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ACTIVE SYNTHESIS OF EPIDERMAL GROWTH FACTOR IN HUMAN MAMMARY GLANDS*

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Background. Human milk contains considerable number of growth factors, including epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1). There are no data comparing the EGF and IGF-1 levels in the serum and milk of breast-feeding women. Therefore, the aim of our study was to assess a possible relationship between the concentrations of these growth factors.
Material and methods. Thirty-nine women in child-birth were included in the study. All women provided blood and milk samples during the first six hours after delivery. EGF (by immunoenzymatic method) and IGF-1 (by radioimmunossay method) concentrations were measured in both media.
Results. EGF breast milk concentrations ranged from 3.18 to 4.51 ng/ml and on average were significantly higher ($p < 0.0001$) than those found in the women's serum (from 0.02 to 0.13 ng/ml). The opposite distribution was found for IGF-1 levels. Its milk concentrations ranged from 8.8 to 61.9 ng/ml and on average were significantly lower ($p < 0.0001$) than the serum concentrations (from 192.6 to 595.3 ng/ml). No correlation was found between the serum and milk concentrations of both growth factors.
Conclusion. EGF seems to be synthesized locally in mammary glands, whereas IGF-1 probably permeates into the milk from the vascular bed.

Key words: epidermal growth factor, insulin-like growth factor-1, human milk, serum

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INTRODUCTION

Epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) are trophic peptides present in human milk. These growth factors stimulate the growth, differentiation and maturation of different human cells. Their action accelerates a number of developmental processes, e.g. maturation of the lungs and nervous system. So far, the best known effect of these growth factors is related to the development of the gastrointestinal tract [Murphy 1998, Wong and Wright 1999, Ziegler et al. 1999, Playford et al. 2000, Burrin 2002]. A local action of EGF on the gastrointestinal mucosa plays a crucial role [Thompson 1999 a, b]. An EGF-stimulated increase in mitotic activity has been observed in cultures of human duodenal mucosal cells. An acceleration in the rate of cell division and accretion of mucosal mass has also been confirmed [Chang and Chao 2002]. In addition to morphological changes, functional adaptation such as an increase in water, electrolytes and glucose absorption has also been documented [Chang and Chao 2002, Troyer et al. 2001]. Thompson et al. [1999 b] have shown an inhibiting action of EGF on the processes of enterocyte apoptosis induced by somatotropin and its analogues, particularly in intestinal crypts.

IGF-1 not only acts locally, but also systemically. This factor is one of the major modulators of somatic development [Kornhauser et al. 2002, Yang and Kim 2000]. It has been documented that IGF-1 strongly stimulates DNA synthesis in fetal chondroblasts and chondrocytes [Orbak et al. 2001]. IGF-1 increases the production of proteins, including collagen and sulphate proteins amongst others. It also stimulates the division of fat cells [Orbak et al. 2001, Vatten et al. 2002]. Clinical trials have confirmed an important role of IGF-1 in the regulation of fetal and neonatal growth [Kornhauser et al. 2002, Yang and Kim 2000, Ochoa et al. 2001, Cooley et al. 2004].

Several reports on levels of growth factors in human milk have been published [Playford et al. 2000, Orbak et al. 2001, Cooley et al. 2004, Dvorak et al. 2003]. However, there are no data comparing EGF and IGF-1 levels in the serum and milk of breast-feeding women. Therefore, the aim of our study was to assess a possible relationship between the concentrations of these growth factors in human milk and serum on the first day of lactation.

MATERIAL AND METHODS

Thirty-nine women in child-birth, aged between 18 and 38 (mean ±SEM: 26.0 ±0.8), were included in the study. The mean gestational age of the infants was 39.6 ±0.2 weeks and the average birth weight was 3495 ±83 g. Blood samples were taken and milk samples were collected from all the women.

The inclusion criteria comprised the delivery of a healthy, full term infant; non-complicated course of the pregnancy; and the possibility of milk and blood collection during the first six hours after delivery. The exclusion criteria comprised of any complications in the mother or/and infant revealed within the in-patient period.

Approximately 3 ml of venous blood was taken from the elbow vein. The blood was centrifuged for 10 min at 5000 r.p.m., and the serum was stored at –20°C until the assay was performed. 3 ml of breast milk was collected by hand massage into a test-tube. The milk was spun in a refrigerated centrifuge (at +4°C) for 20 min at 12 000 r.p.m. After removing the fat, the centrifuged milk was stored at –20°C until the analysis.

EGF concentrations in the breast milk and blood serum were measured by an immunoenzymatic method (ELISA; Biosource, Belgium) [Carpenter and Cohen 1990]. IGF-1 concentrations were measured by a radioimmunossay method (RIA), using Biosource isotopes (Belgium) [Nagashima et al. 1990].

The statistical differences between serum and breast milk concentrations of the growth factors (non-paired data) were calculated by the Whitney-Mann test. The relationship between serum and milk concentrations of the growth factors was assessed using the Spearman rank correlation. Unless stated otherwise, values are expressed as mean ±SEM. The level of significance was set at $p < 0.05$.

RESULTS

EGF breast milk concentrations ranged from 3.18 to 4.51 ng/ml and on average were significantly higher ($p < 0.0001$) than those found in the women's serum (0.02-0.13 ng/ml). The opposite distribution was found for IGF-1 levels. Its milk concentrations ranged from 8.8 to 61.9 ng/ml and on average were significantly lower ($p < 0.0001$) than the serum concentrations (192.6-595.3 ng/ml). The breast milk and serum EGF and IGF-1 levels are summarized in Table 1. No correlation was found between serum and milk concentrations of both growth factors.

Table 1. EGF and IGF-1 serum and breast-milk concentrations

Growth factors	Serum	Breast milk	Statistical significance
	mean ±SEM	mean ±SEM	
EGF	0.05 ±0.003	3.94 ±0.06	$p < 0.0001$
IGF-1	312.2 ±15.5	33.2 ±2.2	$p < 0.0001$

DISCUSSION

In recent years special attention has been paid to the role of growth factors contained in human milk in the maturation processes of the gastrointestinal tract [Burrin 2002, Yang and Kim 2000]. Hirai et al. [2002] documented in vitro a strong trophic action of peptide growth factors such as EGF, IGF-1, FGF, HGF and TGF-α in human fetal small intestine cells. Chang and Chao [2002] found an increased number of intestinal Caco-2 cells, as well as higher DNA, RNA and protein content in these cells by applying EGF stimulation. An EGF-stimulated increase of such intestinal enzymes as saccharase, amylase, lactase, trehalase, alkaline phosphatase, enterokinase and gamma glutamyltranspeptidase is documented [Murphy 1998, Troyer et al. 2001]. After a series of animal studies [Wong and Wright 1999, Ziegler et al. 1999, Thompson 1999 b, Hirano et al. 1995], EGF has also been recognised as a factor in accelerating regeneration of the gastrointestinal mucosa. The role of IGF-1 in the alimentary tract is less evident than that of EGF [Ma and Xu 1997, Burrin et al. 2001]. In this study we have documented that the EGF concentration in breast milk is almost 80 times higher than in serum. This finding potentially indicates a dominating role of the local synthesis of EGF in

mammary glands. We did not find any correlation between EGF levels in serum and breast milk. It also points to factors other than serum concentration influencing EGF levels in human milk. On the other hand, breast milk IGF-1 levels were found to be almost 10 times lower than those documented in serum. Similarly, no significant correlation was found between serum and breast milk concentrations. These results suggest that there is little or no synthesis of IGF-1 in the mammary glands.

CONCLUSIONS

EGF seems to be synthesized locally in mammary glands, whereas IGF-1 probably permeates into the milk from the vascular bed.

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SYNTEZA NABŁONKOWEGO CZYNNIKA WZROSTU W MLEKU LUDZKIM

Wstęp. Ludzkie mleko zawiera znaczącą liczbę czynników wzrostowych, w tym nabłonkowy czynnik wzrostu (epidermal growth factor – EGF) oraz insulinopodobny czynnik wzrostu (insulin-like growth factor-1 – IGF-1). Dotychczas nie porównano poziomów EGF i IGF-1 w surowicy i mleku karmiących kobiet. Dlatego też celem badania była ocena potencjalnego związku pomiędzy stężeniami tych czynników wzrostowych.
Materiał i metody. Badaniami objęto 39 rodzących kobiet, u których próbki krwi oraz mleka pobrano w okresie 6 godzin od porodu. W obydwu mediach oceniono stężenia EGF (metoda immunoenzymatyczna) i IGF-1 (metoda radioimmunoenzymatyczna).
Wyniki. Stężenia EGF w mleku kobiecym wynosiły od 3,18 do 4,51 ng/ml i były znacząco większe ($p < 0,0001$) niż w surowicy (od 0,02 do 0,13 ng/ml). Odwrotny rozkład wartości stwierdzono dla IGF-1, stężenia czynnika wzrostowego w mleku wynosiły od 8,8 do 61,9 ng/ml i były znacząco mniejsze ($p < 0,0001$) niż w surowicy (od 192,6 do 595,3 ng/ml). Nie wykazano występowania żadnych zależności pomiędzy stężeniami badanych czynników wzrostowych w mleku i surowicy.
Wnioski. Wydaje się, że EGF jest syntetyzowane miejscowo w gruczołach mlecznych, natomiast IGF-1 najprawdopodobniej przechodzi do mleka z łożyska naczyniowego.

Słowa kluczowe: nabłonkowy czynnik wzrostu, insulinopodobny czynnik wzrostu, mleko ludzkie, surowica

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