

Protective role of lipoic acid on methotrexate induced intestinal damage in rabbit model

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Abstract Methotrexate (MTX), a folate antagonist agent, is mainly used in treatment of malignant tumors and autoimmune diseases and affects not only tumor cells, but also gastrointestinal mucosa. The present study was undertaken

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to determine whether lipoic acid (LA) could ameliorate methotrexate-induced oxidative intestine injury in rabbits. Twenty-one rabbits were randomly assigned into three groups: Group 1 (control group), Group 2 (received 20 mg/kg MTX), Group 3 (received MTX plus LA 75 mg/kg orally). On the 6th day rabbits were anesthetized and intestinal tissue sampled for pathologic and biochemical assessment. The intestinal tissue injury index and malondialdehyde (MDA) levels were lower in MTX+LA group as compared to the MTX group, and tissue glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity were higher in MTX+LA group than in the MTX group ($p < 0.05$). These findings suggest that co-administration of LA with MTX is associated with reduction in oxidative injury and tissue damage in the intestine. We suggest that lipoic acid may have a protective role in the MTX-induced oxidative injury.

Keywords Intestine · Methotrexate · Thiocetic acid

Introduction

Methotrexate (MTX), a folate antagonist agent, is mainly used in the treatment of malignant tumors; it has also been found to have a major therapeutic role in non-neoplastic diseases as an anti-inflammatory and immunosuppressive agent. However, the clinical application of this agent is often limited by side effects such as nausea, vomiting, diarrhea, stomatitis, gastrointestinal ulceration and mucositis. These side effects are probably due to inhibition of the synthesis of dihydrofolate reductase which is essential to maintain the cellular tetrahydrofolate pool during purine and thymidine synthesis [1]. Being a high affinity inhibitor of dihydrofolate reductase, MTX is a pro-oxidant com-

found that causes depletion of the dihydrofolate pool and directly affects the synthesis of thymidilate, suppressing DNA synthesis. Therefore MTX affects not only tumor cells, but also rapidly-dividing cells such as crypts of gastrointestinal mucosa where it inhibits epithelial proliferation and induces apoptosis in the small intestinal crypts [2]. This is an important complication for patients who are undergoing cancer chemotherapy. Various efforts have been made to minimize the side effects of MTX by reducing mucosal damage and stimulating the tissue repair system of the intestine. However, these have failed to reduce the MTX-induced gastrointestinal toxicity [3]. Recently, it was demonstrated that MTX causes significant reduction in the antioxidant enzyme levels, sensitizing the cells to reactive oxygen species (ROS) [4]. Antioxidants have been used to ameliorate MTX injury in the intestine, and there are reports showing the use of MTX together with antioxidants such as vitamin E, vitamin A, garlic extract, N-acetyl cysteine and sodium tungstate.

Alpha-lipoic acid (LA) or thioctic acid (chemical name: 1,2 dithiolane-3-valeric acid or 6,8-dithio-octanoic acid) is a natural dithiol compound which is known to be a co-factor in the α -ketoacid dehydrogenase mitochondrial complex and for its potent antioxidant properties. The antioxidant effects of LA are attributed to direct radical scavenging and metal chelation [5]. The present study was performed to determine whether LA could ameliorate methotrexate-induced oxidative intestine injury in rabbit.

Methods

Twenty-one male rabbits were used in this experiment. The experimental protocol was approved by the medical ethics committee, and all animals received humane care in compliance with the guidelines of Tabriz Medical University. The rabbits were randomly assigned into three groups of seven rabbits each. Group 1 (control group): rabbits in this group received normal saline. Group 2 (MTX-treated group): rabbits received a single

intraperitoneal dose of MTX (20 mg/kg) on day one. Group 3 (MTX+LA-treated group): rabbits received a single intraperitoneal dose of 20 mg/kg on day one, and also received 75 mg/kg LA orally starting 3 days prior to MTX injection until 6 days after MTX administration. On the 6th day after MTX administration rabbits were anesthetized using ketamin 50 mg/kg and xylazine 10 mg/kg, and decapitated with a decapitator device. The small intestine was excised and stored at -70°C for measuring tissue glutathione peroxidase (GPx), superoxide dismutase (SOD) and malondialdehyde (MDA) levels. A part of the intestinal tissue was fixed in formalin 10% for studying with light microscopy.

GPx and SOD activities and MDA levels in intestinal tissue were measured as described previously [6–8].

Small intestinal tissue specimens excised from jejunum were fixed in 10% formalin, embedded in paraffin wax on the oriented edge, and cut into 5 μm thick sections for histological examination. All tissue sections were stained with hematoxylin and eosin. Intestinal damage was assessed by scoring each of the following histological observations: (a) villous shortening and fusion; (b) epithelial atrophy; (c) crypt loss; (d) inflammatory infiltrate in the lamina propria and (e) goblet cell loss as 0, none; 1, mild; 2, moderate; 3, severe. Thus, the maximum total score was 15 [9].

Statistical analysis

All values are presented as median (range). Differences were considered to be significant at $p < 0.05$. Statistical analyses were performed using Mann–Whitney U test for comparing the result between groups by SPSS version 16.

Results

Data on the enzyme activities of the control group, MTX treated group and MTX+LA group are shown in Table 1. The intestinal tissue of rabbits showed significant reduction of GPx and SOD activity after MTX treatment comparing

Table 1 Antioxidant enzyme activities, lipid peroxidation level and intestinal tissue injury in the intestine of rabbits

	Control	Methotrexate	Methotrexate + Alpha-lipoic acid
Glutathione peroxidase (U/mg wet weight of tissue)	1.69 (1.56–1.91)	0.80 (0.50–1.10)	1.30 (1.10–1.40)
Superoxide dismutase (U/mg wet weight of tissue)	1.93 (1.90–2.20)	1.10 (0.80–1.30)	1.50 (1.30–1.60)
Malondialdehyde (U/mL)	0.95 (0.83–1.20)	3.70 (3.10–3.90)	2.20 (1.90–2.50)
Intestinal tissue injury index	1.0 (1.0–2.0)	10.0 (8.0–11.0)	7.0 (6.0–9.0)

Values are as median (range). The differences among all three groups were significant ($p < 0.05$)

to the saline group ($p=0.009$). GPx and SOD levels were higher in MTX+LA group compared to the MTX group ($p=0.011$ and $p=0.012$). MDA levels in intestinal tissue increased after MTX administration ($p=0.009$), but MDA level was lower in MTX+LA group as compared to the MTX group ($p=0.009$).

Animals from control group had no evidence of mucosal damage at any histology. The intestine specimens of the MTX administered groups showed histological alterations such as villous shortening and fusion, epithelial atrophy crypt loss, inflammatory infiltrate in the lamina propria and goblet cell loss. Histological intestinal damage scores of the groups are summarized in Table 1. Intestinal damage score was significantly higher in MTX administered rabbits than those of MTX+LA group ($p=0.026$).

Discussion

The results of the present study indicate that MTX treatment causes oxidative tissue damage, as assessed by increased lipid peroxidation and decreased GPx and SOD activity in intestinal tissue, while LA treatment prior to starting MTX protects against oxidative injury. SOD and GPx enzyme activities, were lower in groups receiving MTX.

Under physiological conditions, the damaging effects of superoxides are prevented by SOD, which converts superoxide to H_2O_2 ; then, GPx converts H_2O_2 to water [10]. However, during MTX treatment, these natural defenses may be inhibited by the excessive generation of ROS. On comparison of SOD and GPx activities between the MTX group and MTX+LA group, we found that LA enhanced SOD and GPx activities, suggesting that this agent modulates antioxidative capacity in the intestinal tissue.

It is possible that LA may scavenge singlet oxygen by a higher singlet oxygen-quenching capacity, and that it may also scavenge superoxide and peroxy ROS. Previous reports suggest that LA participates in the recycling of biologic antioxidants, such as vitamins E and C and glutathione, especially during oxidative stress [11]. In our study, pre-treatment with LA improved MTX-induced GPx and SOD suppression; this suggests that LA protected against MTX-induced suppression of intestinal antioxidant enzymes activity.

Lipid peroxidation, mediated by oxygen free radicals, is an important cause of destruction and damage to cell membranes and may be a contributing factor in the development of MTX-mediated tissue damage. In the present study, MDA level in MTX+LA group was lower than that in the MTX group, suggesting that LA treatment

had protective effect on MDA production after MTX administration.

The side effects of MTX are marked especially on rapidly proliferative cells of the hematopoietic and gastrointestinal system. Gao and Horie [3] showed that MTX inhibits the de novo purine synthesis and thymidine kinase in the salvage pathway of pyrimidine synthesis in crypt cells of the small intestine, leading to villous degeneration. Li et al. [12] reported that MTX treatment increases the cell death and decreases cell number in the intestinal epithelium. In our study, MTX treatment was associated with damage to intestinal tissue in the form of villous shortening, epithelial atrophy, crypt loss, inflammatory cells infiltration in the lamina propria and goblet cell loss. The degree of intestinal tissue injury in the MTX-LA group was less than in the MTX group suggesting that LA had a protective effect.

The present study indicates that MTX-induced oxidative injury in the intestine is ameliorated by LA treatment. Simultaneously, morphological changes in the injured tissues due to MTX were also improved by LA.

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