Epidermal growth factor enhances repair of rat intestinal mucosa damaged by oral administration of methotrexate

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Abstract: To examine the trophic effect of epidermal growth factor on the rat small intestine, we measured diamine oxidase and ornithine decarboxylase activities in intestinal mucosa injured by methotrexate. Methotrexate was infused orally via a gastric tube at a dose of 10mg/kg per day on 3 successive days (days 1–3). Epidermal growth factor was injected intraperitoneally at a dose of 40μg/kg per day on 4 successive days following methotrexate infusion (days 4–7). Methotrexate caused a marked decrease in diamine oxidase activity; this decrease returned to a normal level on day 13 in controls. In rats injected with epidermal growth factor, diamine oxidase activity began to recover earlier than in the controls, and returned to a normal level on day 11. Epidermal growth factor enhanced the increase of ornithine decarboxylase activity in mucosa injured by methotrexate. When the increase of ornithine decarboxylase activity was suppressed by α-difluoromethylornithine, epidermal growth factor failed to facilitate the repair of intestinal mucosa. These results indicate that epidermal growth factor enhances intestinal repair following methotrexate infusion, and that this effect is mediated, at least in part, by ornithine decarboxylase. It is proposed that epidermal growth factor can be used clinically as a means to enhance mucosal repair of the intestine after chemotherapy with methotrexate.

Key words: ornithine decarboxylase, α-difluoromethylornithine, diamine oxidase, chemotherapy

Introduction

Ornithine decarboxylase (ODC), which catalyzes the formation of putrescine, is the first rate-limiting enzyme in the biosynthesis of polyamines, and both polyamines and ODC play an important role in mucosal growth and repair in the small intestine.1,2 Repair of the mucosa of the rat small intestine damaged by stress,3 cytosine arabinoside,4 burn injury,5 and ischemia-reperfusion6 is attenuated when the increase of ODC activity is suppressed by α-fluoromethylornithine (DFMO), a suicide inhibitor of ODC.

It has been well demonstrated that epidermal growth factor stimulates the repair of gastric mucosa7-9 and, in fact, a clinical study of epidermal growth factor for the treatment of peptic ulcer has begun.9 Several studies have demonstrated that epidermal growth factor stimulated ODC activity and the biosynthesis of polyamines in rat small intestine,10-13 and that epidermal growth factor had a trophic effect not only on gastric mucosa but also on the mucosa of the small intestine.13-16

Methotrexate, a folic acid analog, is used for the treatment of leukemia, choriocarcinoma, and other malignant diseases.17 Major lesions resulting from its cytotoxic action occur in bone marrow and in the intestinal tract.17 The aims of the present experiments were to establish: (1) whether epidermal growth factor stimulates the restoration of rat small intestine mucosa damaged by methotrexate, (2) whether epidermal growth factor stimulates ODC activity in damaged intestinal mucosa, and (3) whether restoration of the intestinal mucosa induced by ODC is attenuated when the increase in ODC activity is suppressed by DFMO.
Materials and methods

Animals

Male Sprague-Dawley rats (240–280g) were used in this study. The animals were housed in wire-bottomed cages placed in a room illuminated from 08:00h to 20:00h (12-h light-dark cycle) and maintained at 21 ± 1°C.

Administration of methotrexate. Methotrexate (Lederle Japan, Tokyo), dissolved in distilled water, was administered orally via a gastric cannula at 10:00h on 3 successive days (days 1, 2, and 3), being infused at doses of 1, 5, or 10 mg/kg per rat on each of the 3 days. Controls were infused orally with vehicle only.

Injection of epidermal growth factor. Human epidermal growth factor, generously provided by Hitachi Chemical Co. (Tokyo, Japan), was injected intraperitoneally at 11:00h on 4 successive days (days 4, 5, 6, and 7) following the oral administration of methotrexate. Epidermal growth factor was dissolved in physiological saline, and was injected at doses of 10, 20, 40, or 80 μg/kg per day on each of the 4 days. Controls were injected intraperitoneally with vehicle only.

Injection of DFMO. DFMO was generously furnished by Merrell Dow Research Institute (Cincinnati, Oh.). The animals were injected intraperitoneally with DFMO (200 mg/kg), every 8 h on days 4, 5, 6, and 7.

ODC and diamine oxidase (DAO) activities in jejunum

The animals were anesthetized with halothane and then sacrificed at 15:00h, 4 h after the epidermal growth factor injection and before the rats normally began feeding. The jejunum was sectioned for histological examination. The jejunal mucosa was also obtained, by scraping with a glass slide over an ice-cold plate, for the determination of ODC and diamine oxidase (DAO) activities.

ODC assay. ODC activity was assayed by a radiometric technique in which the amount of 14CO2 liberated from L-[1-14C]ornithine (52.3 mCi/mmol; New England Nuclear, Boston, Mass.) was measured. Mucosal scrapings from the jejunum (200 mg wet weight) were placed in 2 ml of 0.1 M Tris buffer (pH 7.4), containing 1 mM ethylene diamine tetraacetic acid (EDTA), 50 μM pyridoxal phosphate, and 5 mM dithiothreitol. The tissues were homogenized and centrifuged at 30,000 g for 30 min, protein content was determined, and 200-μl aliquots of the supernatant were incubated in stopped vials in the presence of 3.5 nmol of L-[1-14C]ornithine for 15 min at 37°C. The 14CO2 liberated by the decarboxylation of ornithine was trapped on a piece of filter paper, impregnated with 20 μl of 2 N NaOH that was suspended above the reaction mixture. The reaction was terminated by the addition of 0.3 ml of 10% trichloroacetic acid. The radioactivity of the 14CO2 trapped in the filter paper was measured in an aqueous miscible scintillant (Opti-Fluor; Packard Instrument, Downers Grove, Conn.).

DAO assay. To assay DAO activity, [1,4-14C]putrescine (Amersham, Arlington Heights, Ill.) was used as the substrate. Mucosal scrapings (200 mg) were homogenized in 3 ml of 0.2 M phosphate-buffered saline (pH 7.6), and centrifuged at 48,000 g for 30 min. [14C]Putrescine was mixed with unlabeled putrescine to yield a specific activity of 0.22 μCi/μmole and this was then mixed with phosphate-buffered saline to form a 4.5 mM putrescine solution. Fifty μl of the putrescine solution and 200 μl of supernatant from the mucosal homogenate were mixed and incubated at 37°C for 30 min; the reaction was terminated by the addition of 200 μl of 1.7 M perchloric acid. One ml of 1 N NaOH was added, after which the incubation mixture was extracted with 6 ml toluene that included 0.35% 2,5-diphenyloxazole. The radioactivity of the toluene phase was then determined for 5 min in a liquid scintillation spectrometer (460 CD; Packard Instruments).

Experiment 1: Effect of methotrexate on intestinal mucosa

Methotrexate was infused orally to the rats at doses of 1, 5, or 10 mg/kg per day. The animals were sacrificed on day 8, and the ODC and DAO activities in the jejunal mucosa were measured. There were five animals in each group.

Experiment 2: Effect of epidermal growth factor on mucosal ODC and DAO activities

Either methotrexate (10 mg/kg per day) or vehicle only was administered to the rats orally. Epidermal growth factor was injected intraperitoneally at doses of 10, 20, 30, or 80 μg/kg per day. The animals were sacrificed on day 8 for the measurement of jejunal ODC and DAO activities. There were five animals in each group.

Experiment 3: Effect of epidermal growth factor on repair of jejunal mucosa injured by methotrexate

Methotrexate was administered to the rats orally at a dose of 10 mg/kg per day. Either epidermal growth factor (40 μg/kg per day) or vehicle only was injected intraperitoneally. Animals were sacrificed on each day from days 4 to 14, and ODC and DAO activities in the jejunal mucosa were measured. In addition, histological examination of the jejunum was performed in...