The Effect Of Ascorbic Acid On Protection Of Hepatic Injury Consequence Effective Isomers Of Pentanedioic Acid In Rats

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ABSTRACT: Pentanedioic acid derivatives such as Methotrexate are anti-neoplastic agents and are widely used in treatment of auto immune disorders and malignant diseases, but their use is limited because of several side effects including hepatotoxicity. As one of the important mechanisms involving in the pathogenesis of methotrexate toxicity, some of anti-oxidant agents have been used to reduce its side effect. Ascorbic acid (Vit. C) is an important anti-oxidant drug and in this research we aimed to study vitamin C effect on methotrexate induced hepatotoxicity. Thirty male Wistar rats were divided into three groups (n=10). First group was control group, the second group was Methotrexate group (represent pentanedioic acid derivatives) in which received, and the third group was methotrexate plus vitamin C group whom received methotrexate and vitamin C. On 6th day, the rats were anesthetized and liver tissue removed for pathologic studying and plasma isolated for measuring ALT and AST ($P<0.05$). Results showed that vitamin C treatment decreased liver tissue injury and elevation of ALT and AST after methotrexate administration. Regarding increasing use of methotrexate and the results of this research Vitamin C may be helpful in suppressing methotrexate induced hepatotoxicity and it is suggested to do this research on other animal models and study the underlying mechanisms.
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Key words: hepatotoxicity; ascorbic acid; pentanedioic acid derivatives; Methotrexate; Rat

1. INTRODUCTION

Methotrexate is one of the acid folic antagonists which are used widely as chemotherapy agent to treatment of kinds of leukemia and other malignancies. One of its side effects is hepatotoxicity. Of its action mechanism in creation of the damages can be mention to the production of the free radicals consequence use of methotrexate, therefore, several antioxidant agents have been used to reduce its side effects. Vit C is one of the strong antioxidant agents and showed that cause reduce of free radicals. The objective of this study was to determination of the methotrexate effects on ALT and AST and hepatic injuries and Vit. C effect on them. Methotrexate is an antimetabolite and antineoplastic agent used mainly in childhood acute lymphoblastic leukemia (16) and other forms of carcinoma. Successful treatment of leukemia with meningeal infiltration requires therapy directed at the central nervous system, since the drug cannot cross the blood brain barrier (9). Neurotoxicity reported (3,5,6) in children undergoing this treatment is of serious concern. The neurotoxicity of methotrexate includes headache, meningeal irritation, tremor, paraplegia, seizures, encephalopathy and even death. Methotrexate is known to reduce intelligence quotient scores, impaired memory in children (11, 13). Severe polyneuropathy and motor loss after intrathecal administration. The details of these effects have not been studied extensively.

Methotrexate, a folic acid antagonist, interferes - among other actions – with the methylation of deoxyuridylate to form thyuridylate. This inhibition is brought about by blocking the action of the enzyme dihydrofolate reductase and preventing the formation of tetrahydrofolate, the latter being the coenzyme (as 5,10-methylene tetrahydrofolate) in the conversion of deoxyuridylate to thyuridylate. Tetrahydrofolate is also essential for the de novo synthesis of the purine moiety of inosinic acid, the precursor for adenylic and guanylic acid. Folinic acid (5-formyltetrahydrofolate) will counteract the inhibitions caused by methotrexate (1,2).

2. Materials and methods:

30 male Sprague-Dawley rats (300±10 g) were selected for the study. Animal care and experiments confirmed with the Guide for the Care and Use of Laboratory Animals and approval of the ethics committee of Islamic Azad University was obtained before the commencement of the study. The animals were housed under standard environmental conditions (23 ± 1 °C, with 55 ± 5% humidity and a 12 h light/12 h dark cycle) and maintained with free access to water and a standard laboratory diet *ad libitum*. 30 wistar rats were allocated into the 3 groups of 10 rats. Group

1, as control group received normal saline. Group 2 received MTX at the dose of 20 mg/kg as ip. Group 3 beside of MTX received vitamin C at the dose of 25 mg/kg as oral. This group, 3 days before administration of the MTX received vit C. after six days, rats were euthanized and their liver was achieved to pathologic studies. Also, serum samples to measurement of AST and ALT were obtained. The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean ± SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. P<0.05 was considered statistically significant.

3. Results

Results showed that AST and ALT serum levels and hepatic injury in that group received methotrexate were higher than control group and ALT serum level in that group received vit. C plus methotrexate was more than control group and was less than group 2 (p<0.05). Table 1.

Table 1. ALT serum value in groups at the end of the experiment

Groups	ALT mg/dl
1: control	25.00±5.18
2: methotrexate only	113.50±13.36
3: methotrexate plus vit. C	80.90±9.14

ALT: Alanine Amino Transferase

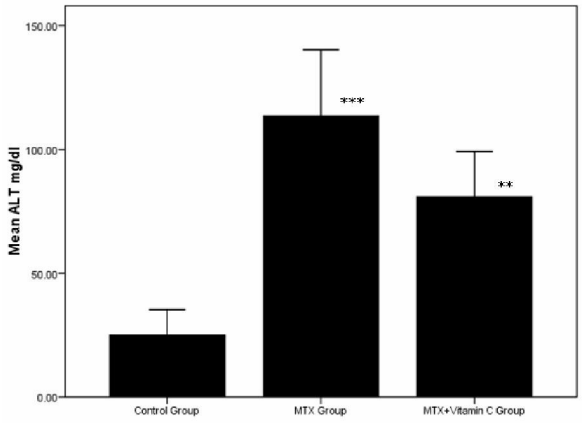

Fig. 1. The comparison amount of ALT between control group, MTX group, MTX+Vit.C group. Results are expressed as Mean±SE. *** p<0.001, **P<0.01 significantly different from control group

AST serum value in that group received methotrexate was higher than control group and AST serum value in group 3 was more than control group and was less than group 2 (p<0.05). Table 2.

Table 2. AST serum value in groups at the end of the experiment

Groups	AST mg/dl
1: control	30.60±6.99
2: methotrexate only	139.30±8.24
3: methotrexate plus vit. C	92.80±1.48

AST: Aspartate Amino Transferase

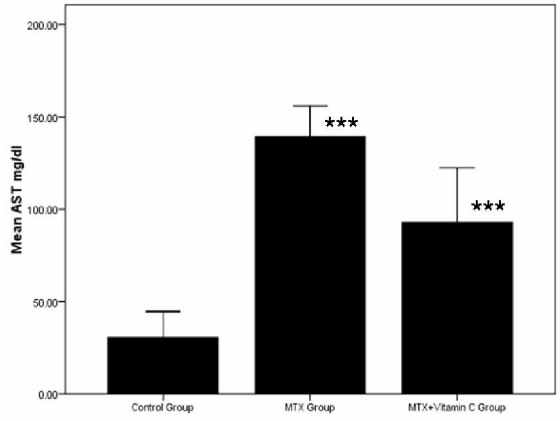

Fig. 2. The comparison amount of AST between control group, MTX group, MTX+Vit.C group. Results are expressed as Mean±SE. *** p<0.001 significantly different from the control group

Finally, results showed that hepatic injury in group 2 was more than control group and in that group received methotrexate plus vit. C however was more than the group had not received methotrexate but was less than the group that received methotrexate alone (p<0.05). Table 3.

Table 3. Hepatic injury index in groups at the end of the experiment

Groups	hepatic injury index
1: control	0.30±0.48
2: methotrexate only	10.60±2.11
3: methotrexate plus vit. C	7.30±1.88

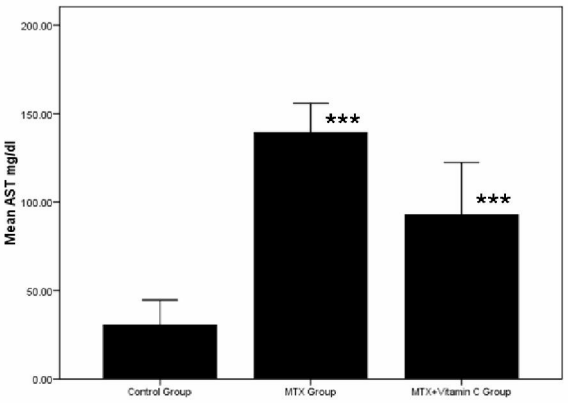

Fig. 3. The comparison rate of hepatic injury between control group, MTX group, MTX+Vit.C group. Results are expressed as Mean±SE. *** p<0.001 significantly different from the control group.

Table 4: Comparative assessment among groups

Dependent Variable		(I) groups	(J) groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
PATHO	Tukey HSD	1	2	-10.40000*	.74087	.000	-12.2369	-8.5631
			3	-7.10000*	.74087	.000	-8.9369	-5.2631
		2	1	10.40000*	.74087	.000	8.5631	12.2369
			3	3.30000*	.74087	.000	1.4631	5.1369
		3	1	7.10000*	.74087	.000	5.2631	8.9369
			2	-3.30000*	.74087	.000	-5.1369	-1.4631
ALT	Tukey HSD	1	2	-88.50000*	4.39141	.000	-99.3881	-77.6119
			3	-55.90000*	4.39141	.000	-66.7881	-45.0119
		2	1	88.50000*	4.39141	.000	77.6119	99.3881
			3	32.60000*	4.39141	.000	21.7119	43.4881
		3	1	55.90000*	4.39141	.000	45.0119	66.7881
			2	-32.60000*	4.39141	.000	-43.4881	-21.7119
AST	Tukey HSD	1	2	-108.70000*	4.74232	.000	-120.4582	-96.9418
			3	-62.20000*	4.74232	.000	-73.9582	-50.4418
		2	1	108.70000*	4.74232	.000	96.9418	120.4582
			3	46.50000*	4.74232	.000	34.7418	58.2582
		3	1	62.20000*	4.74232	.000	50.4418	73.9582
			2	-46.50000*	4.74232	.000	-58.2582	-34.7418

*The mean difference is significant at the 0.05 level.

4. Discussion

This study showed that vitamin C may reduce liver damage in rats induced by methotrexate. Results showed that vitamin C reduced the levels of ALT and AST which are important factors of liver function that may increase after methotrexate application in rats. Vitamin C also reduced liver damage after use of methotrexate in rats. There are many mechanisms reported about hepatic injuries consequence methotrexate usage that of them can be mentioned to the role of free radicals (7).

Free radicals are highly reactive molecules that are produced internally by our own human organism. A healthy person is equipped to face the presence of free radicals by defending the body with an anti-free radical system. This internal anti-free radical system consists of enzymatic and non-enzymatic mechanisms including superoxidedismutase, catalase, carotenoids, polyphenols, and anthocyanines among others (10). However, most people are not able to effectively handle excess free radical activity. If the quantity of free radicals produced by the human body is superior to the physiological and biological processes, the end result is oxidative stress causing cellular damage. There are 5 major free radicals responsible for causing cellular damage: peroxyl radical – hydrophilic, peroxyl radical – lipophilic, hydroxyl radical, peroxynitrite, and singlet oxygen (O+). For the purpose of the clinical study, we will be looking specifically at the singlet oxygen free radical (12).

Recent studies had been showed that vitamin C in plasma increase dose-dependent resistance to lipid peroxidation (8).

However, reported that low serum levels of vitamin C in high-risk individuals can yields to gastric metaplasia or chronic gastritis (4).

In summary, laboratory studies have been shown that vitamin C prevents lipid peroxidation dependent on iron and copper. In contrast, vitamin C can build Hydroxyl radical-dependent metal ion in biological fluids only under the non-physiologic conditions (15). Studies on the anti-apoptotic activity of vitamin C have shown that vitamin C has a role in regulating the immune system (14). The development of chemotherapy drugs, including methotrexate and its side effects with regard to the results of this study suggests that in future studies mechanisms involved in the pathogenesis of liver damage of methotrexate and vitamin C be reviewed.

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