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Modulation of Intestinal Alkaline Phosphatase and Lactase Activities in Organ Culture by Growth Factors

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Colostrum and milk are rich sources of nutrients and biologically active substances. Among these substances growth factors are present that are crucial for the proper development of neonates. These growth factors include EGF, SCF, aFGF, bFGF, TNF-alpha and several others. The exact biological role of the latter factors is still under investigation. We have hypothesized that EGF, SCF, TNF-alpha, aFGF and bFGF could influence the activity and distribution of two important intestinal enzymes namely lactase and intestinal alkaline phosphatase. Using biochemistry protocols to measure the total enzyme activities we determined different types of dependence between the growth factors and the enzymes studied.

Key words: growth factors, intestinal alkaline phosphatase, lactase.

Introduction

Colostrum is the first milk that is produced after parturition. It contains high levels of growth factors and components with immunomodulatory properties. These bioactive substances have different origin – some are produced by the mammary epithelium, some are produced by cells within milk or originate from the maternal serum [5]. Some of the growth factors like tumor necrosis factor-alpha (TNF-alpha) could be classified as cytokines – substances with autocrine or paracrine functions. It has been shown that over time their levels in mature milk lower [13]. The precise role of cytokines found in human milk remains under investigation. They are linked to the recruitment of neutrophils, and to the process of intestinal development. Other growth factors in colostrum and milk as epithelial growth factor (EGF), stem cell factor (SCF) or fibroblast growth factors (FGFs) are linked to maturation and healing of the intestinal mucosa [1], or are potent mitogen agents for diverse cell types [6].

Two of the key enzymes that function at the neonatal intestine are lactase and intestinal alkaline phosphatase (iAP). They are considered marker enzymes for the stage of development of enterocytes – absorptive cells that line the lumen of the small intestine. Lactase is a neutral β-galactosidase that hydrolyzes lactose to glucose and galac-

tose that are absorbed by intestinal enterocytes into the bloodstream. Lactase is present on the apical surface of enterocytes. Its activity in most mammals decreases following weaning. iAP is a hydrolytic enzyme crucial for the intestinal homeostasis. It regulates lipid absorption in enterocytes, limits bacterial passage through intestinal epithelium and detoxifies bacterial endotoxins. Dietary components were shown to modulate the expression and activity of iAP [8]. The modulation of lactase activity was detected to be thyroxine dependent [4].

Little information could be found regarding the possible effect of growth factors derived from colostrum or milk on the activity of intestinal enzymes.

The aim of the present work was to follow up the changes in intestinal alkaline phosphatase and lactase enzyme levels provoked by several growth factors from milk: EGF, SCF, TNF-alpha, aFGF and bFGF in organ culture.

Materials and Methods

Organ culture

Small intestinal organ explants (1.0 cm long pieces of duodenum, jejunum and ileum) from 5-day-old Balb/c mice (weaning period) were cultivated separately with different milk growth factors: EGF (50 ng/ml, 48 h), SCF (20 ng/ml, aFGF (10 ng/ml, 24 h), bFGF (10 ng/ml, 24 h) or TNF-alpha (30 pg/ml, 48 h) in RPMI1640 at 37 °C, 5% CO_2 and 95% humidity.

Biochemical assay

After the cultivation, the explants were homogenized in buffer solutions suitable for the respective enzyme (see below) for 10 min on electrical homogenizer. Protein content of homogenates was assessed after Dawson et al. [2] by measuring absorption of the samples at 260 nm and 280 nm on spectrophotometer Specol 1500 (Analitjk, Jena) and calculations, as follows: mg protein/ml = 1.55×A280 – 0.76×A260.

Enzyme activity

The enzyme activities were determined by incubation of tissue homogenates with substrate solutions containing the following substrates:

– for iAP: 20 mM 4-nitropheyilphosphate disodium salt in 0.1 M Tris/HCl, pH 10.00;

– for Lactase: 20 mM 2-Nitrophenil-β-D-galactopyranoside in 0.1 M citrate/phosphate buffer, pH 6.0.

Aliquots were gathered every 5 min (for iAP) or every 30 min (for lactase) in which the reaction was stopped by applying stopping solutions: for iAP – 0.5 M NaOH (10:1), and for lactase – 4% K_2CO_3 (1:1). Spectrophotometric measurements of the samples were performed at 410 nm for iAP; and at 450 nm for lactase using the same spectrophotometer as above. The curves showing the quantity of enzymatically released colorful product per min were built using SigmaPlot 9.0. The enzyme activity was obtained from the curves by regression analysis and was expressed as mg colorful product per min per mg protein.

Results and Discussion

An increasing body of evidence has indicated that growth factors play an important role in cell growth and differentiation. EGF, aFGF and bFGF have crucial role in the development of mammary epithelial cells. Their concentrations in colostrum and milk are dependent on the physiological stage of development [9]. TNF-alpha is also linked to mammary gland development but at the same time it is able to induce apoptotic cell death [14, 10]. SCF is linked to the process of normal development of mammary gland and its loss is associated with malignant transformations [12]. Once introduced into the developing gut, those growth factors enhance the maturation of intestinal cells. Little is known about the role of growth factors in the activation/deactivation of intestinal enzymes. Studies have shown that only high physiological levels of EGF could recover lactase activity after infections [15]. Other studies reported that epithelial growth factor had vague effect on the activity of iAP [3]. Our results indicated that after stimulation of small intestinal explants with EGF in organ culture the activities of both iAP and lactase were augmented (as seen in **Fig. 1** and **Fig. 2)**.

A possible explanation could be the ability of EGF to modify gene expression outside of the crypt region. TNF-alpha release in murine gut was shown to be dependent on iAP levels in inflammatory bowel disease [11]. Lactase activity was augmented in a study dealing with intestinal maturation in rats [7]. In our study iAP activity was increased in duodenum but not in jejunum or ileum. The activity of lactase was decreased in all three parts of the small intestinal specimens. These results suggest that this inflammatory cytokine could cause enzyme deficiencies by down regulating lactase activities. In our study SCF lowered the activity of iAP in all parts of gut explants, whereas for lactase we noticed increased activity for jejunum and for ileum specimens only. Unfortunately, no data is available to compare our results with. Stimulation with aFGF showed increased activity of iAP in duodenum and decreased activity of the enzyme in

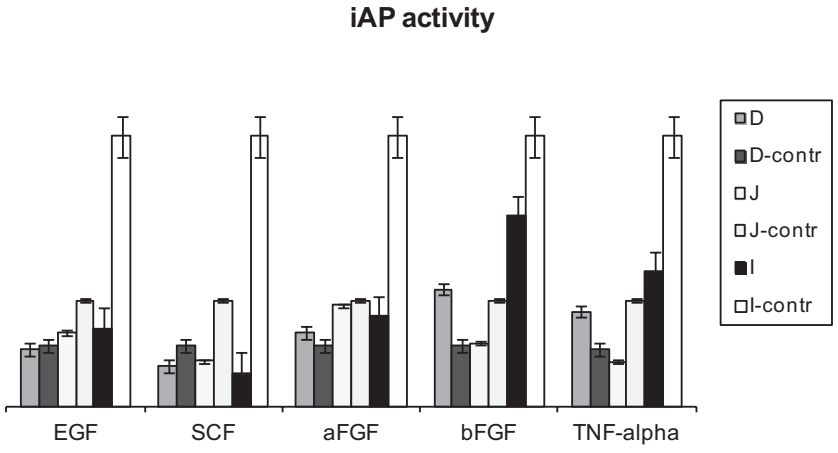

Fig. 1. Intestinal alkaline phosphatase activity in neonatal murine intestinal explants after stimulation with EGF, SCF, aFGF, bFGF and TNF-alpha

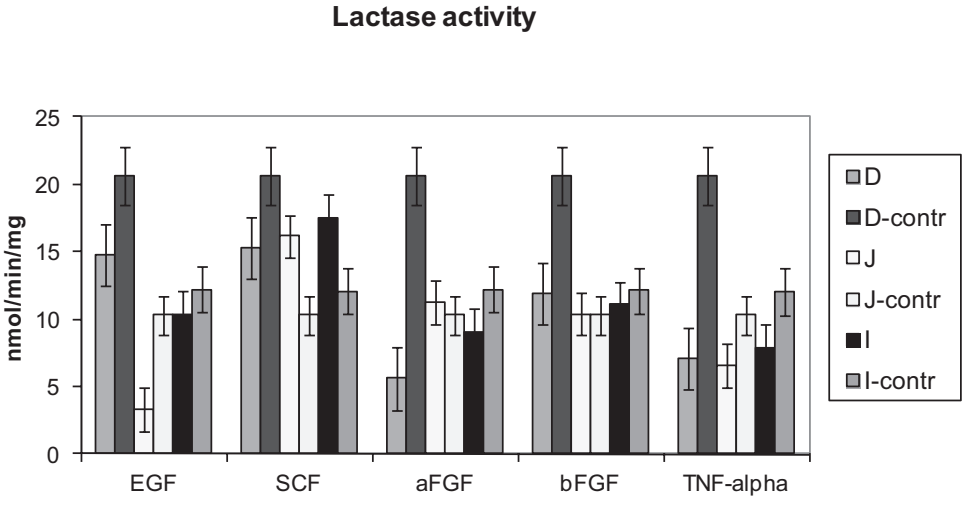

Fig. 2. Lactase activity determined after stimulation of neonatal murine gut explants with EFG, SCF, aFGF, bFGF or with TNF-alpha

jejunum and in ileum. Lactase activity was slightly increased for jejunum and decreased for duodenum and for ileum. Enzyme activities of lactase were not affected by the incubation of neonatal murine gut explants in presence of bFGF whereas iAP levels were increased for duodenal specimens.

Colostrum and milk are dynamic fluids that contain nutrients and bioactive substances needed for the proper development and health of neonates. Growth factors may modulate the activity of key intestinal enzymes in organ culture to different extent. Future studies are needed to investigate the regulatory effect of the growth factors towards the regulation of intestinal cells and the outcomes of their supplementation in infant formulas.

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