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Effect of Alpha-Tocopherol on Tissue Transglutaminase and Reversibility of Thioacetamide-Induced Liver Fibrosis in rats.

[Alfa-Tokoferol'ün Doku Transglutaminazlar Üzerine Etkisi ve Sıçanda Tiyoasetamid Uyarımlı Geridönüşümlü Karaciğer Fibrozu]

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ABSTRACT

Both experimental and clinical studies have suggested the association between the deposition and stabilization of the extracellular matrix (ECM) proteins, and the development of liver fibrosis. Tissue transglutaminase (tTG) is known to stabilize the ECM proteins by its cross-linking activity that may play a key role in the development of liver fibrosis.

This work was designated to evaluate the curative and/or the protective effect of α-tocopherol in relation to the activity of tTG in liver fibrosis. To establish this goal the effects of α-tocopherol on oxidative stress (measured as malondialdehyde, MDA), liver fibrosis (evaluated histologically and as hepatic collagen), and the activity of tTG were investigated in liver fibrosis generated in rats by intraperitoneal injection (i.p.i.) of thioacetamide (TAA).

The data showed that α-tocopherol, significantly decreased the level of hepatic malondialdehyde when it was administrated after or before TAA treatment ($P < 0.001$ and $P < 0.001$, respectively). Similar changes in the level of hepatic collagen were observed, where the hepatic collagen, reduced when α-tocopherol was administrated after and partially before TAA treatment ($P < 0.001$ and $P > 0.05$, respectively). Although, TAA increased the tTG activity, α-tocopherol, variably lowered the enzyme activity when it was taken after, during or before TAA treatment. The histopathologic score of fibrosis was lower in rats treated with TAA then α-tocopherol compared with TAA only.

The data suggest that TAA-induced liver fibrosis was reversed by α-tocopherol after the fibrotic inducer was eliminated. Also, α-tocopherol largely protects against the subsequent oxidative stress and liver fibrosis and slightly decrease the tTG activity after, during or before TAA treatment. The data indicate that, α-tocopherol functions as a potent fibrosuppressant and antioxidant, and may be a therapeutic choice.

Key Words: Liver fibrosis, tissue transglutaminase, α-tocopherol, thioacetamide.

ÖZET

Deneysel ve klinik çalışmalar sonucu ekstrasellüler matriks (ESM) proteinlerinin konumlanıp sabitlenmesi ile karaciğer fibrozunun gelişimi arasında bir bağ olduğu gösterilmiştir. Doku transglutaminazları (tTG) çapraz bağlayıcı aktivitesi ile ESM proteinlerini sabitleyerek karaciğer fibrozu gelişiminde kilit rol oynayabilir.

Bu çalışma karaciğer fibrozundaki tTG aktivitesine α-tokoferol'ün koruyucu/ iyileştirici etkisini ölçmek üzere tasarlanmıştır. Bu amacı gerçekleştirmek için sıçanlarda intra-peritonal enjeksiyon (i.p.i) tiyoasetamid (TAA) sonucu oluşturulan karaciğer fibrozu oluşturulmuş ve α-tokoferol'ün oksidatif stress üzerine etkileri (malondealdehid, MDA, ölçümü) karaciğer fibrozu (hepatik kollajen ve histolojik değerlendirme) ve tTG aktivitesi incelenmiştir.

Veriler, TAA uygulamasından önce veya sonra verilen α-tokoferol'ün belirgin olarak karaciğer kökenli malondealdehid seviyesini belirgin olarak düşürdüğünü ($P < 0.001$ ve $P < 0.001$) göstrmektedir. Karaciğer kökenli kollajen seviyesinde de benzer değişimler gözlenmiştir. α-tokoferol uygulamasından sonra ($P < 0.001$) ve kısmi olarak da TAA uygulamasından önce ($P > 0.05$) karaciğer kökenli kollajen azalmıştır. TAA, tTG aktivitesini arttırmasına rağmen, α-tokoferol TAA uygulaması sırasında, öncesinde veya sonrasında değişken olarak enzim aktivitesinin düşürmüştür. Fibrozun histopatolojik değerlendirme derecesi TAA uygulamasını takiben α-tokoferol verilen sıçanlarda sadece TAA uygulanan sıçanlara göre daha düşüktü.

Veriler fibrotik uyarıcı ortadan kaldırıldığında, TAA uyarımlı karaciğer fibrozunun α-tokoferol ile geri dönüşüm olabileceğini öne sürmektedir. Ayrıca α-tokoferol, takip eden oksidatif hasar ve karaciğer fibrozuna karşı koruma sağlar ve TAA uygulamasından önce, sonra ve sırasınca tTG aktivisini bir miktar azaltır. Veriler α-tokoferol'ün güçlü bir fibro-baskılayıcı ve antioksidan olarak görev yapar ve terapötik bir seçim olabilir.

Anahtar Kelimeler: Karaciğer fibrozu, doku transglutaminazı, α-tokoferol, tiyoasetamid

Introduction

Liver fibrosis is participated by a variety of etiologies leading to sustained cellular injury. Lipid peroxidation is closely involved in liver fibrogenesis [1,2], where the generated free radicals activate the hepatic stillate cells (HSC) and increase the deposition of extracellular matrix (ECM) components. Also, the fibrogenesis appears to involve several events mediated by proinflammatory and cytotoxic cytokines, such as tumor necrosis factor (TNF), interleukins and transforming growth factor (TGF) [3,4]. Early studies have documented the association between fibrogenesis and the excessive deposition of collagen, elastin, laminin [5] and hyaluronan [6] in the ECM. Additionally, factors involved in stabilization or degradation of these proteins may play a potential role in the progression or reversibility of fibrosis. Tissue transglutaminases (tTG), for example catalyzes the specific cross linking of ε-amines and γ-glutamyl residues among amino acids [7]. This activity has been implicated in the cross-linking of ECM proteins leading to increase the deposition [8] and the resistance of such proteins to proteolytic enzymes leading to tissue fibrosis [9,10]. Several studies, specifically described the role of tTG in cross-linking of fibronectin, osteonectin, osteopontin, laminin and other extracellular matrix components [7]. In addition to its cross-linking activity, tTG has many other functions including guanosinetriphosphatase activity, where it acts as the GTP-binding, G_h subunit, which couples the $_1$-adrenergic receptor to a unique form of phospholipase C [11]. Such data implicate tTG in a variety of signal transduction events. In liver fibrosis induced in rats by carbon tetrachloride (CCl_4) and in human patients with acute liver disease, Mirza and his coauthors [12] found a dramatic rise in tTG activity. These findings suggested that tTG could participate in the deposition of excess ECM seen in fibrotic diseases.

Alpha-tocopherol, vitamin E, on the other hand, is traditional antioxidant known to react with reactive oxygen species (ROS) blocking the propagation of radical reactions in a wide range of oxidative stress situations. Therefore, it would be reasonable to investigate the effect of such antioxidants on both the reversibility of oxidative stress-induced liver fibrosis and the activity of tTG. In the present work liver fibrosis was generated in rats by TAA. This model was used to investigate the effect of α-tocopherol on both liver fibogenesis and the accompanying tTG activity.

Materials and methods

Experimental animals and groups

This study was carried out on 80 Albino rats, 50 (62.5%) males and 30 (37.5%) females, weighing 130–200 g (mean ±SD: 182.4 ±39.3 g). According to the treatment with the liver fibrotic and/or the antioxidant agents (TAA and α-tocopherol, respectively), rats were, initially categorized into 8 groups (10 rats each). Group I included untreated rats, group II included rats treated with TAA twice/week for one month, group III included rats treated with TAA twice/ week for one month then treated with α-tocopherol daily for extra one month. Groups IV and V included rats treated, simultaneously with TAA twice a week and with α-tocopherol, either daily or day after day for one month, respectively. In group VI rats were treated with α-tocopherol daily for 15 days then treated with TAA twice/week for extra one month. Groups VII and VIII included rats treated only with the vitamin either daily or day after day for a month, respectively. Animals were housed in breeding cages and received a similar basic care in compliance with international ethical standards during the study period. Liver fibrosis was induced by i.p.i of 200 mg/kg TAA (Sigma-Aldrich Chemical Co., USA) as previously described by [13], whereas 2 mg/rat of α-tocopherol (Sigma-Aldrich Chemical Co., USA), dissolved in olive oil, were supplemented by i.p. injection. After the study period of each group, rats were sacrificed, where both blood and liver tissues were immediately harvested and labeled. Blood samples were left to clot and centrifuged at 5000 rpm and the recovered sera were kept at –20°C. A portion of liver was kept on 10% neutral buffered formalin, until processed for histology analysis. The rest of liver tissues were kept at –80°C until investigated. Liver tissue homogenate (10% w/v) was prepared by homogenizing a portion of liver in phosphate-buffered-saline (PBS) (0.02 M sodium phosphate buffer with 0.15 M sodium chloride, pH 7.4) using glass tissue homogenizer with a Teflon pestle (Jencons, Cat. No 361092).

Analytical Methods

Total malondialdehyde was estimated in deproteinized liver homogenate according to the method of **Ohakawa and Ohishi** [14], where the absorbance of the color developed due to the reaction of malondialdehyde (MDA) with thiobarbituric acid was measured at 535 nm.

Histological analysis of liver scarring was determined on formal method, where specimens of liver tissue were fixed in 10% neutral buffered formalin, processed into paraffin blocks, sectioned and stained with hematoxylin and eosin. Histopathological slides were examined blindly and the severity of liver fibrosis was scored. Biochemically, fibrosis was investigated by determining the collagen in liver tissue. Briefly, hydroxyproline in liver homogenate was hydrolyzed with 12N HCl at 110°C for 18 hours then oxidized into pyrrole followed by coupling with p-dimethyl-amino-benzaldehyde and the developed red color was measured spectrophotometrically at 456 nm. The concentration of hydroxyproline was multiplied by a correction value (31.25 µg/g liver tissue) then the corresponding hepatic collagen content was determined by multiplying the value of hydroxyproline by 7.46 [15,16].

Tissue transglutaminase activity was estimated in 100

μl liver homogenate, by a direct spectrophotometric method developed by De Macedo and co-authors [17]. The method uses N,N-dimethyl-1,4-phenylenediamine (DMPDA) (Sigma-Aldrich Chemical Co., USA) as a γ-glutamyl acceptor substrate and carbobenzyloxy L-glutamylglycine (Z-Gln-Gly) (Sigma-Aldrich Chemical Co, USA) as a peptide γ-glutamyl donor substrate. The resulting anilide substituted with a strong electron-donating group is a chromophore that absorbs light at 278 nm. Thus, the transamidation activity of transglutaminase could be determined kinetically over 7 minutes period by measuring the increase in absorbance.

Routine laboratory methods were used in determination of protein in liver homogenate assayed according to Lowry et al., [18] using bovine serum albumin as a standard. Both serum alanine aminotransferase (ALT) activity and γ-glutamyl transferase (γ-GT) activity were estimated using Rnadox reagents following the manufacturer instruction. All spectrophotometric measurements were performed using a SpectraMax Plus spectrophotometer (Molecular Devices).

Statistical analysis

Results are given as mean ±(SD) standard deviation. Due to rat's mortality, particularly in groups from 2 to 8, only data from 7 rats from each group were considered and statistically processed. Differences between different groups were determined by nonparametric analysis of variance (ANOVA) (Kruskal-Wallis test) followed by Dunn's Multiple comparison test. Correlations between variables were determined by Pearson correlation coefficients. Probability of <5% was considered significant.

Results

Malodialdehyde (MDA) was investigated in liver homogenate as a marker of oxidative stress. Treatment of rats with TAA (group II), insignificantly increased the level of MDA from 3.61 ±1.06, in the control group, to 5.15 ±0.42 nmol/mg protein. Also, simultaneous treatment with both the TAA and α-tocopherol daily or day after day, insignificantly increased the level of MDA to 5.09 ±0.9 and 5.76 ±0.29 nmol/mg protein, respectively. Treatment of rats with α-tocopherol alone (groups VII and VIII) did not induce oxidative stress, where the MDA levels were maintained in concentrations comparable to the untreated rats (Table 1). Treatment of rats with α-tocopherol after (group III) or before (group VI) TAA treatment, significantly reduced the level of MDA compared with the TAA group ($P < 0.001$ and $P < 0.01$, respectively).

The degree of TAA-induced fibrotic lesion was monitored biochemically through the estimation of hepatic collagen levels. The highest collagen concentration (732.7 ±90.2 μg/g liver tissue) was observed in TAA-treated rats (group II). Compared with this group, different treatments with α-tocopherol (in groups III, IV, V and VI) led to a variable degrees of reduction of hepatic collagen content (Table 1). The vitamin, significantly decreased the collagen concentration, only when it was taken after ceasing of TAA injection (group III) ($P < 0.001$) or during TAA treatment in group IV. No significant decrease, however was observed in other rats treated with the vitamin before (group VI) or during TAA treatments (groups V). Compared with the collagen concentration of the untreated rats (group I)

Table 1. Effects of TAA and/or vitamin α tocopherol on both hepatic collagen and malondialdehyde.

Group		MDA (nmole/mg protein)	Collagen (μg/g liver tissue)
No.	**Treatment**		
I	No treatment (control group).	3.61 ±1.06	331.59 ±45.72
II	TAA twice a week form one month	5.15 ±0.42	732.70 ±90.17[a]
III	TAA then α tocopherol twice/w.	2.49 ±0.79[b]	382.57 ±40.24[b]
IV	TAA and α tocopherol twice/w.	5.09 ±0.90	563.08 ±62.55[a,b,c]
V	TAA and α tocopherol d after d.	5.76 ±0.29	562.86 ±59.34[a]
VI	α-tocopherol (15 d) then TAA (30 d).	2.89 ±0.82[b]	599.18 ±66.49[a]
VII	α-tocopherol daily for one month	3.99 ±1.03	404.99 ±44.32[b]
VIII	α-tocopherol d after d. for one month	3.52 ±0.13	369.54 ±31.41[b]

Abbreviations: MDA: Malondialdehyde, TAA: thioacetamide, d: day, w: week.

- Results are presented as mean ±SD of 8 groups, 7 rats each.
- Small letters, if present, refer to statistically significant differences between groups. (a): compared to the untreated rats (group I), (b): compared to the TAA treated rats (group II) and (c) compared to group V.

Table 2. Effects of TAA and/or α tocopherol treatments on both hepatic tissue transglutaminase, serum alanine aminotranseferase and γ-glutamyltranseferase.

Group		tTG activity (anilide/umol/mg protein/min.)	ALT (U/l)	γ-GT (U/l)
No.	Treatment			
I	No treatment (control group).	1.54 ±0.40	61.2 ±3.2	1070.8 ±25.4
II	TAA twice a week form one month	2.89 ±0.87[a]	73.0 ±6.7	1888.1 ±47.6[a]
III	TAA then α tocopherol	2.07 ±1.03	58.0 ±2.7[b]	1169.2 ±37.6[b]
IV	TAA and α tocopherol twice/w	2.41 ±0.71[a]	75.3 ±11.6[a]	1256.5 ±54.5[a]
V	TAA and α tocopherol d after d.	1.67 ±0.34[b]	80.7 ±7.1	1374.5 ±25.1
VI	α-tocopherol (15 d) then TAA (30 d).	1.98 ±0.48	62.0 ±5.4	1508.9 ±85.7
VII	α-tocopherol daily for one month	1.65 ±0.42[b]	67.0 ±7.7	1053.7 ±62.6[b]
VIII	α-tocopherol d after d. for one month	0.73 ±0.08[a,b]	61.0 ±9.1	1100.9 ±83.9[b]

Abbreviations: TAA: thioacetamide, d: day, w: week.

- Results are presented as mean ±SD of 8 groups, 7 rats each.
- Small letters refer to, statistically significant differences between groups. (a): compared to the untreated rats (group I) and (b): compared to the TAA treated rats (group II).

(331.59 ±45.7 μg/g liver), administration of α tocopherol daily (group VII) or day after day (group VIII) for one month did not affect the concentration of hepatic collagen. Histologically, a marked fibrosis was observed in TAA-treated rats (group II) (grade 3, stage 3), where portal inflammation, portal, bridging fibrosis and necrosis, preportal cholestases, foci of hepatocytolysis, mild cloudy cells, prominent nucli and prominent Kupfer cells were observed. This histological pattern was improved in rats treated with the vitamin after TAA treatment (group III), where a moderate inflammation, less fibrosis and less cholestases (grade 2, stage 1) were observed after the vitamin treatment (Table 3).

Table 3. The histopathologic score of fibrosis

Group		Score	
No.	Treatment	Grade	Stage
I	no treatment	0	0
II	TAA twice a week for one month	3	3
III	TAA for one month then vitamin E	2	1
IV	TAA and vitamin E twice a week	4	3
V	TAA and vitamin E day after day	2	2
VI	15 day vitamin E then 30 day TAA	3	3
VII	Vitamin E daily	0	0
VIII	Vitamin E day after day	0	0

The degree of cross-linking of ECM proteins was evaluated by the tTG activity in liver. Compared with the untreated rats, the tTG activity, significantly increased from 1.54 ±0.4 to 2.89 ±0.87 and to 2.41 ±0.71 anilide/μmol/mg protein/min in rats treated with TAA (group II) (P <0.01) and in rats treated, simultaneously with both TAA and α-tocopherol daily (group IV) (P <0.05), respectively. Other groups, however did not show significant changes in the enzyme activity. Also, treatment with the vitamin alone, reduced the tTG activity particularly in rats treated with the vitamin day after day (Table 2). On the other hand, compared with the liver-fibrotic rats (group II) a significant decrease in the tTG activity was observed in rats treated with both TAA and the vitamin day after day (group V) (P <0.05). Additionally, the enzyme activity was correlated with severity of oxidative stress (Figure 1).

The level of serum ALT was mildly changed among different groups. Compared with the untreated rats, a significant increase in the ALT levels was seen only in group treated, simultaneously with TAA and the vitamin (groups V) (P <0.001). Compared with the fibrotic rats (group II), a significant decrease in the ALT levels was observed in rats treated with the vitamin after TAA treatment (group III) (P <0.01). Also, a decrease (but insignificant) in ALT level was observed in rats treated with the vitamin before the TAA treatment (group VI).

Serum γ-GT in contrast showed a marked sensitivity compared with the ALT level, where all rats treated with TAA and the vitamin (after, during or before TAA) showed an increase in γ-GT levels compared to the untreated or fibrotic rats (Table 2), particularly in group

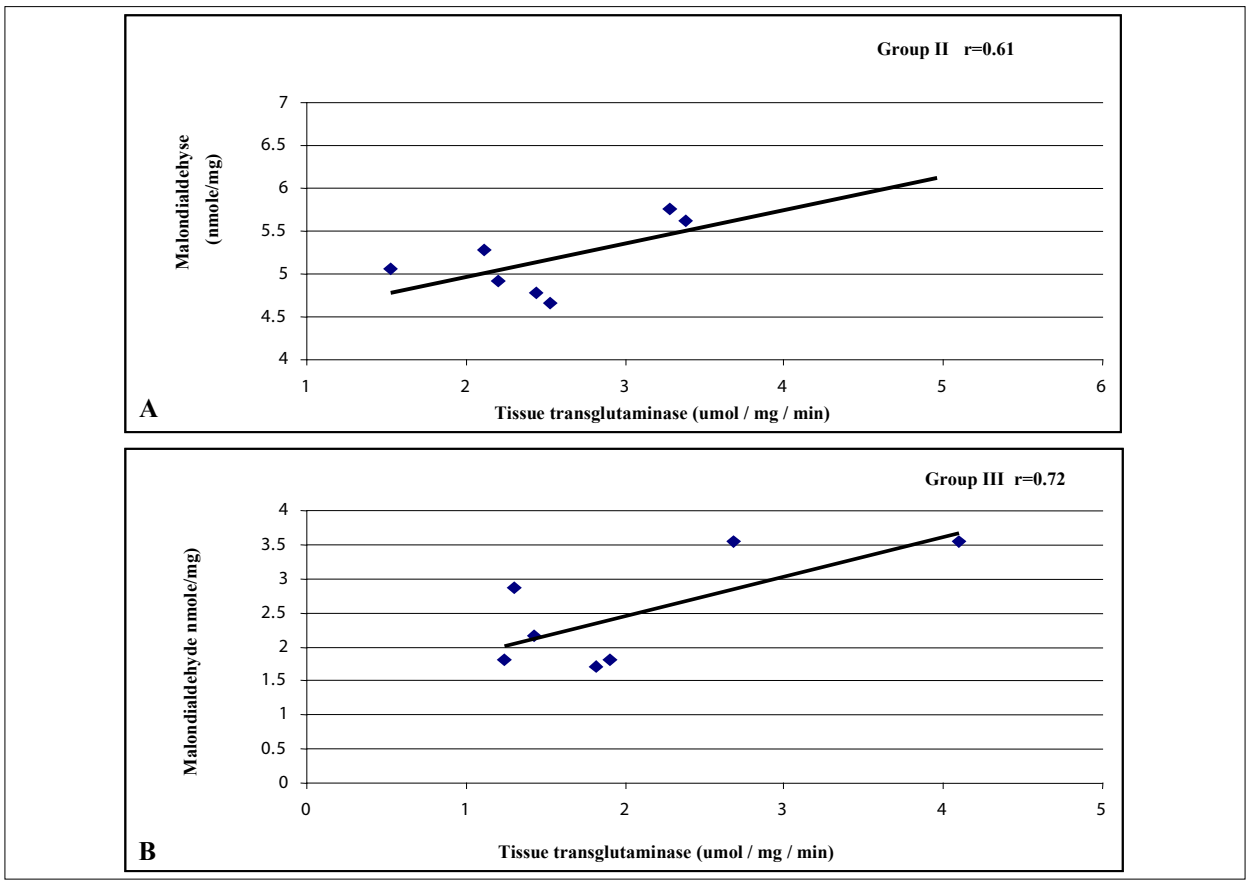

Figure 1. Pearson correlation between tissue transglutaminase activity and hepatic malondialdehyde in rats treated with TAA for one month (group II) (A) and in rats treated with TAA for one month then with vitamin E daily for extra one month (group III) (B).

IV ($p < 0.001$). Also, compared with TAA treated group (Group II), a significant decrease in γ-GT levels was observed in group III ($P < 0.01$). Rats treated with the vitamin daily (group VII) or day after day (group VIII), on the other hand, did not show significant change in the enzyme level compared with the untreated normal controls (Table 2).

Discussion

In order to investigate the effect of α-tocopherol on both the reversibility of oxidative stress-mediated liver fibrosis and tTG activity, a model of liver fibrosis was generated in rat by i.p. injection of TAA. Among various hepatotoxins, TAA is known to be the most potent because of its rapid elimination and cumulative injury [18]. In addition to the oxidative stress it generates, TAA decreases the level of some endogenous antioxidants including α-tocopherol [19]. Consequently, it is anticipated that supplementation of rats with α-tocopherol, could compensate the depletion of the vitamin and challenge the TAA effect. This explains the usage of TAA rather than other hepatotoxicants. Because this study was concerned with fibrosis, rather than the subsequent histopathological stages, the progression to cirrhosis was avoided by injection of a group of rats (n = 10) with 200 mg TAA mg/kg twice a week. Serial monitoring of liver histology over 12 weeks revealed that treatment of rats with TAA twice a week for one month led to the development of marked hepatic fibrosis. Although MDA, an end product of lipid peroxidation, is constitutively broken down by aldehyde dehydrogenases, its production, however, is accelerated by oxidative stress such that it may escape the detoxification process. In a similar work [19] a marked oxidative stress was observed, where the levels of MDA were significantly increased in rats livers after TAA treatment for one month. This lipid peroxidation-mediated oxidative stress was suggested to be generated by TAA-S-oxide, which is derived from the biotransformation of TAA by the liver microsomal FAD-containing monooxygenase (FMO) and cytochrome P450 systems [1,20]. In agreement with Altavilla [21], α-tocopherol was able to reverse the overproduction of MDA after ceasing of treatment with TAA (group III). Also, administrating the vitamin, daily for 15 days before induction of fibrosis, significantly prohibited the development of the oxidative stress. In contrast, concomitant treatment with TAA, the vitamin was unable to inhibit the oxidative stress. This refers to the protective effect of α-tocopherol in maintaining normal liver homeostasis after and before (but not during) the toxic insult.

As a consequence of lipid peroxidation, HSC, which is the central mediator in the pathogenesis of fibrosis, are known to be activated by free radicals and overwhelm

the ECM with collagen [22]. This may explain the doubling of the hepatic collagen level in TAA treated rats compared with the normal controls. Similarly, α-tocopherol reversed the condition when it was taken after TAA treatment. In a similar condition exposure of cultured HSC to a pro-oxidant system, ascorbate/iron, increased the expression of procollagen type I, the principal collagen in fibrotic liver, and this fibrogenic effect was almost completely abolished when the cells were treated with α-tocopherol [2]. Such finding indicated that α-tocopherol may reduce both reactive oxygen species (ROS) production and collagen accumulation in liver, only when it was taken after TAA. Conversely, a mild (insignificant) decrease in collagen production was observed in rats simultaneously treated with TAA and the vitamin. The accumulation of collagen observed in rats treated only with the vitamin may be due to the inhibitory effect of the vitamin on the collagenase expression [23]. In parallel to the changing pattern of both the oxidative stress and the collagen content, a histological improvement was observed in rats administered with the vitamin after cessation of TAA treatment.

The activity of tTG and the level of its substrates, particularly collagen in liver ECM are limiting factors in the development or treatment hepatic scar. If oxidative stress is the underlying mechanism of fibrosis, one may anticipate that the drug of choice should be an efficient antioxidant, able to decrease or maintain the normal cross-linking activity of the tTG and minimize the stability of ECM proteins. This signifies the investigation of both the effects of α-tocopherol as an antioxidant and the accompanying activity of tTG activity. The constitutive existence of tTG in the ECM [24] fulfills its involvement in matrix assembly and basement membrane stabilization [25,26]. In a previous work, in fibrotic liver, tTG was found with higher activity than in normal liver and tTG-mediated cross-linking occurred during the early inflammatory stage of fibrosis [27]. Similarly, the data of the present study suggested that tTG activity was higher in rats with fibrosis and this increase was paralleled with oxidative stress and liver dysfunction, indicated by the elevation of ALT and γ-GT, and the overproduction of collagen. Such conditions favor the stability of ECM proteins and the development of fibrosis. In the oxidative stress, tTG may be activated as a consequence of GSH depletion and mitochondrial dysfunction [28]. Another mechanism explaining the increase in tTG activity in TAA-treated rats is the increased binding of the nuclear factor-kappaB (NF-κB) to the NF-κB motif of the tTG promoter, where tTG gene expression increase during hepatic injury and fibrosis [12]. The concomitant increase of both hepatic collagen and tTG activity may be explained by the dual effect exerted by the NF κB, which is induced by oxidative stress and inhibited by vitamin E [29]. Nevertheless, the association between tTG activity and fibrosis may involve other factors such as the transforming growth factor-beta (TGF-β), a major fibrogenic growth factors, where tTG has been known to activate the latent TGF-β1, which in turn led to de novo synthesis of tTG [30,31]. In a similar condition (renal fibrosis) tTG was associated with the accumulation of the ECM, both indirectly via TGF-β1 activation and directly by the formation of ε- (γ-glutamyl) lysine dipeptide bonds within the ECM [32]. Also, the data of the present work showed that oxidative stress, markedly increased the tTG activity, where the increase of the MDA was accompanied with an increase of the enzyme activity. The enzyme activity, however was slightly (insignificantly) decreased in rats treated with TAA followed by α-tocopherol. This observation was accompanied with an improvement in the liver function, regression of oxidative stress and decreased collagen production. Surprisingly, when α-tocopherol was taken in 4 doses a week alone or during TAA treatment, the activity of tTG was comparable with the corresponding control. This suggested the possible direct effect of α-tocopherol on the activity of tTG. Away from the role of α-tocopherol as a scavenger for the free radicals, the list of nonantioxidant functions of α-tocopherol is expanding**.** Although, there is no clue explaining how α-tocopherol maintain (or decrease) the tTG activity, many studies, however, have implicated the role of the vitamin in modulating cell signalling and the gene expression. Among the affected genes, some known to be involved in the synthesis and processing of ECM proteins such as matrix metalloproteinase-19, collagenase [33] and liver collagen α1 [34]. Such effects may reflect specific interactions of the vitamin with enzymes, structural proteins and transcription factors.

Although, currently available serum markers of fibrosis are not reliable in discriminating mild, moderate and progressive degrees of fibrosis, both ALT and γ–GT levels were changing in harmony with the reversal effect of the vitamin on the pre-induced fibrosis. However, γ-GT was more sensitive, where rats treated with TAA and α tocopherol in different formats showed a variable decreasing levels compared with the fibrotic controls. This supported previous reports suggesting that serum γ-GT, even within its normal range, might be an early and sensitive marker of oxidative stress. The sensitivity of γ-GT may be attributed to its direct involvement in GSH metabolism, which is generally associated with antioxidant properties [35]. The relation between γ–GT and the level of α-tocopherol is not clear. Few reports have detected an inverse relation between the enzyme level and some antioxidants such as α-carotene, β-carotene, β-cryptoxanthine, and vitamin C [36]. It seems that α-tocopherol behaves differently, where rats treated with 2 or 4 doses a week almost had γ–GT levels similar to the control animals. Thus, both ALT and γ-GT were informative in monitoring the liver dysfunction and the reversal effect of the vitamin.

In summary, the data suggested that TAA induces the oxidative stress leading to a fibrogenic environment

within the liver through a combination of ECM collagen overproduction, and increased the tTG activity. α-Tocopherol, however, improved the liver histology, diminished both the oxidative stress, collagen overproduction, mildly lowered the tTG activity and improved the liver function. The best results were obtained when the vitamin was taken after (curatively) or before (protectively) the induction of fibrosis. This approach refers to the importance of both the initial elimination of factors stimulating the overproduction of ECM components and investigating the factors involved in ECM processing in parallel to the antifibrotic potential of the investigated drug.

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