

ORIGINAL ARTICLE

Antisecretory factor counteracts secretory diarrhoea of endocrine origin

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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.

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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 antiseecretory 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 \pm 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

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 \pm SD	<i>n</i>	<i>P</i>
Baseline	5.6 \pm 2.6	7	<0.01
AF-egg	4.2 \pm 2.4	7	
CC	4.0 \pm 1.9	5	<0.05
SPC	2.6 \pm 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 \pm SD	<i>n</i>	<i>P</i>
Baseline	0.02 \pm 0.05	6	<0.01
AF-egg	0.25 \pm 0.08	6	
CC	0.12 \pm 0.10	3	<0.05
SPC	0.74 \pm 0.33	3	

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).

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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References

- Johansson E, Lönnroth I, Lange S, Jonsson I, Jennische E, Lönnroth C. Molecular cloning and expression of a pituitary gland protein modulating intestinal fluid secretion. *J Biol Chem* 1995; 270: 20615–20620
- Tateishi K, Misumi Y, Ikehara Y, Ikehara Y, Miyasaka K, Funakoshi A. Molecular cloning and expression of rat antisecretory factor and its intracellular localization. *Biochem Cell Biol* 1999; 77: 223–228
- Lange S, Lönnroth I, Skadhauge E. Effects of the antisecretory factor in pigs. *Pflügers Arch* 1987; 409: 328–332
- Göransson L, Lange S, Lönnroth I. Post weaning diarrhoea: focus on diet. *Pigs News Inf* 1995; 16: 89N–91N
- Björck S, Bosaeus I, Ek E, Jennische E, Lönnroth I, Johansson E, Lange S. Food-induced stimulation of the antisecretory factor can improve symptoms in human inflammatory bowel disease. A study of a concept. *Gut* 2000; 46: 824–829
- Bosaeus I, Jennische E, Johansson E, Lundgren BK, Lönnroth I, Lange S. Food-induced stimulation of antisecretory factor is related to small bowel length in patients with diarrhoea after intestinal resection. Submitted to *Scand. J. Gastroenterology*
- Lange S, Lönnroth I. The antisecretory factor: synthesis, anatomical and cellular distribution and biological action in experimental and clinical studies. *Int Rev Cytol* 2001; 210: 39–75
- Wängberg B, Westberg G, Thylén U et al. Survival of patients with disseminated midgut carcinoid tumours after aggressive tumour reduction. *World J Surg* 1996; 20: 892–899
- Samaan N A, Schultz P N, Hickey R C. Medullary thyroid carcinoma: prognosis of familial versus sporadic disease and the role of radiotherapy. *J Clin Endocrinol Metab* 1988; 67: 801–805
- Lange S, Lönnroth I, Martinsson K. Concentrations of antisecretory factor in eggs and in chicken blood plasma. *Br Poult Sci* 1994; 35: 615–620
- Lange S. A rat model for an in vivo assay of enterotoxic diarrhoea. *FEMS Microbiol Lett* 1982; 15: 239–242
- Johansson E, Lange S, Lönnroth I. Identification of an active site in the antisecretory factor protein. *Biochim Biophys Acta* 1997; 1362: 177–182