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The Protective Effect of α-Tocopherol and\or N-acetylcysteine against Methotrexate-Induced Hepatotoxicity in Chronically-Treated Patients with Rheumatoid Arthritis: Clinical Study

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- Methotrexate
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- Liver function tests

Abstract

Long-term methotrexate (MTX) administration has the potential to cause hepatotoxicity that is claimed to be due to decrease the folate levels and/or oxidative stress.

Objectives: To study the protective effect of α-tocopherol and\or N-acetylcysteine against methotrexate-induced hepatotoxicity in chronically-treated patients with rheumatoid arthritis.

Subjects and methods: The study was carried on 250 subjects, whom were divided into five groups (50 subject each) as follows: Group I: Normal apparently healthy, Group II: Newly diagnosed as rheumatoid patients whom will be treated with MTX alone over one year, Group III: Newly diagnosed as rheumatoid patients whom will be treated with MTX with α-tocopherol over one year, Group IV: Newly diagnosed as rheumatoid patients whom will be treated with MTX with N-acetylcysteine over one year, Group V: Newly diagnosed as rheumatoid patients whom will be treated with MTX with α-tocopherol + N-acetylcysteine over one year. All subjects were investigated for liver function tests (ALT, AST, ALP, GDH) in the beginning of the study and every 3 months of the period of the study.

Results: It was found that long-term MTX therapy induced significant elevation of GDH started from 6th month of treatment while the other parameters (ALT, AST & ALP) started to be significantly increased from the 9th month of therapy. Co-administration of α-tocopherol or N-acetylcysteine induced significant improvement of all parameters. Co-administration of both anti-oxidants lead to improvement which was statistically significant more than each drug alone.

Conclusion: The co-administration of MTX with α-tocopherol and/or N-acetylcysteine may protect against MTX-induced hepatotoxicity in chronically-treated patients with rheumatoid arthritis which seems to be multifactorial.

INTRODUCTION

Since 1950s, low-dose methotrexate (MTX) therapy has been approved as a medical intervention for many inflammatory diseases such as psoriasis, sarcoidosis, systemic lupus and rheumatoid arthritis [1].

However, long-term MTX administration has the potential to cause diverse organ toxicities, including hepatotoxicity [1-2]. MTX may lead to liver hepatotoxicity, including steatosis, cholestasis, fibrosis, and cirrhosis [3].

Vitamin E is not a single nutrient, but a group of compounds which consists of 4 tocopherol isomers (α-, β-, γ- and δ-tocopherol) and 4 tocotrienol isomers (α-, β-, γ- and δ- tocotrienol),

and functions as a lipophilic antioxidant that prevents lipid peroxidation.1 Of all the natural isomeric forms of vitamin E, α-tocopherol was the most extensively studied, probably because it is the most predominant form in plasma and tissues [4]. Alpha-tocopherol is a fat-soluble antioxidant which is located within the phospholipid bilayer of cell membranes to prevent formation or limit the effects of free radicals. The major biologic role of vitamin E is to protect poly unsaturated fatty acids (PUFAs), low-density lipoprotein (LDL) and other components of cell membrane from oxidation by free radicals. Alpha-tocopherol acts as a peroxyl radical scavenger, disabling the production of damaging free radicals in tissues, by reacting with them to form a tocopheryl radical, which will then be reduced by a hydrogen donor (such as vitamin C) and thus return to its reduced state [5].

N-acetylcysteine (NAC) is a well-established cytoprotective drug that has proven efficacy against drug acetaminophen induced hepatotoxicity [6]. NAC exerts effective antioxidant and anti-inflammatory functions as it acts as an acetylated precursor of glutathione (GSH) and by inhibiting H_2O_2 formation [7].

The current study was designated to study the protective effect of α-tocopherol and\or N-acetylcysteine against methotrexate-induced hepatotoxicity in chronically-treated patients with population rheumatoid arthritis**.**

SUBJECTS AND METHODS

The current study was carried out at Minia university Hospitals, Minia city, Egypt (longitude 29.79°, latitude 28.25°) during the period from January, 1St, 2016, to December, 31st, 2016. The study was approved by the Research Ethical Committee 0f Minia Faculty of Medicine. The study was carried on 250 subjects aging from 25 to 50 years old of both sexes, whom were referred by the rheumatology, rehabilitation and physical medicine department. The patients were divided into five groups as follows (50 subjects each):

Group I: Normal apparently healthy subjects.

Group II: Newly diagnosed as rheumatoid patients whom will be treated with MTX alone with a starting dose of 7.5 to 10 mg, or 3-4 pills, taken all together once a week [8], over one year.

Group III: Newly diagnosed as rheumatoid patients who will be treated with MTX as in group II with α-tocopherol (350 mg/day) [9] over one year.

Group IV: Newly diagnosed as rheumatoid patients whom will be treated with MTX as in group II &III with N-acetylcysteine (600 mg/day) [10] over one year.

Group V: Newly diagnosed as rheumatoid patients whom will be treated with MTX with α-tocopherol+N-acetylcysteine in the previous doses over one year.

Inclusion\exclusion criteria:

The subjects of the study were:

- All urban.
- Aging from 25 to 50 years old of both sexes.
- Body mass index: 18.5 – 24.9 [11].
- Newly diagnosed (according to the revised criteria of the American College of Rheumatology (ACR) in 1987(Arnett and others 1988) and reclassified according to ACR/European League Against Rheumatism (EULAR) criteria in 2010 [12].
- No other immunologic or systemic diseases.
- No pregnancy or lactation.
- No previous therapy for rheumatoid arthritis or any disease (hypertension, DM, etc).

All subjects were investigated for liver function tests: Alanine transferase (ALT), Aspartate transferase (AST) and alkaline phosphatase (ALP) in the beginning of the study and every 3 months of the period of the study.

Biochemical investigations

Liver function tests: Alanine transferase and Aspartate transferase (ALT and AST): were measured spectrophotometrically using Spekol II Carl-Zeiss spectrophotometer [13,14]. Serum alkaline phosphatase level (ALP) measured by colorimetric method according to Donald & Ralph, 1993 [15].

Glutamate dehydrogenase (GDH): is measured using a Hitachi 917 automated clinical chemistry analyzer (Roche Diagnostics Limited, Lewes, UK).

Statistical analysis

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 10. Data were expressed as Mean + Standard Deviation (SD). ANOVA test was used to compare between the means where probability (P); $P < 0.05$: was considered significant.

RESULTS

It was found that long-term MTX therapy induced a significant elevation of GDH level that became evident after the 6th month of continuous MTX therapy.

On the other hand, it reported that the other parameters including ALT, AST and ALP started to be significantly increased from the 9th month of MTX therapy.

Co-administration of α-tocopherol-alone or N-acetylcysteine-alone with MTX induced significant improvement of all parameters, when compared to the MTX-treated group, with no statistical difference between the two co-administrated drugs.

Co-administration of both drugs (α-tocopherol and N-acetylcysteine) together with MTX lead to significant improvement of all parameters, when compared to the MTX-treated group. This improvement was statistically significant more than each drug alone (Tables 1-4 and Figures 1-4) **6.**

DISCUSSION

The results of the current study reported that long-term MTX therapy induced hepatotoxicity which was reflected by the significant elevation of GDH, ALT, AST and ALP levels. These findings are in accordance with many previous studies that stated that the therapeutic applications of MTX are usually limited by its severe hepatotoxicity [16-19].

In addition, it was reported that the significant elevation

Table 1: Values of glutamate dehydrogenase enzyme (GDH) and comparison between the different studied groups throughout the different periods of the study (ANOVA test).

GDH (IU/L)	I	II	III	IV	V	P value
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
0	23.2 ± 6.1	22.8 ± 4.7	22.7 ± 6.7	23.4 ± 6.4	21.9 ± 6.4	0.772
3 months	23.9 ± 4.8	25.5 ± 7.9	24.8 ± 5.2	25.8 ± 8.6	24.3 ± 8.1	0.643
6 months	22.9 ± 5.7	41.7 ± 14.5	31.8 ± 9.4	30.9 ± 14.1	26.1 ± 9.8	**< 0.001***
9 months	21.7 ± 7.6	59.1 ± 17.8	39.3 ± 18.3	37.8 ± 18.6	28.6 ± 6.3	**< 0.001***
12 months	23.8 ± 6.4	69.7 ± 26.2	54 ± 14.6	51.4 ± 15.4	42.7 ± 11.9	**< 0.001***

P < 0.05: was considered significant. GDH : Glutamate Dehydrogenase; IU/L : International units per liter; SD: **S**tandard Deviation

Table 2: Values of alanine transferase enzyme (ALT) and comparison between the different studied groups throughout the different periods of the study (ANOVA test).

ALT (mg/dl)	I	II	III	IV	V	P value
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
0	28.1 ± 5.72	26.9 ± 7.5	27.1 ± 5.5	27.7 ± 4.5	28.2 ± 6.5	0.759
3 months	28.38 ± 5.6	28.1 ± 5.9	29.6 ± 7.8	28.5 ± 6.8	29.9 ± 7.1	0.582
6 months	29.92 ± 6.1	29.6 ± 6.4	29.9 ± 8.9	29.8 ± 8.3	29.7 ± 11.4	0.999
9 months	29.2 ± 5.65	58.3 ± 13.5	49.6 ± 11.4	47.1 ± 10.1	42.6 ± 8.5	**< 0.001***
12 months	28.6 ± 5.3	61.8 ± 11.7	53.5 ± 16.9	51.9± 7.5	46.4 ± 16.7	**< 0.001***

P < 0.05: was considered significant. ALT: Alanine Transferase; mg/dl : milligrams per deciliter; SD : Standard Deviation

Table 3: Values of aspartate transferase enzyme (AST) and comparison between the different studied groups throughout the different periods of the study (ANOVA test).

AST (mg/dl)	I	II	III	IV	V	P value
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
0	30.7 ± 4.25	32.6 ± 4.9	31.9 ± 6.9	32.5 ± 5.7	31.4 ± 4.2	0.347
3 months	31.1 ± 3.95	33.4 ± 8.7	32.5 ± 8.2	32.1 ± 9.2	31.1 ± 6.4	0.499
6 months	32.3 ± 5.3	34.3 ± 12.2	32.8 ± 12.7	33 ± 11.7	32.9 ± 9.9	0.915
9 months	32.5 ± 5.9	64.3 ± 16.7	46.2 ± 8.8	45.3 ± 7.4	40.5 ± 9.7	**< 0.001***
12 months	31.85 ± 4.7	69.2 ± 15.7	49.6 ± 9.6	47.9 ± 8.1	42.2 ± 5.3	**< 0.001***

P < 0.05: was considered significant. AST: Aspartate Transferase; mg/dl=milligrams per deciliter; SD: **S**tandard Deviation

Table 4: Values of alkaline phosphatase enzyme (ALP) and comparison between the different studied groups throughout the different periods of the study (ANOVA test).

ALP (mg/dl)	I	II	III	IV	V	P value
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
0	46.3 ± 9.8	46.5 ± 7.9	45.8 ± 7.6	46.2 ± 6.4	45.5 ± 5.9	0.967
3 months	47.7 ± 8.1	46.2 ± 11.7	45.2 ± 9.9	45.4 ± 8.9	44.1 ± 6.8	0.383
6 months	47.2 ± 7.9	49.8 ± 10.3	49.3 ± 13.6	48.3 ± 11.2	47.5 ± 10.7	0.714
9 months	46.8 ±11.2	59.7 ± 9.6	54.7 ± 7.9	53.9 ± 6.4	49.2 ± 7.5	**< 0.001***
12 months	48.1 ± 8.9	79.6 ± 19.4	59.6 ± 14.9	57.8 ± 15.6	50.9 ± 8.8	**< 0.001***

P < 0.05: was considered significant. ALP: Alkaline Phosphatase; mg/dl: milligrams per deciliter; SD: **S**tandard Deviation

of GDH level became evident after the 6th month of continuous MTX therapy, while the other parameters including ALT, AST and ALP started to be significantly increased from the 9th month of MTX therapy. The reported early increase of serum GDH is in accordance with O'brien and his fellows, 2002, who reported that GDH was increased several-fold in most rats treated with either dexamethasone or cyproterone despite having no histopathological evidence of hepatocellular necrosis [20]. This could be explained by the fact that GDH is synthesized in the cytoplasm [21], so, there will be at least residual GDH available for release into plasma. Moreover, blebbing, the primary mechanism by which the hepatocellular release of enzymes from prelethally-injured cells into plasma occurs, is thought only to release cytosolic content. Furthermore, blebs may contain small amounts of mitochondrial material, which may be released into the plasma [22].

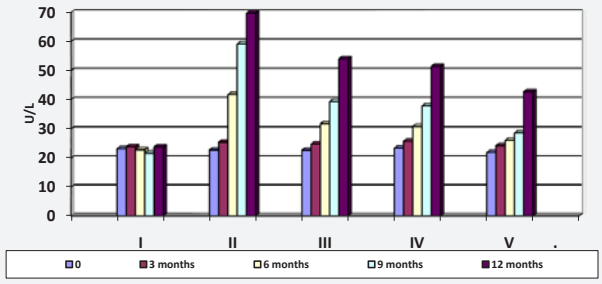

Figure 1 Values of GDH of the different studied groups throughout the different periods of the study.

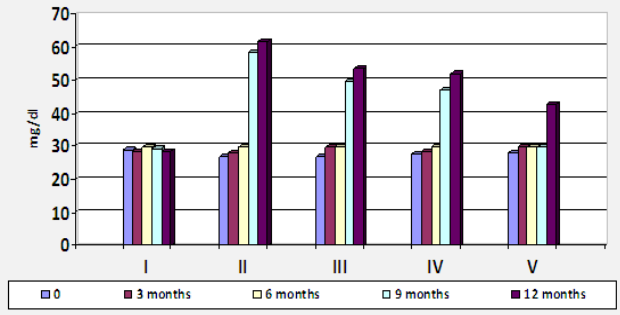

Figure 2 Values of ALT of the different studied groups throughout the different periods of the study.

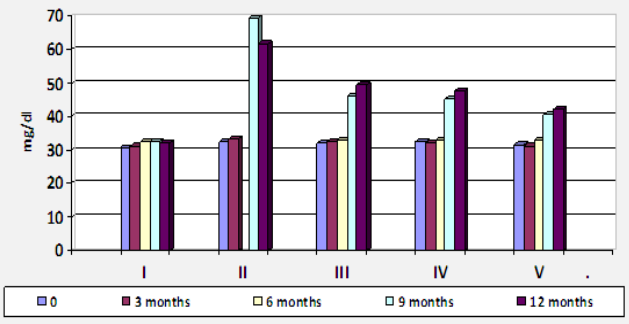

Figure 3 Values of AST of the different studied groups throughout the different periods of the study.

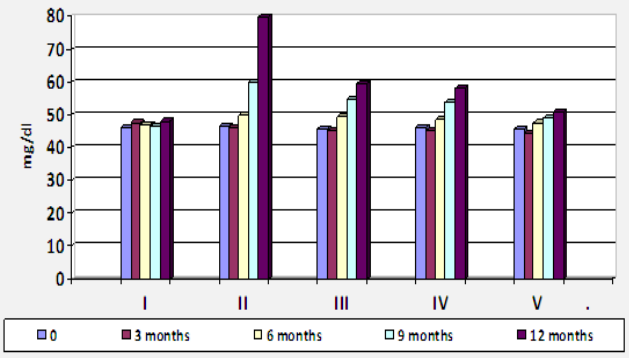

Figure 4 Values of ALP of the different studied groups throughout the different periods of the study.

The mechanism(s) of MTX-induced hepatotoxicity is unclear and was (were) not the concern(s) of the present study. However, it is claimed that accumulation of MTX inside the liver cells in a polyglutamated form which decrease the folate levels with subsequent liver cell damage. In addition, it is well known that oxidative stress plays an important role in MTX-induced hepatotoxicity [3-23].

On the other hand, the present study revealed that co-administration of α-tocopherol-alone or N-acetylcysteine-alone with MTX induced significant improvement of all parameters, when compared to the MTX-treated group, with no statistical difference between the two co-administrated drugs. These reported results are in agreement with previous experimental studies [24-26], but, to our knowledge, the current study is the first one conducted on humans.

Moreover, co-administration of both drugs together with MTX induced significant improvement of all parameters, when compared to the MTX-treated group, which was statistically significant more than each drug alone. These findings could be referred to the multifactorial mechanisms of MTX-induced liver damage and the different mechanisms by which both drugs produce their antioxidant activity [5-7].

In conclusions, the results of the present study demonstrate that chronic administration of MTX induces hepatotoxicity and that α-tocopherol and NAC are capable of reducing MTX-induced oxidative liver injury. These data indicate that both drugs may be of therapeutic use in preventing hepatotoxicity in patients receiving MTX.

In addition, as the number of patients was relatively low (50 patients of each groups) which may be considered as a limitation factor, it is advised to conduct a next multi-centers study on a larger patients' sample to assure the current study results,

The future clinical trials should be arranged to investigate the actual effect of α-tocopherol and N-acetylcysteine in a large sample of human patients and the possibility of considering these medical drugs as potential additive to MTX therapy.

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