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Human Milk-Derived Growth Factor Prevents Duodenal Ulcer Formation

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ABSTRACT. Human milk was fractionated to obtain a partially purified growth factor preparation. The growth factor in this fraction, designated as human milk growth factor III, exhibits chromatographic and biological characteristics similar to epidermal growth factor-urogastrone. Pretreatment of mice with human milk growth factor III significantly reduces the incidence, number, total length, and severity score of cysteamine-induced duodenal ulcers. (*Pediatr Res* 19: 916–918, 1985)

Abbreviations

HMGF, human milk growth factor
EGF, epidermal growth factor

Human milk is a source of growth factor activity (1) and contains at least three species of growth factors (2). The predominant one, designated as HMGF III, accounts for about 75% of the total growth factor activity in milk and is an acid-stable polypeptide with a mol wt of about 6000 and an isoelectric point of between 4.4 and 4.7 (2–4). These structural characteristics, along with Carpenter's (5) finding that about 70% of the growth factor activity in human milk can be neutralized by antibody directed against human EGF, strongly suggest that EGF or a closely related polypeptide is the major growth factor in human milk. EGF, a polypeptide first isolated from submaxillary glands (6), was found to be structurally similar to urogastrone, a polypeptide prepared from urine (7). EGF-urogastrone is a potent mitogen for a variety of cells (8) and is known to stimulate the growth and maturation of the intestinal mucosa (9, 10). Besides being a mitogen, EGF-urogastrone is an inhibitor of gastric acid secretion (11) and has been used to promote healing of ulcers (12). Moreover, EGF-urogastrone has been localized by immunostaining in the duodenal (Brunner's) glands (13, 14). Specific receptors for EGF-urogastrone have been demonstrated in intestinal epithelial cells (15). Such findings suggest that EGF-urogastrone plays a role in the secretion and growth of the gastrointestinal tract. Recently, Kirkegaard *et al.* (16) reported that EGF significantly inhibits the formation of cysteamine-induced duodenal ulcer in the rat. A similar activity in human milk would be of physiological interest since it would suggest that defined macro-molecular factors in human milk are capable of having an effect on the gastrointestinal tract of the human infant. This report demonstrates that a partially purified HMGF III is capable of protecting mice against the formation of duodenal ulcers induced by cysteamine in a dose-dependent manner.

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MATERIALS AND METHODS

Human milk was kindly provided by Dr. Cutberto Garza of the Baylor College of Medicine, Houston, TX. HMGF III was prepared as previously described (2). In brief, about 100 ml of human milk was centrifuged at 13,000 × *g* for 60 min at 4° C. The fat at the top of the centrifuge tube and the cells and debris at the bottom were discarded. The skimmed milk was acidified by adjusting the pH to 4.3 with HCl and centrifuged at 30,000 × *g* for 60 min to remove precipitate. The supernatant fraction, which contained about 1.5 g of protein determined by the method of Lowry *et al.* (17), was dialyzed against distilled H_2O and lyophilized. The lyophilized sample was then resuspended in 20 ml of 0.1 M NaCl and 0.01 M sodium acetate, pH 4.3 and chromatographed in a Sephadex G-100 column (5 × 90 cm) equilibrated with the same buffer. Fractions of 18 ml were collected, measured for absorbance at 280 nm, and tested for the ability to stimulate DNA synthesis in confluent quiescent monolayers of BALB/c 3T3 cells as previously described (18).

The molecular weights of HMGF III and purified mouse EGF (Collaborative Research, Waltham, MA) were determined by size exclusion chromatography on high-pressure liquid chromatography TSK 3000 columns (7.5 mm ID × 50 cm, Varian, Palo Alto, CA) equilibrated with 6 M guanidine-HCl, 5 mM dithiothreitol and 0.02 M 2-(N-morpholino) ethane sulfonic acid, pH 6.5. About 4 mg of HMGF III prepared by Sephadex G-100 gel filtration was resuspended in 100 μl of equilibration buffer and applied to the column. High-pressure liquid chromatography was carried out at room temperature at a flow rate of 1 ml/min. Fractions of 0.85 ml were dialyzed against distilled H_2O and tested for the ability to stimulate DNA synthesis in BALB/c 3T3 cells. Molecular weight markers used were blue dextran (mol wt, 2×10^6), bovine serum albumin (mol wt, 67,000), ovalbumin (mol wt, 43,000), myoglobin (mol wt, 17,800), ribonuclease (mol wt, 13,700) trasylol (mol wt, 6,200), and insulin (mol wt, 5,800).

Duodenal ulcers were induced in male CD-1 mice (body weight 20–25 g, Charles River, Wilmington, MA) by two subcutaneous injections of cysteamine HCl (350 mg/kg), the second injection being given 7 h after the first. Cysteamine HCl (Aldrich, WI) was freshly prepared in saline at a concentration of 35 mg/ml. The mice were randomly divided into groups of about 12. Ten minutes prior to each cysteamine injection, groups of mice were pretreated with subcutaneous injections of either saline (10 ml/kg), atropine sulfate (10 mg/kg), or HMGF III in various concentrations (0.5, 2, 8, 32, and 128 mg/kg). Twenty-four hours after the first cysteamine injection, the mice were sacrificed by rapid cervical dislocation and their duodena were removed. The duodena were opened along the antimesenteric side and examined under a dissecting microscope (Nikon, Japan) for mucosal lesions. The following four parameters of duodenal ulceration were measured: 1) The incidence of mucosal lesions was determined in each group. 2) The number of mucosal lesions was counted in each mouse. 3) The mucosal lesions were measured at their greatest length and the total length of lesions was esti-

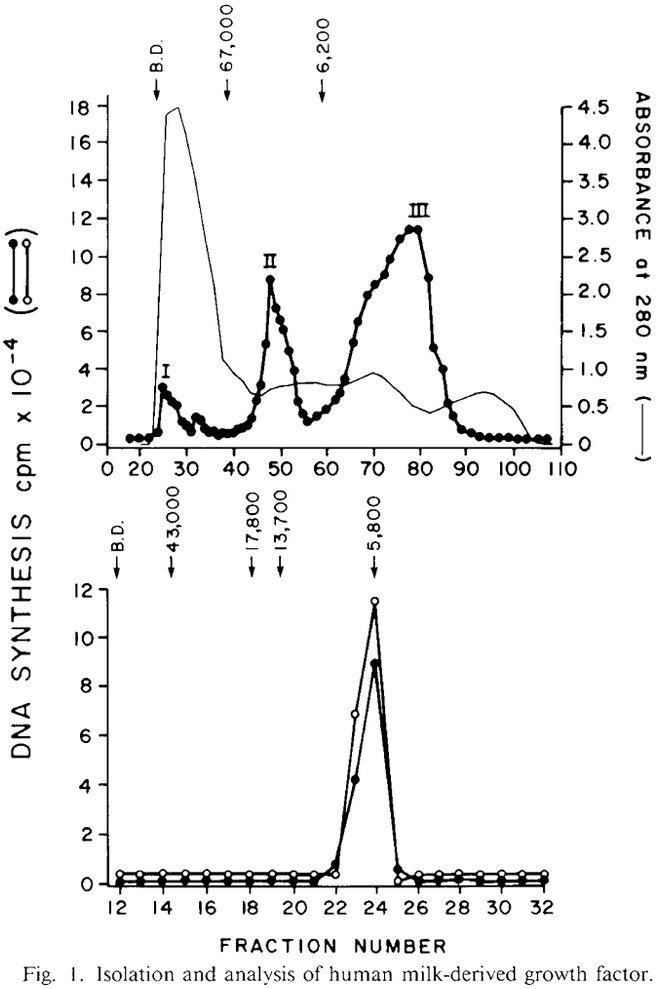

Fig. 1. Isolation and analysis of human milk-derived growth factor. Defatted, acellular milk was acidified to pH 4.3 and the precipitate formed was removed by centrifugation. The supernatant fraction was chromatographed on a Sephadex G-100 column (*top panel*) as described in "Materials and methods." Fractions were collected, measured for absorbance at 280 nm, and tested for the ability to stimulate DNA synthesis in 3T3 cells. Partially purified HMGF III (4 mg/100 μl, obtained from fractions of the activity peak labeled III) was further analyzed on a high-pressure liquid chromatography size exclusion column (*bottom panel*) as described in "Materials and methods." Purified mouse EGF (20 μg/100 μl) was also analyzed in the same manner for comparison. Fractions were collected, dialyzed, and tested for the ability to stimulate DNA synthesis in 3T3 cells. HMGF III (●—●); mouse EGF (○—○).

mated for each mouse. 4) The severity of ulcers was assessed using the scoring system of Smith (19): 0–normal; 1–superficial mucosal erosion; 2–hemorrhagic ulcer; and 3–perforation. The incidence of duodenal ulcers was evaluated using a χ^2 test. The number, total length, and severity of ulcers were analyzed with a Student's *t*-test. The ulcer index was calculated by adding the severity score to the incidence of duodenal ulcers multiplied by 2 (20).

RESULTS

When defatted, acidified human milk was fractionated by gel filtration on Sephadex G-100 column (Fig. 1, *top panel*) three peaks of growth factor activity were observed. The third peak in descending order of mol wt contained about 75% of the total growth factor activity in human milk and was designated as HMGF III. The molecular weight of HMGF III was more accurately determined by high-pressure liquid chromatography on a size exclusion column (Fig. 1, *bottom panel*). It had a mol wt of about 6000 and comigrated with mouse EGF. HMGF III, partially purified by Sephadex G-100 column chromatography, was tested as an antiulcer agent.

Two subcutaneous injections of cysteamine separated by an interval of 7 h caused duodenal lesions in 62.5% of the 16 mice treated (Table 1). These lesions, varying from superficial mucosal erosions to marked hemorrhagic ulceration, were located in the anterior duodenal wall and close to the pylorus. Pretreatment of mice with atropine prior to cysteamine injections significantly protected them from cysteamine-induced duodenal ulceration. All parameters of ulceration were markedly reduced and the ulcer index was 0.72 compared to 2.77 for those mice which received cysteamine without atropine pretreatment. These results demonstrated that cysteamine-induced ulcers were responsive to a well known antiulcer agent (21, 22).

HMGF III significantly reduced the incidence, number, and total length, as well as the severity score, of cysteamine-induced duodenal ulcers in a dose-dependent manner (Table 1). At 32 mg/kg all parameters were significantly reduced. HMGF III at this level appeared equally as effective as atropine as an antiulcer agent. There was no mortality among the 86 mice treated.

DISCUSSION

This report demonstrates that, in mice pretreated with HMGF III, all parameters of the cysteamine-induced duodenal ulcers are significantly reduced in a dose-dependent manner. These results are in agreement with those reported earlier by Kirkegaard *et al.* (16). They were able to demonstrate that intraduodenal infusion of exogenous EGF inhibits the formation of cysteamine-induced duodenal ulcer in the rat. The cysteamine-induced duodenal

Table 1. *Effects of HMGF III on cysteamine-induced duodenal ulcers in mice**

Pretreatment with two subcutaneous injections of	*n*	Duodenal ulcers				
		Incidence (%)	Number of ulcers	Total length (mm)	Severity score	Ulcer index†
Saline						
10 ml/kg	16	62.50	1.19 ± 0.29	1.15 ± 0.23	1.52 ± 0.33	2.77
Atropine sulfate						
10 mg/kg	11	18.18‡	0.18 ± 0.12§	0.25 ± 0.17‖	0.36 ± 0.24§	0.72
HMGF III (mg/kg)						
0.5	12	66.67	1.08 ± 0.30	1.33 ± 0.36	1.51 ± 0.33	2.84
2	12	58.33	1.00 ± 0.30	0.88 ± 0.27	1.14 ± 0.31	2.31
8	12	33.33	0.33 ± 0.14‡	0.35 ± 0.19‡	0.83 ± 0.37	1.50
32	11	18.18‡	0.27 ± 0.19§	0.26 ± 0.18§	0.32 ± 0.22§	0.68
128	12	16.67§	0.25 ± 0.18‡	0.29 ± 0.19§	0.33 ± 0.22§	0.66

* The values of number of ulcers, total length, and severity score are means ± SEM.

† Ulcer index = the sum of the arithmetic mean of the severity score in a group and the incidence of duodenal ulcer multiplied by 2.

‡ $p < 0.05$, § $p < 0.02$, ‖ $p < 0.01$ when compared with the corresponding values in saline control.

ulcer in the rat has been used widely as a model of peptic ulcer disease. It resembles duodenal ulcer in man in its characteristic location, histological appearance, and some aspects of pathophysiology (20, 23). It has been reported that mice are slightly less sensitive to cysteamine ulcerogenesis than rats, but the ulcers induced in mice have a similar location, histopathology, and drug responses to those induced in rats (19). However, the cysteamine model in mice has the advantage of lower drug consumption and, therefore, has been advocated as a screening test for antipeptic ulcer activity in novel compounds. In our experiments, partially purified HMGF III was used because not enough pure growth factor was available to treat the large number of mice. Therefore, there is no conclusive proof as yet that it is the growth factor that prevents ulcers. However, the structural similarities of HMGF III to EGF-urogastrone, which is an anionic polypeptide with a mol wt of about 6000 and a well known antiulcer reagent (24), strongly suggests that it is the growth factor responsible for the antiulcer effect. Whether HMGF III and EGF-urogastrone are identical or structurally similar polypeptides will require amino acid sequencing analysis.

HMGF III was given to mice subcutaneously rather than orally because subcutaneous administration is a much more efficient technique for growth factor delivery. However, the resistance of HMGF III to inactivation by HCl, pH 1 (3), and the presence of trypsin inhibitors in human milk (25) make it plausible that this growth factor could survive passage through the gastrointestinal tract. Thus the antiulcer effect of HMGF III might be one of the protective mechanisms that human milk is postulated to have for the breast-fed infant.

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