Kaohsiung Journal of Medical Sciences (2016) 32, 339–347

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.kjms-online.com

ORIGINAL ARTICLE

The effects of tadalafil and pentoxifylline on apoptosis and nitric oxide synthase in liver ischemia/reperfusion injury

Sibel Bektas [a,*], Kemal Karakaya [b], Murat Can [c], Burak Bahadir [d], Berrak Guven [c], Nilsen Erdogan [a], Sukru Oguz Ozdamar [d]

[a] *Department of Pathology, Gaziosmanpasa Taksim Training and Research Hospital, Gaziosmanpasa, Istanbul, Turkey*
[b] *Department of General Surgery, Bulent Ecevit University, School of Medicine, Kozlu, Zonguldak, Turkey*
[c] *Department of Biochemistry, Bulent Ecevit University, School of Medicine, Kozlu, Zonguldak, Turkey*
[d] *Department of Pathology, Bulent Ecevit University, School of Medicine, Kozlu, Zonguldak, Turkey*

Received 2 February 2016; accepted 8 May 2016
Available online 2 June 2016

KEYWORDS
Ischemia/reperfusion;
Liver;
Pentoxifylline;
Tadalafil

Abstract The aim of this study was to investigate the effects of tadalafil (TDF) and pentoxifylline (PTX) on hepatic apoptosis and the expressions of endothelial and inducible nitric oxide synthases (eNOS and iNOS) after liver ischemia/reperfusion (IR). Forty Wistar albino rats were randomly divided into five groups ($n = 8$) as follows: sham group; IR group with ischemia/reperfusion alone; low-dose and high-dose TDF groups received 2.5 mg/kg and 10 mg/kg TDF, respectively; and PTX group received 40 mg/kg PTX. Blood was collected for the analysis of serum alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transferase, uric acid, malondialdehyde (MDA), and total antioxidant capacity (TAC). MDA and TAC also were measured in liver tissue. Histopathological examination was performed to assess the severity of hepatic injury. Apoptosis was evaluated using the apoptosis protease-activating factor 1 (APAF-1) antibody; the expressions of eNOS and iNOS were also assessed by immunohistochemistry in all groups. Serum alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transferase, uric acid, MDA, and TAC, tissue MDA and TAC levels, hepatic injury, and score for extent and for intensity of eNOS, iNOS, and apoptosis protease-activating factor 1 were significantly different in TDF and PTX groups compared to the IR group. High dose-TDF and PTX have the best protective effect on IR-induced liver tissue damage. This study showed that

Conflicts of interest: All authors declare no conflicts of interest.

* Corresponding author. Gaziosmanpaşa Taksim Training and Research Hospital, Department of Pathology, Karayolları Gaziosmanpaşa, İstanbul 34255, Turkey.
E-mail address: sibel_bektas@yahoo.com (S. Bektas).

http://dx.doi.org/10.1016/j.kjms.2016.05.005

TDF and PTX supplementation may be helpful in preventing free oxygen radical damage, lipid peroxidation, hepatocyte necrosis, and apoptosis in liver IR injury and minimizing liver damage.

Introduction

Ischemia/reperfusion (IR) injury results from prolonged ischemic damage followed by restoration of blood perfusion. IR injury is a serious complication of liver surgery, transplantation, and various forms of circulatory shock. Liver IR injury is a complex cascade of events mediated by numerous inflammatory cells and molecular mediators, resulting in hepatocyte death and systemic inflammatory response. The degree of inflammatory response and organ dysfunction is dependent on the duration of liver ischemia and underlying liver disease [1—3]. A large number of pharmacological agents have been shown to confer protection against IR injury in the liver. These agents include antioxidants, ozone, adenosine agonists, nitric oxide donors, sildenafil, and vardenafil [3—5]. Nevertheless, only a few drugs are currently introduced into clinical practice.

Tadalafil (TDF) is a potent and selective inhibitor of phosphodiesterase type-5 (PDE5), which was originally studied as a potential antianginal agent, but became popular for its use in treatment of erectile dysfunction and pulmonary arterial hypertension. Hepatic vascular resistance is regulated by contraction or relaxation of smooth muscle cells in terminal arterioles. By contrast, perisinusoidal stellate cells regulate sinusoidal tonus depending on concentration of nitric oxide (NO) synthesized by sinusoidal endothelial cells. TDF is a specific inhibitor of the NO/cyclic guanosine monophosphate (cGMP) pathway in vascular smooth muscle and platelets, which results in vasodilation of peripheral arteries and veins and inhibition of platelet aggregation respectively. NO generates cGMP, which induces cellular response such as vasodilation. cGMP is inactivated to GMP by phosphodiesterases. Inhibition of PDE augments and prolongs the cellular responses of vasodilation induced by NO and its products. [6,7]. NO is synthesized by one of three NO synthase (NOS) isoforms. The expression of neuronal NOS is mostly limited to neural tissue. Inducible NOS (iNOS) is not expressed under normal circumstances but is upregulated during inflammatory conditions in hepatocytes, endothelial cells, biliary cells, Kupffer cells, neutrophils, and T lymphocytes. Endothelial NOS (eNOS) is constitutively expressed in many cell types, including liver endothelial cells and hepatocytes. Several studies have revealed the protective effect of TDF on renal, myocardial and testicular IR injury [8—10].

Pentoxifylline (PTX) is a nonspecific type-4 PDE inhibitor that displays vasodilatory effects on peripheral blood vessels, particularly in the liver [11]. Some studies have indicated that PTX treatment restores depressed cardiac output and improves hepatic perfusion and intestinal blood flow after hemorrhage and resuscitation [3,12,13]. PTX has been reported to suppress the production of tumor necrosis factor-α, interleukin (IL)-1, IL-6, and IL-12 and to reduce oxidative stress and inflammatory indices [14,15]. Although TDF and PTX have demonstrated beneficial effects, the mechanisms by which these drugs exert protective effects are not fully understood in liver IR injury.

Several mechanisms exist to inhibit IR injury and many drugs have also shown protective effects. The protection mechanisms against IR-induced injury are multifactorial. A number of mechanisms have been proposed, including the elimination of free radicals, inhibition of free radical production, neutrophilic inhibition, and reduction of lipid peroxidation [6,7,12—14]. Although the basic pathological mechanism underlying hepatic injury is not completely understood, it has been shown that reactive oxygen species, formed during IR and apoptosis play an important role in this process. This is the first experimental study in which the effect of two different PDE inhibitors on liver IR injury was investigated. The purpose of this study was to establish the impact of TDF and PTX on hepatic apoptosis and the expressions of eNOS and iNOS in IR-induced liver tissue damage.

Methods

The experimental protocols were conducted with the approval of the Animal Research Committee at Bulent Ecevit University, Kozlu, Zonguldak. All animals were maintained in accordance with the recommendations of the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Animal model

Forty female Wistar rats weighing 250—300 g were housed individually in cages, and were allowed free access to standard rat chow and water before the experiments. The animal rooms were windowless with temperature ($22 \pm 2^{\circ}C$) and lighting controls. The animals were fasted overnight before the experiments but were given free access to water. They were anesthetized by 100 mg/kg ketamine intraperitoneally. All surgical interventions were performed under sterile conditions by the same surgical team at the same period and environment. After midline laparotomy, the left and median liver lobes were rendered ischemic with a microvascular clamp. The successful occlusion of the hepatic artery and portal triad branch in question was