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Induction of anti-secretory factor in human milk may prevent mastitis

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Aim: The aim of the study was to try to induce anti-secretory factor (AF) in human milk and possibly prevent mastitis. *Methods:* Forty mothers who had normal deliveries and healthy full-term infants were randomly divided into two groups, 3–7 days postpartum. The experimental group received a food inducing AF. The control group received the same type of food, without AF-inducing properties. Milk was tested for AF after the mothers had eaten the cereals for 4–5 wk. AF was determined by intravenous injection of milk samples into rats measuring their capacity to prevent secretion into a gut loop of the rat injected with cholera toxin. *Results:* The median levels of AF differed between the experimental ($n = 12$) and control groups ($n = 16$): 1.1 (0.7–1.25) units vs 0.1 (0.0–0.25) units, $Z = -4.492$, $p < 0.0001$ (11 mothers dropped out and one milk sample is missing from one of the control mothers). The frequency of mastitis in the experimental compared with the control group was reduced ($p = 0.0086$, permutation test). The median AF levels in mothers with or without mastitis differed; 0.0 (0.0–0.1) vs 0.5 (0.2–1.1), $Z = -2.399$, $p = 0.017$.

Conclusion: We suggest a specially treated cereal induces AF in human milk and protects against clinically manifested mastitis.

Key words: *Anti-secretory factor, breastfeeding, mastitis*

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Mastitis in lactating women is a common condition (24–33%) and may range from a relatively short-lasting but painful period of inflammation in the breast to a much worse inflammatory reaction of longer duration and with more severe symptoms (1). When no infection can be confirmed, milk stasis is considered to be one cause of the inflammatory changes. The most severe mastitis is mostly caused by *Staphylococcus aureus* infection (1, 2). These cases require antibiotics. Treatment of the cases of inflammatory and infectious origin is not always immediately effective. In particular, infectious mastitis may lead to interruption of lactation. Recently, it has been suggested that sub-clinical mastitis may result in an increased risk of transfer of HIV-1 from mother to her breastfed offspring (3, 4). Against this background, it is obvious that it would be important to find a way of preventing mastitis. Anti-secretory factor (AF) may occur in milk and seems to have anti-infectious and possibly anti-inflammatory capacities in pigs (5). AF is a protein and is present in most tissues in the human body as well as in the placenta. The anti-secretory activity is exerted by a peptide. The plasma level of AF is increased by enterotoxins, and it has recently been shown that AF might be induced in human body fluids by intake of cereals exposed to a hydrothermal process (5, 6). AF has been isolated and cloned and, in addition, has been shown to modulate intestinal fluid secretion (7). The anti-secretory effect of AF has been demonstrated in patients with secretory diarrhoea of endocrine origin (8). An anti-secretory effect has been suggested in Meniere's disease as well (9). AF may also have an anti-inflammatory effect as illustrated by histological studies of ulcerous colitis. These findings were supported by improvements of inflammatory parameters such as C-reactive protein, erythrocyte sedimentation rate and orosomucoid (10, 11). The aim of the study was to determine whether AF can be induced in human milk with such cereals and possibly affect the appearance of clinical mastitis.

Patients and methods

Data were collected April–August 2002 at the Karolinska Hospital, Stockholm, Sweden. All mothers were Swedish or raised in Sweden, had had normal deliveries and healthy full-term infants. When visiting the outpatient clinic for check-up, they were asked to parti-

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cipate in the study. All women had early discharge (within 36 h postpartum) from the delivery ward and they were fully breastfeeding. Their babies were 3–7 d old. The Ethics Committee at the Karolinska Hospital, Stockholm and the Animal Ethics Committee of the University of Göteborg approved the study.

Participation and procedures

During the pre-determined study days, 77 mothers who fulfilled the inclusion criteria were consecutively approached (KS). Thirty-seven mothers declined, mainly because they did not want to come back to the hospital, or that they did not like to eat cereals, or felt tired and stressed when they were asked to participate.

Mothers who agreed to participate were randomly assigned (sealed envelops opened consecutively) to one of two groups. The randomization was blocked for time. The experimental group received hydrothermally processed cereals (HPC) (6). The control group received the same type of food, but with the HPC replaced by ordinary cereals without specific AF-inducing properties (placebo food). Active and placebo food had the same taste, appearance, and content of energy and nutrients. Both mother and researcher were blind to the content of the bags. The randomization sequence was concealed until data had been obtained and laboratory analyses had been performed. The mothers were instructed to have a daily intake of 1dl (approximately 50 g) of the advised cereals for every breakfast for 5 wk. All mothers received 5 bags of cereals each containing approximately 450 g.

When entering the study, the mothers were interviewed about their background data, any signs of allergy and habits of eating cereals. The mothers were checked for breastfeeding status; squeezed nipple after breastfeeding, sore nipples, or nipples with wounds, milk stasis and mastitis. Incorrect infant sucking technique was corrected. Mastitis was diagnosed when a mother had a breast with one or many resistances, localized redness, swelling, pain and, in addition, had influenza-like symptoms with or without fever. The mothers were asked to make note in a protocol day by day of their cereal consumption. When the mothers had eaten the cereals for 4–5 wk, they were asked to collect 5 ml of milk into a sterile propan screw-top jar and to keep it frozen at −20°C until the next appointment at the clinic.

At a second visit, when the mothers had taken the cereals for 5 wk, they were interviewed about their intake of cereals and how much cereal remained. An additional interview was performed to determine breastfeeding outcome. Exclusive breastfeeding was registered when the mother gave only breast milk and no other liquid or food, apart from prescribed drugs. The mothers were thoroughly interviewed about symptoms of mastitis and diagnosed according to the symptoms above.

Out of the 40 mothers who agreed to participate, 11 dropped out during the first 2 wk. Three mothers in the experimental group and two in the control group left the study due to a medical complication unrelated to mastitis. Two mothers in the experimental group, who did not like the taste of the cereal, also left the study. Two mothers in the experimental group and one in the control group left for unknown reasons. One mother in the experimental group, who baked cereal into cake, was excluded because the process had damaged the capacity to induce AF. Finally, the experimental group consisted of 12 mothers and the control group of 17. In addition, one mother in the control group failed to provide a milk sample.

Preparation of active cereals

The cereals of the HPC were treated in a process similar to malting. The content of sugars and amino acids in the cereals at the end of the hydrothermic process has previously been described (6). After processing, the cereals were dried to 10% moisture. The active material, as well as the placebo food, was available in the form of cereals produced by BioDoc AB, Stockholm, Sweden.

Determination of AF activity in milk

AF was measured in a biological test (5). One millilitre of the clear, intermediate layer of centrifuged milk was diluted with 0.5 ml of phosphate buffered saline (PBS), and the total amount of 1.5 ml was used for intravenous injection into a rat under anaesthesia with Isofluran® (Baxter Medical AB, Kista, Sweden) inhalation via a Univentor 400 Anaesthesia Unit (AgnTho AB, Lidingö, Sweden). Within 20–30 s after the intravenous injection of milk, the rat was challenged with 3 μg cholera toxin in a 15–20-cm long ligated jejunal loop. Immediately after the toxin injection, the intestine was put back and the abdominal wall closed. The rat was sacrificed 5 h later, the ligated loop removed and the fluid secreted into the loop determined by the loop's weight-to-length (mg/cm) ratio. The AF activity of the milk was measured as the inhibition of the cholera toxin-induced fluid secretion. Thus, a 50% reduction of secreted fluid in relation to control rats injected intravenously with the vehicle alone (PBS) was assigned an AF value of 1 unit. There was one animal used for each milk sample; however, for a few samples more rats were used, but never more than three rats per sample.

Statistical analysis

Chi-square test, Mann-Whitney U-test, as well as permutation test were used to analyse differences. Median and quartiles were employed as measure of central tendency and dispersion. A p-value of ≤ 0.05 was considered significant. The statistical program StatView 5.0 was used.

Table 1. Maternal and obstetrical background data for the experimental and the control group.

	Experimental group ($n = 12$)	Control group ($n = 17$)	t-test	p-value
Age (y) median (25th–75th Q)	30.0 (27.5–34.5)	31.0 (29.0–32.0)	−0.071 χ^2	0.9442
Education (n)				
compulsory school	1	0	2.131	0.3446
secondary school	3	7		
university	8	9		
primipara (n)	5	9	2.430	0.2967
multipara (n)	7	8		
Delivery (n)				
partus normalis	11	16	2.127	0.3452
vacuum extraction	0	1		
breach	1	0		
Anaesthesia (n)				
epidural block	6	7	0.221	0.6379
N_2O-O_2	9	14	0.232	0.6302
non-medical	3	2	0.864	0.3527
Child sex (n)				
female	6	6	0.627	0.4284
male	6	11		
			Z-value	p-value
Birthweight (g) median (25th–75th Q)	3440 (3338–3570)	3600 (3296–3709)	0.531	0.5952

Results

Background data and breastfeeding status on entering the study

There were no differences between the experimental group and the control group regarding maternal background data or obstetrical data such as age, education, parity, type of maternal anaesthesia during delivery, child sex and birthweight (Table 1). There was no difference between the groups in the habit of eating cereals. No mothers were allergic to cereals. Four mothers in each group had nipples with wounds at the first visit. Two mothers in the experimental group and one mother in the control group had squeezed nipples after breastfeeding, indicating suboptimal sucking technique. No mother had signs of mastitis.

Frequency of mastitis

One mother in the experimental group was diagnosed with mastitis versus six in the control group. Three of those in the control group had mastitis twice and one had mastitis three times ($p = 0.0086$, permutation test). The mother in the experimental group who had mastitis had eaten cereals only 5 d per week. Misunderstanding the instructions, she did not eat them on Saturdays and Sundays. All mothers who had mastitis had fever >38.4°C. Although no culture was done, one mother was clinically diagnosed with an infected mastitis as she had had nipples with sores that did not heal during the weeks preceding the mastitis. She was treated with antibiotics.

There was no significant relation between nipple complications when entering the study and mastitis during the first 6 wk.

Content of AF in milk samples

There was a significant difference in the median levels of AF between the experimental ($n = 12$) and control group ($n = 16$): 1.1 (0.7–1.25) units vs 0.1 (0.0–0.25) units, $Z = -4.492$, $p < 0.0001$ (Fig. 1). When comparing the median AF levels in mothers with or without mastitis, there was a significant difference: 0.0 (0.0–0.1) vs 0.5 (0.2–1.1), $Z = -2.399$, $p = 0.017$.

The mother in the experimental group who got mastitis and had eaten the HPC only 5 d per week had an AF level of 0.5, which was the lowest level among the mothers in that group.

Most mothers in the experimental group (11 out of 12) and in the control group (15 out of 17) breastfed exclusively during the study period. The others

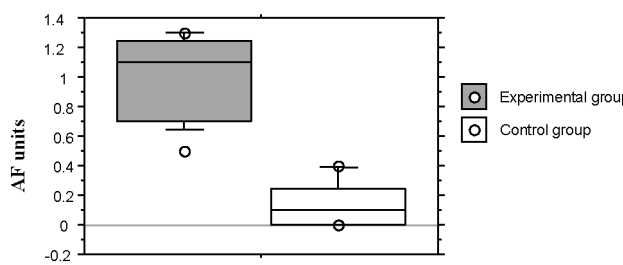

Fig. 1. Level of AF units in the experimental ($n = 12$) and control groups ($n = 16$). There was a significant difference in median levels of AF between the two groups: 1.1 (0.7–1.25) units vs 0.1 (0.0–0.25) units, respectively, $Z = -4.492$, $p < 0.0001$.

breastfed partly. The two mothers in the control group who partly breastfed got mastitis. Their AF levels were 0.1 and 0.0, respectively.

There was no relation between the mothers' AF levels at 4–5 wk and their habits of eating cereals when entering the study, or their parity.

Discussion

Mastitis is a frequent and painful condition for women, and seldom is there adequate treatment. In this small study, originally planned to test the effectiveness of a specially treated cereal to induce AF in human milk, we found a potential preventive effect against mastitis. The frequency of mastitis in our control group was the same as reported in a current review article on this phenomenon (1). The additional finding regarding the positive effect of the cereals will have to be confirmed in a larger study, but the significant preventative aspects of the cereal prompted us to present the data. Further studies are needed to define the likely protective level of AF in human milk and to determine the optimal dose and timing of giving the special cereal to reach such levels. In our study, it seems that an AF level >0.5 in human milk may be protective against mastitis. Previous studies on animal herds have indicated that AF values of more than 0.5 in the milk are correlated to reduction of diarrhoeal disease in the offspring (5). Induction of AF in human milk may possibly also add to the protection against diarrhoea in the offspring as suggested from animal experiments (5). This may support the significant protection provided by secretory IgA antibodies and other components of the milk and would be of special importance in poor countries where diarrhoeal diseases are still a major problem (12). It should be added that the special cereal is an inexpensive product which might well be used in developing countries.

The active component in the hydrothermally treated cereals remains to be identified. However, the process is similar to malting, and degradation of starch, lignin and proteins takes place. Hydrothermally processed wheat has recently been shown to improve the survival in colorectal cancer (13). Neither the active components nor their mechanisms have been identified in this cereal preparation.

In the future, it will be useful to determine whether intake of the cereal will be acceptable in different cultures. The latter point is especially useful if it can be determined that the levels of AF reached in human milk can also prevent the sub-clinical mastitis that, in African studies, has been related to an increased risk of transfer of virus from an HIV-1 positive mother to her breastfed offspring (14).

In conclusion, we present results suggesting that a specially treated cereal induces AF in human milk and prevents clinical mastitis.

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