Aged Garlic Extract Protects against Methotrexate-Induced Apoptotic Cell Injury of IEC-6 Cells\textsuperscript{1,2}

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ABSTRACT Gastrointestinal toxicity is one of the most serious side effects of methotrexate (MTX) treatment. The side effects often disrupt the cancer chemotherapy. We previously reported that aged garlic extract (AGE) protects the small intestine of rats from MTX-induced damage. In this study, the protection of AGE against MTX-induced damage of IEC-6 cells originating from the rat jejunum crypt was investigated. MTX decreased the viability of IEC-6 cells, but this effect was prevented by AGE (0.5%). The MTX-induced apoptosis of IEC-6 cells was depressed by AGE. These results indicated that AGE protects IEC-6 cells from the MTX-induced damage. AGE may be useful in cancer chemotherapy with MTX because it reduces MTX-induced intestinal damage. J. Nutr. 136: 861S–863S, 2006.

KEY WORDS: • aged garlic extract • methotrexate • IEC-6 cells • intestinal damage

Methotrexate (MTX) is a folate antagonist used not only as an antitumor drug but also as an antirheumatoid drug. The use of MTX in cancer chemotherapy is often limited by various side effects, including vomiting, diarrhea, mucositis, and decrease of nutrient absorption (1). The toxic effects are not considered to be a result of direct action on the gastrointestinal tract tissues but rather to be the consequence of an inhibition in dihydrofolate reductase synthesis (2). This enzyme is required to maintain the intracellular pool of tetrahydrofolate during purine and thymidine synthesis. It affects not only tumor cells but also rapidly dividing host cells such as crypt cells of the gastrointestinal mucosa. It is characterized histologically by villus atrophy or crypt loss (3). MTX treatment decreases the surface area of the small intestine because of the damaged and shortened villi, components of the small intestinal mucosa like proteins and lipids, and the number of crypt cells. Cellular edema and bleb formation occur during treatment (4). The treatment also changes the physical structure of brush-border membranes (5).

The chemical and morphologic changes in the small intestine may be triggered by crypt cell damage (6). To minimize the side effects in patients undergoing chemotherapy, it is important to reduce mucosal damage and stimulate tissue repair (6). Such intestinal damage is reported to be prevented by some kinds of nutrients and growth factors. For example, keratinocyte growth factor (7) and insulin-like growth factor-1 (8) stimulate regrowth of the damaged intestine and protect mice from gastrointestinal injury. We have also demonstrated a protective effect of retinol (5), docosahexaenoic acid (9), and synthetic analogs of prostaglandin E\textsubscript{1} (4,10) on the MTX-induced damage of the small intestine.

Garlic derivatives have various biologic properties such as antimicrobial and anti thrombotic activities, immune system enhancement, and antitumor potential (11). Aged garlic extract (AGE) and its constituents prevent oxidative injury in endothelial cells (12) and suppress cancer growth (13). AGE also protects the small intestine of rats from MTX-induced damage (14). Thus, it has been suggested that AGE has different effects on tumor cells and normal intestinal cells.
MTX induces apoptosis in the small intestine (15). We found that MTX induced apoptosis of IEC-6 cells, an immortalized epithelial cell line derived from neonatal rat ileum. In the present study, we investigated the effect of AGE on MTX-induced cytotoxicity, using IEC-6 cells, and showed that AGE inhibits the MTX-induced apoptosis in IEC-6 cells.

MATERIALS AND METHODS

Materials. MTX was donated by Wyeth Lederle Ltd. (Tokyo, Japan). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and other chemicals were obtained from Sigma Chemical. AGE was prepared by Wakanaga Pharmaceutical as described elsewhere.

Cell culture. IEC-6 was obtained from American Type Culture Collection. IEC-6 cells were grown in DMEM (Sigma) containing 4.5 g/L glucose, 1.5 g/L sodium bicarbonate, 5% fetal bovine serum (FBS), 100 units/L bovine insulin (Wako Pure Chemical Industries), and 20 mg/L gentamicin sulfate (Nacalai Tesque). Cells were incubated at 37°C in 5% CO₂ and 95% air. Culture media were changed every 2 d.

MTT assay. The viability of IEC-6 cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, IEC-6 cells were plated on a 96-well multiplate and treated with MTX in the presence or absence of AGE for 72 h. MTT solution (5mg/mL, 1:10) was added to each well. Following 4 h of incubation at 37°C, the produced formazan was dissolved with acid-isopropanol solution (0.04 N HCl/isopropanol). The absorbance at 570 nm (reference at 630 nm) was determined by a microplate reader Multiskan JX (Themo LabSystems).

RESULTS AND DISCUSSION

IEC-6 is an immortalized epithelial cell line derived from neonatal rat ileum. IEC-6 cells have the characteristics of crypt-type intestinal cells and have been extensively used as an in vitro intestinal model for gastrointestinal regeneration (16) and for the study of folate and its transport derivatives, including MTX (17). In the present study, we investigated MTX-induced gastrointestinal toxicity, using IEC-6 cells.

IEC-6 cells were treated with MTX (0.01–100) for 72 h (Fig. 1). The viability of IEC-6 cells decreased with the increase of MTX concentration. The MTX-induced loss of viable IEC-6 cells was prevented by the presence of 0.5% AGE (Fig. 2). We have recently found in chromatin condensation, DNA fragmentation, caspase-3 activation, and cytochrome c release in IEC-6 cells with MTX. These changes were returned to the control levels by the presence of AGE (unpublished results). Thymidine incorporation into IEC-6 cells incubated with MTX for 24, 48, and 72 h was markedly increased (Fig. 3). This finding suggested that the salvage pathway that used the added thymidine contributed to the increase of thymidine incorporation, because MTX inhibited the de novo pathway of thymidylate synthesis. The presence of AGE in IEC-6 cells with MTX suppressed the increase of thymidine incorporation, which suggested that AGE prevented the MTX-induced inhibition of dihydrofolate reductase and/or activated the de novo pathway, resulting in suppression of the requirement of extracellular thymidine.

The results indicate that AGE inhibits MTX-induced toxicity, which suggests that AGE may be useful in cancer chemotherapy by reducing the intestinal damage induced by antitumor drugs.

LITERATURE CITED