnology, Stockholm, Sweden), the agarose-adsorbed AF was eluted with 1 M methyl-α-D-glucoside. The eluate was dialyzed against PBS for 24 h at 4°C, and then stored at −20°C until analysis.

The AF activity of the purified fraction was determined by the ligated loop assay in rats using cholera toxin (CT) as a secretagogue (9). Thus, an AF preparation with 50% inhibition of fluid secretion was assigned an AF value of 1.0. Previous studies on animal herds have indicated that AF values of more than 0.5 are correlated with reduction of diarrhoeal disease (4).

Intestinal output

The patients recorded the daily number of bowel movements (passage of faeces or emptying/change of ileostomy bag) during three periods:

for 7 days just before treatment, for 13 days during the 2 weeks of treatment, and for 7 days starting 2 to 4 weeks after termination of treatment with the SPC.

The control subjects did not register bowel movements, but were asked to record any symptoms or subjective changes in bowel habits.

Statistical analyses

Data are presented as mean±standard deviation (SD). Means were compared using Student's *t* test. A p-value of 0.05 or less was considered significant. Linear regression was used to explore the relation between small bowel length and AF activity, and the relation between change in bowel movements per day and AF activity. All calculations were made using SPSS version 10.0 (SPSS, Chicago, IL).

TABLE 1. *Age, sex and intestinal status of the patient group*

Patient	Age	Sex	Small intestine	Large intestine
P1	37	F	300 cm	no
P2	45	F	200 cm	no
P3	43	M	90 cm	yes, ending in sigmoidostomy
P4	42	M	150 cm	no
P5	62	F	130 cm	yes, ending in sigmoidostomy
P6	53	M	400 cm	yes
P7	34	M	190 cm	yes
P8	40	M	80 cm	yes

RESULTS AND DISCUSSION

Blood values for AF activity before, during and after the SPC period for the eight patients and six healthy controls are shown in Table 2.

AF baseline levels varied somewhat in the healthy controls and several subjects had detectable activity. In response to SPC intake the AF activity in plasma samples increased from basal 0.28±0.37 (mean±SD) units to 1.28±0.23 units after the 2 weeks of SPC diet ($p<0.05$, Table 2). In all healthy subjects, AF levels were maintained for at least 4 weeks after termination of SPC (1.28± 0.39 units).

The AF activity in all patients was low before the test period (0.04±0.07 units) in spite of chronic diarrhoea. Thus, this type of diarrhoea does not seem to elicit an AF response, in contrast to diarrhoea of toxic aetiology, which in experimental models as well as in infected humans induces an AF response that may con-

TABLE 2. *AF activity in patients and controls before, during and 4 weeks after consumption of SPC. "C" denotes control subject, "P" patient, ND=not determined*

Subject	AF at baseline	AF during SPC	AF after SPC
C1	0.3	1.0	1.1
C2	0	1.0	0.6
C3	0.2	1.4	1.4
C4	1.0	1.5	1.7
C5	0	1.5	1.4
C6	0.2	1.3	1.5
P1	0	1.0	0
P2	0	0.5	0
P3	0	0.5	0
P4	0	0.3	ND
P5	0.1	0.5	ND
P6	0.2	1.6	1.1
P7	0	0.7	ND
P8	0	0.2	ND

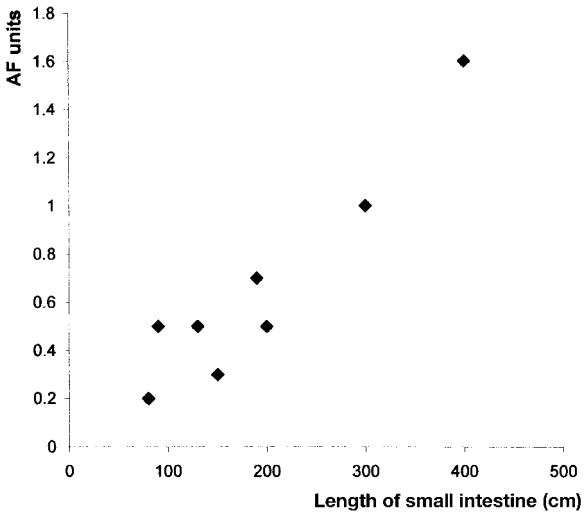

Fig. 1. Correlation between length of remaining small intestine and AF activity after SPC treatment, patients 1–8.

tribute to the self-limiting nature of the disease (10, 11).

After 2 weeks of the SPC diet the average AF activity in the patients had increased to 0.66±0.45 units. Only two patients, P 1 and P6 (Table 2), reached levels comparable to those of the healthy controls, i.e. they responded to the SPC intake with an AF induction of 1.0 units or more. Both these patients had a comparatively long remaining small intestine. Regression analyses of data from the eight patients showed that there was a significant relation between the length of the small intestine and plasma AF during the diet period (Fig. 1, Pearson correlation coefficient of 0.94, $p<0.01$).

Two patients had undergone total colectomy, four patients had an intact large intestine, and two patients had sigmoidostomy (Table 1). There was no apparent effect of the presence or absence of large intestine on the ability to induce AF activity.

For technical reasons AF values 4 weeks after termination of the SPC diet were only available for four patients. Three patients again had undetectable AF values. The remaining patient, P6, maintained an AF value comparable to that registered in the healthy subjects.

These data suggest that the bowel length influences not only the ability to induce AF but also the ability to maintain an increased AF level after termination of the AF-inducing diet.

The healthy control group registered no changes in bowel habits in response to the SPC intake and the ensuing increased AF activity during the test period.

The response to SPC was quite variable in the patients and no average change in number of bowel movements occurred (7.7±2.4 daily bowel movements before treatment and 7.1±5.6 after the treatment period). There was no significant correlation between AF activity after treatment and change in intestinal output ($r=0.39$, ns).

The pathogenesis of diarrhoea in patients with small intestinal resections is multifactorial and AF can at present only be assumed to affect the secretory component of the diarrhoea. This secretory component is predominant in endocrine diarrhoea in patients suffering from intestinal carcinoid tumours and this type of diarrhoea is consequently decreased by AF-inducing therapy (6). Thus, an evaluation of the clinical value of SPC treatment of patients with diarrhoea after intestinal resections demands further studies.

The results of the present study show that a certain length of small intestine and/or a certain passage time for SPC is necessary to induce AF activity. The mechanism by which SPC can induce AF activity is not known. Previous clinical and experimental studies indicate that several cell types in the intestinal mucosa express AF. It is possible that these tissue components react directly on SPC stimulation by increased AF

synthesis, or alternatively that SPC after interaction with receptor molecules induce secondary responses increasing AF synthesis in other tissues.

In conclusion, all control subjects responded to SPC intake with increased plasma AF activity. In patients with intestinal resections the magnitude of the plasma AF activity was significantly correlated with the length of the remaining small intestine. Furthermore, the AF induction did not appear to be influenced by the presence or absence of the large intestine.

Financial support was provided by the Swedish State under the LUA agreement (grant no. I 33 913), by AS Faktor AB, Nectin AB, and by Anna-Lisa and Björn Björnssons Forskningsfond.

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