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Milk-borne epidermal growth factor modulates bilirubin levels in neonatal rats

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SUMMARY

Objectives: Breast milk jaundice (BMJ) is commonly of undetermined etiology. Recently, it has been shown that the degree of BMJ was associated with the increased levels of milk-borne EGF (Epidermal Growth Factor). The aim of the present study was to test the effects of enteral administration of EGF on the development of hyperbilirubinemia in neonatal rats.
Methods: Pups were divided into three groups: Group 1; artificially fed with formula, Group 2; artificially fed with formula supplemented with 500 ng/ml rat EGF, Group 3; fed by their mothers. Hyperbilirubinemia was induced by an intraperitoneal injection of unconjugated bilirubin suspension once daily for 5 consecutive days. Bilirubin levels, length of ideal villi, and EGF-R expression in the intestine was determined at postnatal day 6.
Results: In the groups of 2 and 3, serum bilirubin levels were significantly higher when compared to animals in the group 1. There was no significant difference in terms of serum bilirubin levels between the Group 2 and Group 3. Length of ileal villi was significantly longer in the Group 2 and Group 3 than the Group 1 animals. The pattern of EGF-R expression in the Group 2 was similar to that seen in Group 3 animals. In contrast, animals given formula without EGF had markedly decreased expression of the EGF-R.
Conclusion: Results suggest that EGF supplementation in newborn rats leads to a significant increase in intestinal mucosal proliferation and a significant decrease in bilirubin elimination. These data suggest that EGF possibly increases intestinal bilirubin absorption

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and may have a role in development of breast milk jaundice. Further studies are needed to confirm this hypothesis.

1. Introduction

Breast milk jaundice (BMJ) is characterized by prolonged unconjugated hyperbilirubinemia in otherwise healthy infants. Even the mechanism of breast milk jaundice is not completely clear, several substances present in milk are involved in the pathogenesis: pregnan-3α-20β-diolon, hepatic glucuroniltransferase inhibitor, inflammatory cytokines, EGF (epidermal growth factor), which could cause reduced intestinal motility and increased bilirubin resorption [1,2].

The introduction of enteral nutrition initiates significant changes in mucosal structure and function required for the utilization of milk. Physiologic changes in digestive and absorptive processes are well described, but the understanding of the mechanisms by which intestinal growth and epithelial turnover are regulated remains fragmentary [3]. In vivo and in vitro studies showed that human milk stimulates intestinal development, DNA synthesis, and cell proliferation. EGF is one of the most important growth-stimulating peptides that augments intestinal epithelial cell proliferation and differentiation and is secreted by multiple cells throughout the gastrointestinal system into the intestinal lumen. It is supplied by amniotic fluid throughout pregnancy, whereas the infant in the postnatal period relies on the significant concentrations of EGF found in human milk and colostrum [4,5]. Because EGF is detected in several mammalian milks, questions have been raised about its functional significance. Previous studies demonstrated the presence of EGF in the developing gut of suckling rats and showed that intestinal EGF levels are predominantly related to the intake of milk-borne EGF [6]. Studies have demonstrated the ability of EGF to preserve intestinal barrier function, increase intestinal enzyme activity, and improve nutrient transport [7]. The biologic actions of EGF are mediated through binding to its specific receptor, EGF receptor (EGF-R), which is distributed throughout the fetal and neonatal gastrointestinal tract [8]. In a previous clinical study, we have suggested that the degree of BMJ was associated with the increased levels of milk-borne EGF. In the aforementioned study, the concentrations of EGF were higher in the breast milk of the mothers whose infants had BMJ and the milk concentrations of EGF were significantly correlated with neonatal bilirubin and blood EGF concentrations [9].

The aim of the present study was to test the effects of EGF supplementation on the development of hyperbilirubinemia in neonatal rats. We hypothesized that enteral administration of EGF could increase the development of neonatal hyperbilirubinemia and the effect of EGF could be mediated through interaction with the EGF-R at the site of intestinal maturity.

2. Materials and methods

2.1. Animals

This study was performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee of the Dokuz Eylul University, School of Medicine. Twenty-one 1-day-old Wistar albino rat pups were used for all experiments.

2.2. Experimental design

Newborn rats were collected from their mothers immediately after birth to prevent suckling of maternal milk. Animals were weighed and then placed in an infant incubator to control body temperature and assigned to three experimental groups: Group 1; artificially fed with growth factor-free