Pentoxifylline attenuates ischemia-reperfusion injury in rat liver*

Pentoksifilin rat karaciğerinde iskemi-reperfüzyon hasarını azaltmaktadır

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SUMMARY: *This study was performed to determine whether the pentoxifylline improves microcirculation and inhibits the generation of oxygen free radicals after ischemia and reperfusion of rat liver. Hepatic ischemia was induced for 30 minutes. Tissue was reperfused for 24 hours. The placebo group (n=10) received 1 mL of saline intraperitoneally 5 min before the reperfusion. The experimental group (n=11) received 45 mg/kg pentoxifylline in 1 mL of saline intraperitoneally 5 min before the reperfusion. In control group (n=7), no medication was administered or ischemia-reperfusion was induced. Hepatic tissue was assayed for lipid peroxidation, glutathione peroxidase, lactate dehydrogenase and aspartate aminotransferase.*

In placebo group, lipid peroxidation markedly increased ($p<0.001$) and glutathione peroxidase, lactate dehydrogenase and aspartate aminotransferase activities significantly decreased as compared to control group ($p<0.01$, $p<0.001$, $p<0.05$, respectively). The levels of glutathione peroxidase and lactate dehydrogenase in the pentoxifylline group were not different than controls. Also, the lipid peroxidation was lower than the placebo group. Our results confirm that pentoxifylline attenuates free radical mediated ischemia-reperfusion injury of the liver by modification of the activity of enzyme, glutathione peroxidase.

Key words: Glutathione peroxidase, ischemia-reperfusion, lipid peroxidation, liver, pentoxifylline

ÖZET: *Bu çalışma, pentoksifilinin (PTX) rat karaciğer iskemi ve reperfüzyonundan sonra mikrosirkülasyona etkilerini ve serbest oksijen radikallerinin oluşumunu inhibe edip etmediğini araştırmak amacı ile yapıldı. Karaciğer iskemisi 30 dakika uygulandı. Doku 24 saat süreyle reperfüze edildi. Plasebo grubuna (n=10), reperfüzyondan 5 dakika önce intraperitoneal olarak 1 mL serum fizyolojik verildi. Deney grubuna (n=11) ise reperfüzyondan 5 dakika önce 45 mg/kg PTX 1 mL serum fizyolojik içinde intraperitoneal olarak verildi. Kontrol grubuna (n=7) herhangi bir tedavi veya iskemi-reperfüzyon uygulanmadı. Karaciğer dokularında lipid peroksidasyonu, glutatyon peroksidaz, laktat dehidrogenaz ve aspartat aminotransferaz düzeyleri ölçüldü.*

Plasebo grubunda kontrol grubu ile kaşılaştırıldığında lipid peroksidasyonu belirgin olarak artmış ($p<0.001$) ve glutatyon peroksidaz, laktat dehidrogenaz ve aspartat aminotransferaz aktiviteleri belirgin olarak azalmış bulundu (sırasıyla $p<0.01$, $p<0.001$, $p<0.05$). Glutatyon peroksidaz ve laktat dehidrogenaz düzeyleri kontroller ile pentoksifilin grubu arasında bir farklılık göstermiyordu. PTX grubunda lipid peroksidasyonu da plasebo grubundan düşüktü. Sonuçlarımız PTX'in glutatyon peroksidaz enzim aktivitesinde değişiklik ile karaciğerde iskemi-reperfüzyon hasarını azalttığını göstermektedir.

Anahtar sözcükler: Glutatyon peroksidaz, iskemi-reperfüzyon, karaciğer, lipid peroksidasyonu, pentoksifilin

There is growing evidence that oxygen free radicals play a major role in the ischemia-reperfusion injury of the liver (1-3). During the ischemic period, cellular ATP is catabolized to hypoxanthine; ischemia also triggers the conversion of xanthine dehydrogenase to xanthine oxidase. Upon reperfusion, with the presence of molecular oxygen, xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine and uric acid. Superoxide radical arises from this reaction as a byproduct and xanthine oxidase is one of the important oxygen free radical source during ischemia-reperfusion (4). Also the activation of leukocytes and trombocytes by complement, cytokines and some metabolites, are another important factors responsible from the ischemia-reperfusion injury of the liver (1, 3, 5). Previously, it has been shown that, microvascular plugging of capillaries or sinusoids

Geliş tarihi: 7.8.1997 Kabul tarihi: 20.3.1998
Türk Gastroenteroloji Dergisi, 1998

*Presented as a poster and selected for oral discussion in The International Congress on Free Radicals in Health and Disease, September 6-10, 1995, Istanbul.

with leukocytes and platelet aggregates cause a *no-reflow phenomenon* in ischemia-reperfusion of liver (3, 6). Hypoxia also reduces erythrocyte deformability in different species (7).

Pentoxifylline (PTX), a hemorrheologic agent, has been used clinically for years in the treatment of intermittent claudication and cerebrovascular disease to improve peripheral blood flow. PTX improves microcirculatory blood flow and tissue oxygenation by different mechanisms (8-10). Recent investigations demonstrated the beneficial effect of PTX in acute renal failure, oxygen induced lung injury, hemorrhagic and septic shock (7, 9-12).

The purpose of this study was to determine whether the pentoxifylline treatment attenuates cellular injury during the ischemia reperfusion of the liver by means of the activity of antioxidant enzyme glutathione peroxidase (GSHPx). Also lipid peroxide values and the commonly indicators of hepatocellular injury, lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) levels were assayed in the liver tissues, in order to determine the cellular injury after ischemia-reperfusion period.

MATERIALS AND METHODS

Adult male Swiss-Albino rats, weighing 190-250 g, were used in experiments. They were fed standard rat chow and given tap water ad libitum. Animals were randomly allocated into the three study groups: (a) those that received saline solution intraperitoneally (IP), *placebo group* n=10; (b) those that received 45 mg/kg pentoxifylline IP, *pentoxifylline group* n=11; and (c) those that no medication and no ischemia-reperfusion was done, healthy *control group* n=7. The research protocol was reviewed and approved in our institute by The Animal Care Committee.

Placebo group: After general anesthesia induction, a midline laparotomy was performed and hepatic ischemia induced by the total occlusion of the hepaticoduodenal ligament using a noncrushing small vascular clamp for 30 minutes. 5 minutes before the vascular clamp was removed, 1 mL saline solution was infused IP. Reperfusion was achieved by removal of the vascular clamp. The abdomen was closed and rats were allowed to awaken. 24 hours after reperfusion, the animals were sacrificed to remove the liver tissues.

Pentoxifylline group: All surgical procedures were done as in the placebo group. The animals were received intraperitoneally bolus of pentoxifylline in concentration 45 mg/kg, 5 minutes before the reperfusion of the liver.

Control group: A small midline laparotomy incision, large enough to expose the liver was made and the rat was sacrificed. The liver was removed and washed with cold saline solution.

The liver tissues were stored at -20°C until analyzed. The tissues were thawed and homogenized in phosphate buffer (1 M, pH=7.0) for enzyme assays and in potassium chloride buffer (0.15 M) for lipid peroxide analysis with an Ultra Turrax (T25, Janke & Kunkel, IKA Labortechnik). The homogenates were then sonicated with a Bandelin Sonopuls HD 70. After centrifugation for 20 minutes (4°C, 10.000 g), the supernatants were analyzed. Lactate dehydrogenase and aspartate aminotransferase activities were determined by BM-Hitachi 911 automated analyzer using kinetic method of Boehringer Mannheim. Glutathione peroxidase activity was determined by the method of Paglia and Valentine (13). Malondialdehyde (MDA) concentration, the secondary product of lipid peroxidation was assayed according to the method of Ohkawa with some modifications (14). Protein concentrations were analyzed using a commercial Lowry protein kit.

GSH, GSSGR, NADPH, TBA, 1-1-3-3'-tetraethoxypropane and Lowry protein kit were purchased from Sigma Chemical Co. (St.Louis).

All results were presented as the mean ± the standard error of the mean (SEM). Statistical analysis was done using student's t test. p values of less than 0.05 were considered as statistically significant.

RESULTS

Hepatic tissue levels of GSHPx, LDH, AST and MDA were assessed in placebo, pentoxifylline and control groups (Table 1). GSHPx activities were markedly decreased in placebo group ($p<0.01$) as compared to controls. GSHPx values of the PTX-treated group were not significantly different from the controls. Moreover, GSHPx values in PTX-treated group were higher than in placebo group ($p<0.05$). LDH levels were decreased in placebo group ($p<0.001$) as compared to controls. LDH levels of the PTX group were markedly increased ($p<0.01$) compared to the concentration of placebo group and was not significantly different from the

Table 1. Changes in the levels of MDA, GSHPX, LDH and AST in liver tissues of all three groups (Mean ± SEM)

	Placebo	*Pentoxifylline*	*Control*
MDA (nmol/mL)	2.4 ± 0.1[c]	2.1 ± 0.1[c, d]	1.4 ± 0.1
GSHPX (U/mg prot)	370.1 ± 19.9[b]	471.7 ± 35.0[d]	520.9 ± 41.2
LDH (U/mg prot)	2706.6 ± 89.5[c]	3256.7 ± 160.6[e]	3325.7 ± 100.4
AST (U/mg prot)	406.1 ± 8.1[a]	403.7 ± 6.3[a]	442.9 ± 15.4

a: $p<0.05$ as compared with controls; b: $p<0.01$ as compared with controls; c: $p<0.001$ as compared with controls;
d: $p<0.05$ as compared with placebo; e: $p<0.01$ as compared with placebo

levels of the controls. AST values decreased in both placebo and PTX groups as compared to controls ($p<0.05$, for each). There were no differences between the placebo and PTX groups.

To determine whether the effect of PTX in ischemia-reperfusion of liver is due to its hemorrheologic effects on microcirculation and tissue oxygenation, the level of MDA, which is the secondary product of lipid peroxidation, was determined. The level of MDA significantly increased in both placebo ($p<0.001$) and PTX ($p<0.001$) groups as compared with controls. However, PTX treatment decreased MDA levels compared to placebo group ($p<0.05$).

DISCUSSION

Recent studies reported from our laboratory have shown that free radicals were produced during the ischemia-reperfusion of different tissues like heart and kidney, and increased formation of lipid peroxides with a concomitant decrease in protective antioxidants such as superoxide dismutase, catalase and glutathione reductase (15-17).

Our data demonstrates that intraperitoneally administration of pentoxifylline prior to reperfusion, protects the liver from the ischemia-reperfusion injury. Although there are a lot of experimental study on pentoxifylline, we could not find any article dealing with the effects of pentoxifylline on hepatic ischemia-reperfusion injury.

Pentoxifylline, a novel hemorrheologic agent, has been successfully used to improve blood flow in patients with intermittent claudication and cerebrovascular diseases (8, 9). Although the exact mechanism of action of pentoxifylline is not well understood, pentoxifylline may exert its effects by increasing erythrocyte deformability by raising its ATP content (8, 18), stimulating vasodilatory prostaglandin production (7, 19), increasing the intracellular concentration of cAMP (20), reducing platelet aggregation and fibrinogen levels (8, 19), decreasing the activation and adhesiveness of polymorphonuclear leukocytes and increasing polymorphonuclear leukocyte mobility (10, 21). Thus, these effects restore or improve microcirculation and tissue oxygenation.

In experimental hepatic ischemia, free radicals may arise from the electron transport chain, activated leucocytes or Kupffer cells, the enzyme xanthine oxidase and the auto-oxidation of catecholamines (1, 2, 4, 22). These free radicals are superoxide anion, hydroxyl radical and hydrogen peroxide. Glutathione peroxidase, one of the antioxidant enzyme in the cell, catalyzes the reduction of hydrogen peroxide to water. During this reaction, glutathione is oxidized to glutathione disulfide. Glutathione peroxidase activity was decreased in the liver tissue of placebo group, possibly due to excess generation of free radicals. However, pentoxifylline treatment has beneficial effect on glutathione peroxidase activity. It was proposed that pentoxifylline treatment reduces superoxide anion and hydrogen peroxide generation with the resultant restoration of the blood flow in liver and intestine (6, 21).

Free radicals cause cellular damage primarily through the peroxidation of lipids in the membranes of the cell and mitochondrion and hence, the structural integrity and capacity for cell transport and energy production (1, 3). This was more prominent during the reperfusion of ischemic tissues. Pentoxifylline appears to significantly attenuate hepatic ischemia-reperfusion injury. Further, MDA levels, the secondary product of lipid peroxidation were significantly elevated in those ani-

mals not treated and attenuated in those which were. Marubayashi showed that MDA levels were increased more than two fold in 90 min hepatic ischemia followed by 60 min reperfusion (22). Our results are in agreement with the report of Marubayashi.

As the lipid peroxidation increased, the membrane integrity altered and the increased permeability of the cell membrane subsequently allows the cytosolic components, like enzymes, to leak out of the cell. Two such enzymes, AST and LDH are released from the hepatocyte in proportion to the degree of cell injury. Release of endogenous enzymes is the most commonly used indicator of hepatocellular injury (1). Our hepatic LDH and AST levels were markedly decreased in the placebo group. The pentoxifylline treatment increased LDH levels, but did not change AST levels.

In summary, the results of our study show that pentoxifylline significantly attenuated ischemia-reperfusion injury of liver. This was manifested by a reduction of MDA concentrations as well as increased glutathione peroxidase activity compared to placebo group. However, pentoxifylline treatment in ischemia-reperfusion of liver requires further studies to examine the dosage of pentoxifylline and the effect of treatment before the ischemic period.

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