THE CHANGES OF OXIDATIVE AND ANTIOXIDATIVE PARAMETERS IN LIVER CIRRHOSIS INDUCED BY CARBON TETRACHLORIDE

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KARBONTETRAKLORÜRÜN NEDEN OLDUĞU KARACİĞER SİROZUNDA OKSİDAN VE ANTİOKSİDAN PARAMETRELERİN DEĞİŞİMİ

Özet: *Siroz; karaciğerde rejenerasyon nodüllerinin varlığı ve fibrozisdeki artış ile tanımlanan bir hastalıktır. İnvitro ve invivo çalışmalar karaciğer fibrogenezinde oksidatif stresin önemli olduğunu göstermiştir. Ratlarda karbon tetraklorür (CCl4) ile oluşan karaciğer hasarının patogenezinde serbest radikallerin rolü önemlidir. Serbest radikaller hücre proteinlerini oksidatif hasara uğratır ve hücrelerin fonksiyonunu değiştirir. Çalışmada ratlarda deneysel olarak oluşturulmuş karaciğer sirozunun, serum total antioksidan status (TAOS), doku malondialdehid (MDA), doku sodyum potasyum adenozin trifosfataz (Na^+-K^+ ATPaz) ve redükte glutatyon (GSH) düzeyleri üzerine etkilerinin incelenmesi amaçlanmıştır. Çalışmada 20 adet rat'dan oluşan bir deney grubu ile 17 adet rat'dan oluşan bir kontrol grubu oluşturuldu. Siroz oluşturmak için CCl_4 zeytin yağı içinde 3/4 (v/v) oranında karıştırılarak 6 hafta süre ile deri altına uygulandı. Kontrol grubuna ise sadece zeytin yağı uygulandı. Uygulamayı takiben hem kontrol grubu hem de deney grubundaki ratlar dekapite edildi. Ratların karaciğerinin bir kısmı histopatolojik inceleme için ayrıldı. Doku MDA düzeyleri, siroz oluşturulmuş grupta kontrol grubu ile karşılaştırıldığında anlamlı olarak yüksek bulundu ($p<0.001$). Diğer taraftan; serum TAOS düzeyleri, doku Na^+-K^+ ATPaz aktivitesi ve doku GSH düzeyleri kontrol grubu ile karşılaştırıldığında, deneysel olarak siroz oluşturulmuş grupta anlamlı olarak düşük bulundu ($p<0.001$). Sonuçta; CCl_4'ün neden olduğu karaciğer hasarının patogenezinde protein oksidasyonunun önemli bir rol oynadığı bulunmuştur. Bu parametrelerin düzeylerindeki değişimler, CCl_4'ün neden olduğu karaciğer hasarının tanı ve prognozunun bilinmesi açısından önemli olabilir.*

Anahtar Kelimeler: *Siroz, CCl_4, MDA, Antioksidan parametreler*

Summary: *Cirrhosis is characterized with increased fibrosis and presence of regeneration nodules in liver. Invitro and invivo studies indicate that oxidant stress is implicated in liver fibrogenesis. Free radicals have been implicated in the pathogenesis of Carbon Tetrachloride (CCl_4)-induced liver injury in rats. Free radicals can cause oxidative damage to cellular proteins and alter cellular function. In the present study, we aimed to determine the effects of experimentally-induced liver cirrhosis on the serum Total Antioxidant Status (TAOS), tissue malondialdehyde (MDA), Sodium-Potassium Adenosine Triphosphatase (Na^+-K^+ ATPase), and reduced Glutathione (GSH) levels in the rats. Two group of rats were randomized for this study; an experimental (n:20) and a control group (n:17). CCl_4 was dissolved in olive oil (3/4; v/v) and subcutaneously administered to the rats for six weeks in order to induce cirrhosis. Controls received olive oil only. At the end of the study both rats in experimental and control groups were sacrified. A part of the liver in each rat was removed for histopathological examination. Tissue MDA levels was significantly higher in the experimentally-induced cirrhosis group when compared to control group ($p<0.001$).On the other hand, serum TAOS levels, tissue Na^+-K^+ ATPase activity and tissue GSH levels were significantly lower in experimentally-induced cirrhosis group when compared to control group ($p<0.001$). In conclusion; these results showed that protein oxidation may play a role in the pathogenesis of CCl4 induced liver injury that the changes in the level of these parameters may be important for determination and prognosis of CCl4 induced liver damage.*

Key Words: *Cirrhosis, CCl_4, MDA, Antioxidant Parameters*

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INTRODUCTION

Hepatotoxins, such as alcohol, nutritional, genetic and immunological factors as well as drugs, play important roles in the development of liver cirrhosis (1-3). Alteration in liver functions, levels of lysosomal enzymes and trace elements together with microscopic evidence of liver damage have been previously demonstrated in human and rats with liver cirrhosis. Although the exact mechanism of liver cirrhosis is still obscure, liver cirrhosis are characterized by progressive accumulation of connective tissue undergoing fibrotic degeneration (4-7). Evidence of oxidative reactions is often associated with fibrogenesis occuring in liver (8-10).

It has also been found that metabolism of carbon tetrachloride (CCl_4) involves the production of free radicals through its activation by drug metabolizing enzymes located in the endoplasmic reticulum. The production of free radicals stimulate fibrogenesis either directly or through inflammatory stimuli. Free radicals damage intracellular structures such as lysosomes and microsomes. These radicals can interact with polyunsaturated fatty acids of cell membranes and cause tissue damage (6,11-14).

In the present study; we aimed to determine the effects of experimentally-induced liver cirrhosis on serum Total Antioxidant Status (TAOS), tissue Malondialdehyde (MDA), Sodium Potassium Adenosine Triphosphatase(Na^+- K^+ ATP'ase) and Reduced Glutathione (GSH) levels in the rats.

MATERIAL AND METHODS

Wistar albino rats, weighing 300-350 g were used in experimental group (n:20) and control group (n:17). Carbon tetrachloride was dissolved in olive oil (3/4 ; v/v) (Olive oil Sigma Chemical Co.St. Louis, MO. USA) and 0,150 ml/100 g CCl_4 subcutaneously administered to rats in the experimental group three times weekly for six weeks in order to induce cirrhosis. Only received olive oil was administered to rats in control group. At the end, both experimental and control animals were sacrified. Blood and liver tissue samples were obtained from both experimental group and control group. One part of the liver tissue sample were preserved for histopathological examination by embedding in paraffin blocks, after routine preparation procedures in % 10 formol, stained with Hematoxylene-Eosin, examined with light microscope and photographed. The rest of tissue was freezed for the determination of the levels of other parameters. Blood samples were centrifuged to separate serum. Serum TAOS levels were determined colorimetrically using the kits supplied by Randox (Cat No: N X 2332).

A tissue homogenate was promptly prepared for Na^+ - K^+ ATPase study to prevent the deactivation of the enzyme activity. For this purpose, 10% tissue homogenate in a solution of 0.3 M sucrose containing 1mM magnesium, was homogenised by using glass-glass homogenisator. Homogenates were centrifuged at 3000 rpm for 5 minutes and supernatants were separated. Na^+ - K^+ ATPase in supernatant is determined. Measurement of Na^+ - K^+ ATPase activity is based on the principle of measurement of inorganic phosphate that is formed from 3 mM disodium adenosine triphosphate which is added to the medium during incubation period (15). The medium was incubated in a 37°C water bath for 5 minutes with a mixture of 100 mM NaCl, 5 mM KCl, 6mM MgCl2, 0.1 mM EDTA, 30 mM Tris HCl (pH : 7.4). Following the preincubation period, Na2atp, at a final concentration of 3 mM was added to each tube and incubated at 37°C for 30 min. After incubation, the tubes were taken into an ice bath and the reaction was stopped. Subsequently, the level of inorganic phosphate was determined using an Olympus AU-560 autoanalyzer. The specific activity of the enzyme was expressed as nmol Pi/mg protein/hour. The protein determination of supernatant was measured by the Lowry method (16).

In the present study, tissue MDA which is the last product of lipid peroxidation, was determined spectrophotometrically according to the method described by Ohkawa (17). MDA levels were expressed as nmol MDA per mg protein.

The levels of Glutathione (GSH) of liver was measured spectrophotometrically by the method of Ellman (18). The reduced Glutathione levels were expressed as μmol glutathione / g tissue.

Statistical analysis was performed by Student's-t test for a group comparison. The results were expressed as Mean ± SD. Unsuitable values were eliminated during statistical analysis of the data in all groups. Differences at the $p<0.001$ level were considered to be statistically significant.

Table 1. Mean tissue MDA, Na^+-K^+ ATPase, GSH and serum TAOS levels

	MDA (nmol/mg.prot)	Na-K ATPase (nmol Pi/mg prot/h)	GSH (μmol /g tissue)	TAOS (mmol/L)
Control	14,40±1,51 (n:17)	140,56±27,48 (n:11)	24,87±5,33 (n:16)	0,52±0,19 (n:15)
Cirrhosis	25,79±7,80* (n:20)	78,90±17,94* (n:15)	13,19±4,47* (n:18)	0,25±0,05* (n:14)

There was statistically significant difference between each parameter of two groups.
**p<0,001*

RESULTS

The comparison of serum TAOS levels in control group and cirrhosis group are shown in Table 1. Mean serum TAOS levels, were found to be significantly lower in cirrhosis group than in the control group ($p<0.001$). Tissue MDA levels of groups are shown in Table 1. Mean MDA levels were found to be significantly higher in cirrhosis group compared with the control group ($p<0.001$). Mean tissue Na^+-K^+ ATPase and reduced glutathione levels were also found to be significantly decreased in cirrhosis group we compared to the control group as demonstrated in Table 1 ($p<0.001$). The results of histopathological examination are shown in Figure 1 and 2. There were central vein dilation and congestion, severe centrilobular and midzonal damage, disseminated hydropic degeneration in cirrhosis group (Figure 2).

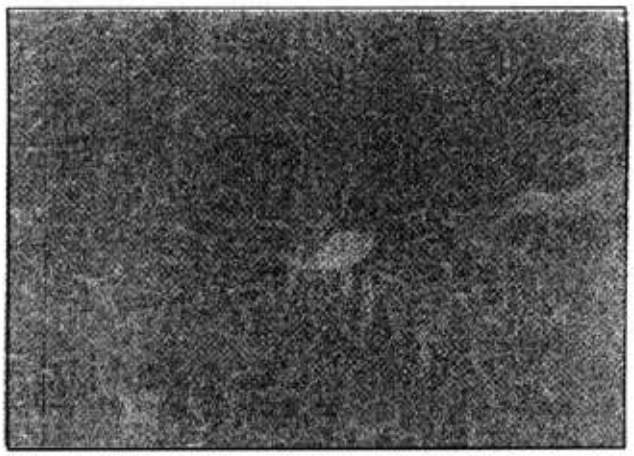

***Figure 1.** Normal hepatocyte pattern. H-E x 200*

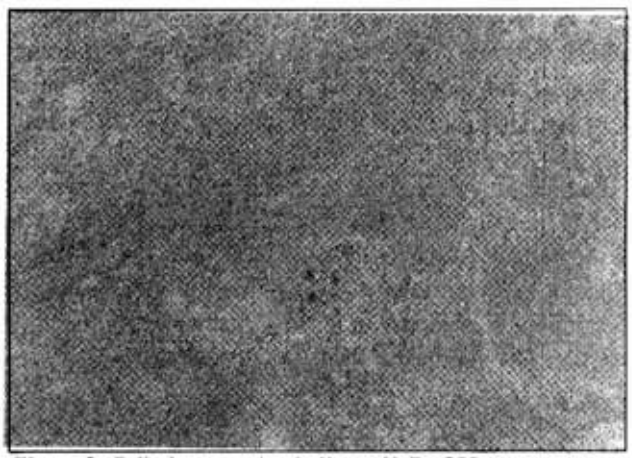

***Figure 2.** Cell degeneration in liver. H-E x 200*

DISCUSSION

The prooxidant activity of carbon tetrachloride (CCl_4) was explored. Upon exposure to carbon tetrachloride, both invivo and invitro, lipid peroxidation as well as generation of free radicals occur in liver and other organs. CCl_4 is oxidized and converted to halo alkane free radicals by the mixed function oxidases. Trichloromethyl and trichloromethyl peroxyl radicals are known to be produced during CCl_4 metabolism (7). These radicals are known to damage intracellular structures such as lysosomes and microsomes and can interact with polyunsaturated fatty acids to initiate a complex series of reactions (9). CCl_4 is activated to trichloromethyl radical which reacts with polyunsaturated fatty acid to cause lipid peroxidation and oxygen free radical formation which in turn leads

to cellular membrane damage (19). In the present study, Tissue MDA levels, a product of lipid peroxidation were found to be significantly higher in cirrhosis group compared with the control group ($p<0.001$).On the other hand, mean serum TAOS levels was significantly lower in the cirrhosis group compared with the control group ($p<0.001$). This finding explains that antioxidative defence system was affected by oxidative stress.

Na^+-K^+ ATPase is a membrane dependent enzyme that enables the transport of sodium and potassium to across the membrane against a concentration gradient by hydrolysis of ATP. The enzyme mainly protects the membrane from increased membrane potential. Many dependent enzymes requires phospholipids for its activity and is very sensitive to free radical reactions and lipid peroxidation (20). It has been reported that the peroxidation of membrane phospholipids and the accumulation of malondialdehyde can cause inhibition of membrane Na^+-K^+ ATPase (21-22). As documented in the present study tissue Na^+-K^+ ATPase activity was decreased in the cirrhosis group .

GSH is continuously produced by the liver cells, released into circulation, and transported to other organs via blood. In our study we observed that tissue GSH levels were found to be significantly lower in the cirrhosis group compared with the control group ($p<0.001$). Gasini et al. have indicated that hepatic lipid peroxidation is directly related to the hepatocellular GSH content (23-25). On the other hand, histopathological examination of the tissue samples in addition to these parameters in damaged tissue provided us to confirm our findings.

In conclusion; carbon tetrachloride–induced liver cirrhosis in rats forms toxic free radicals, thus causes liver tissue damage and initiates membrane lipid peroxidation chain reactions,. Increasing lipid peroxidation will damage antioxidative defence systems and eventually the levels of antioxidative parameters decrease in carbon tetrachloride–induced liver cirrhosis in rats. As a result, the level of these parameters may be useful in the determination and follow-up of carbon tetrachloride–induced liver cirrhosis in rats.

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