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Protective effects of cordycepin on the histopathological changes and oxidative stress induced by hepatic ischemia/reperfusion in rats

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ABSTRACT

Aim: To investigate the effects of cordycepin on the histopathological changes and oxidative stress induced by hepatic ischemia/reperfusion (I/R) in rats.

Method: Forty male Wistar rats were randomly divided into 4 groups as group I (sham, n=10), group II (control, n=10), group III (I/R-untreated, n=10) and group IV (I/R-cordycepin, n=10). Liver ischemia was induced for 30 min then reperfusion was allowed for 1 h. At the end of the experiment, liver specimens and blood samples were taken for histopathological and antioxidant evaluations, and biochemical analysis.

Results: The levels of IL6, IL-1β, and TNFα in the serum and liver tissues were higher in the I/R-untreated group compared to the I/R-cordycepin treated group. In the I/R-cordycepin group, serum MDA levels were decreased compared with the I/R-untreated group. The I/R-cordycepin treated group showed an increase in TAS levels, and a decrease in TOS levels compared with I/R-untreated group. The histopathological injury score were significantly lower in the I/R-cordycepin treated group compared to the I/R-untreated group. In the I/R-untreated group, the integrity of the hepatocyte cell lines deteriorated. Mononuclear inflammatory cells infiltrated the parenchyma regions, the sinuses dilated and there was diffuse congestion Preoperative treatment with cordycepine reduced histopathological abnormalities.

Conclusion: Cordycepin has demonstrated significant hepatoprotective effects against I/R injury induced in rats through TAS elevation and reduction of TOS, MDA and proinflammatory cytokines.

Keywords: Liver ischemia/reperfusion; cordycepin; oxidative stress; proinflammatory cytokines.

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Introduction

Liver ischemia-reperfusion (I/R) is an important pathophysiological condition encountered in such conditions as trauma, shock, and transplantation [1]. Despite the widespread knowledge in the literature on

biochemical and metabolic changes in liver I/R injury, studies in animal models demonstrating cellular and ultrastructural changes often involve in the process [2,3]. Liver I/R damage involves multifactorial complex mechanisms, and reactive oxygen species (ROS) production is one of them which can lead to cell damage and death [4]. Oxidation of intracellular organelles, such as proteins, lipids and DNA, plays a role in both damage to the intracellular organelles and in cell death. In addition, mitochondria are a major producer of both reactive oxygen species (ROS) and primary oxidative stress target organ [5,6]. Furthermore, the release of other pro-inflammatory cytokines such as (TNF-α), interleukin (IL)-1β and interleukin-6 (IL-6) may result in increased inflammatory status and activation in Kupffer cells [7-9].

Cordycepin (3-deoxyadenosine), a functional component of Cordyceps militaris, has a wide biological field with antitumor, anti-inflammatory, antidiabetic and antioxidant properties [10-12]. Recent studies have suggested that cordycepin prevents cellular damage caused by I/R, reducing oxidative damage and increasing free radical scavenging activity [13,14]. In the current literature, we did not find a study that showed the effect of cordycepin on liver ischemia-reperfusion injury. Then, our aim in this experimental study is to investigate the potential effects and possible mechanisms of cordycepin on liver ischemia-reperfusion injury in the rat model.

Material and methods
Animals
Forty male Wistar rats (250–300 g) obtained from Dicle University Dr. Sabahattin Payzin Health Sciences Application and Research Center were used for this experimental study.

The experimental procedures were approved by the Animal Ethics Committee of Dicle University (DUHADEK) and animals were handled following the International Animal Ethics Guidelines. Animals were kept in their personal cages under a 12/12 hour light / dark cycle, with free access to tap water and food. Room temperature and humidity were carried out at 23±1 °C and 55±5%, respectively.

Liver ischemia reperfusion model
All rats were anesthetized with Ketamine/Xylazine (Ketalar, Parke-Davis, Istanbul, Turkey 100 mg/ kg, Rompun®; Bayer AG, Leverkusen, Germany 10 mg/kg, respectively). For liver I/R [15], after midline laparotomy, pedicle composed the portal vein, the hepatic artery, and the bile duct were clamped with an artery clip. During ischemia, normal saline was injected into the abdominal cavity at 37 °C every 10 minutes and the clips were removed after 30 minutes. Reperfusion was applied for 30 minutes. During this time, the body was kept on a hot plate set at 37 °C to maintain heat.

Study protocol
Animals were divided into four experimental groups (n=10) as follows: Group I (Sham): Dissection of hepatoduodenal ligament was performed and no medication was given. Group II (Control): In addition to dissection, cordyceptin (Product NumberC3394; Sigma, St. Louis, MO, USA) was given at a dose of 10 mg/kg by oral lavage 15 min before the experimental study. Group III (I/R-untreated): Thirty minutes after the Pringle maneuver, reperfusion was performed for 30 min and no drug was given. Group IV (I/R-cordyceptin treated): In addition to procedures of Group 3, cordyceptin was given at a dose of 10 mg/kg

by oral lavage 15 min before the ischemia period.

Blood and tissues of liver lobes from sham-control and I/R injury animals were collected. The liver tissues were then divided into two parts: one part was kept in 10% formalin for histopathological evaluation and the other part was immediately flash frozen in liquid nitrogen and kept at − 80 °C for measurement of tissue parameters.

Determination of oxidative stress markers and inflammatory cytokines

Serum TAS and TOS were measured using commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey) and an Abbott Architect C16000 auto analyzer (Abbott Diagnostic Laboratories, Chicago, IL, USA) using the automated colorimetric measurement methods developed by Erel et al. [16]. The TAS results are presented as the micromolar troloxequivalent per liter (μm Troloxequiv. / L). The TOS results are presented as the micro molar hydrogen peroxide equivalent per liter (μmol H2O2 Equiv./L). The MDA content was evaluated spectrophotometrically, as described previously [17], using a method based on the spectrophotometric measurement of the color that develops during a reaction between thiobarbituric acid and MDA.

The serum levels of TNF-α, IL-1β, and IL-6 were evaluated using enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer's instructions. The data obtained from tissue biochemistry experiments were entered in to a form that was prepared in advance. Tissue particles weighing 0.12–0.24 g were irrigated with physiological saline, dried using blotting paper, placed into Eppendorf tubes, and stored at −85°C until use. Once all of the sampling procedures were complete, the tissues were homogenized using an automated tissue homogenizer after thawing. Then, TNF-α, IL-6, IL-1β, TAS, TOS, and MDA levels were evaluated in the liver tissues.

Histopathological examination

Paraffin tissue blocks were all set for sectioning at 4 μm' thickness. The obtained tissue sections were set on glass slides, deparaffinized, stained by Hematoxylin and Eosin (H&E). The hepatic histological damage scale consists of 4 grades (G0–G3): grade 0 indicates normal histological structure; grade 1 indicates mild injury with cytoplasm vacuolization and focal nuclear pyknosis; grade 2 indicates moderate-to-severe injury with extensive nuclear pyknosis, loss of intercellular borders, and mild-to-moderate neutrophil infiltration; grade 3 indicates severe injury with disintegration of hepatic cords, hemorrhage, and severe polymorphnuclear neutrophil (PMN) infiltration [18].

Statistical analysis

Differences for the biochemical and histopathologic results between the groups were assessed using Kruskal-Wallis and Mann-Whitney U tests. P values <0.05 were considered to indicate statistical significance.

Results

A results of the biochemical and histopathological parameters is presented in Table 1. Biochemical analyses revealed that the levels of IL6, IL-1β, and TNFα in the serum and liver tissues were higher in the I/R-untreated group compared to the I/R-cordycepin treated group (p<0.05, p<0.05 and p<0.05, respectively). In the I/R- cordycepin group, serum MDA levels were decreased

compared with the I/R-untreated group (p < 0.05). But there was no difference between the two groups in terms of tissue MDA levels. The I/R-cordycepin treated group showed increase

Table 1. Blood, tissue biochemical parameters and Liver Injury Score of the all groups.

Parameters	Group I (Sham)	Group II (Control)	Group III (I/R-untreated)	Group IV (I/R- Cordycepin)
Blood				
IL-1β (pg/L)	651±90	652±79	3661±408*▲	2142±143*▲†
IL-6 (pg/ml)	38±5	38±5	304±17*▲	317±20*▲
TNF-α (ng/L)	18 ±10	21±2,5	78±11*▲	31±8*▲†
MDA (μM)	12,3±1,05	17,3±2,1	24±3,4	15,6±1,9†
TAS (μm Trolox- equiv./L)	1± 0,1	1±0,5	1,6±0,4*▲	2,3±0,1*▲†
TOS (μm H2O2 equiv./L)	407±160	579±40	2784±424*▲	765±34
Tissue				
IL-1β (pg/ g protein)	122±26	151±33	755±180*▲	212±95†
IL-6 (pg/ g protein)	32±17	41±9	186±30*▲	75±17*▲†
TNF-α (ng/ g protein)	52±27	62±11	92±10*▲	73±7
MDA (μM/g protein)	14,9±2,4	18,3±2,5	27,4±3,3*▲	23±4,4
TAS (μmTroloxequiv./g protein)	0,6±0,1	0,8±0,1	0,4±0,2*	0,8±0,1†
TOS (μm H2O2 equiv/ g protein)	3084±1210	2860±885	10307±2810*▲	4911±1041*▲†
Liver injury score	0,4±0,1	05±0,1	2,18±0,1*▲	1,3±0,4*▲†

I/R: Ischemia reperfusion; TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin-1 beta; IL-6: Interleukin 6; MDA: Malondialdehyde TOS: Total oxidative stress, TAS: Total antioxidant status.*Group I vs. groups III and IV. ▲Group II vs. groups III and IV. † Group IV vs. group III.
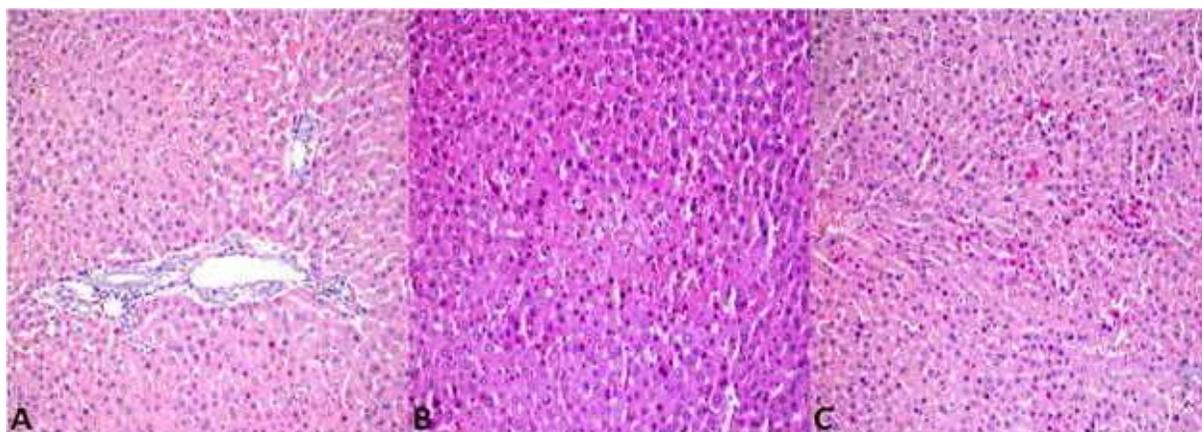

Fig. 1A-C. A) In sham-control group, the structures of hepatocytes and portal areas were observed to be of normal appearance (H&E, x100). B) In the I/R-untreated control group, disruption in the integrity of hepatocyte mononuclear inflammatory cells, sinusoidal dilatation, diffuse congestion and hepatocyte vacuolization were showed (H&E, x100). C) In the liver parenchyma of cordycepin group animals, rare mononuclear inflammatory cells and mild sinusoidal congestion were observed (H&E, x100).

in TAS levels and decrease in TOS levels, compared with I/R-untreated group.

The mean histopathological scores of the groups are given in Table 1. The histopathological injury score were significantly lower in the I/R-cordycepin treated group compared to the I/R-untreated group (p<0.05). The histopathological findings are presented in Figures 1 to 3. Liver tissue sections from the sham-control group were found in normal morphology [Fig. 1A]. In the I/R-untreated group, the integrity of the hepatocyte cell lines deteriorated. Mononuclear inflammatory cells infiltrated the parenchyma regions, the sinuses dilated and there was diffuse congestion [Fig. 1B]. Preoperative treatment with cordycepine reduced histopathological abnormalities compared to the I / R- untreated group [Fig. 1C].

Discussion

ROS plays an important role in I/R mechanisms. ROS, which occurs in increased parameters such as superoxide anion, hydrogen peroxide, hydroxyl radical, and lipid peroxides, occurs in the reperfusion phase following tissue ischemia and causes tissue damage. Tissue MDA is the best predictor of a structural oxidative injury in the cell membrane [19-21]. Cordyceps sinensis is a caterpillar fungus, and their bioactive agent is called cordycepin (3′-deoxyadenosine). It is a natural derivative of the nucleoside adenosine with only one absence of oxygen in the 3′ position of the ribose part [22,23]. The effects of cordycepin on cell biology related to ROS have been reported. For this reason, the anti-tumor, anti-inflammatory, antiangiogenic and anti-oxidant effects of cordycepin have been extensively investigated in various experimental models [24-27]. Cordycepin also acts on specific cell types by decreasing oxidative stress [28]. Dou et al. [14] further suggested that cordycepin treatments scavenged the generation of ROS, upregulated interferon regulatory factor 8 (IRF-8) and suppressed the activity of nuclear factor of activated T cells c1 (NFATc1) during osteoclastogenesis. On the other hand, the anti-tumor effect of cordycepin has been shown to be mediated through ROS-induced apoptosis [27]. Cordycepin has also been reported to stimulate cAMP and cGMP production in platelets [28]. In another study, the production of ROS, O_2^- and H_2O_2, induced by platelet-derived growth factor-BB was abolished by the treatment of cordycepin [29]. In their study, Jeong et al. investigated the apoptotic effects of cordycepin in human leukemia cells, and treatment with cordycepin significantly inhibited cell growth in a concentration-dependent manner by inducing apoptosis but not necrosis. They suggested that this induction was associated with generation of ROS, mitochondrial dysfunction, activation of caspases, and cleavage of poly (ADP-ribose) polymerase protein [27]. Xiao et all. [25] demonstrated that cordycepin could ameliorate albumin-induced epithelial-mesenchymal transition of renal tubular cells by decreasing NADPH oxidase activity and inhibiting ROS production. After I/R, it was found to be an increase in inflammatory mediators such as TNF-a, IL-6 and IL-1β. The release of these proinflammatory cytokines causes aggravation in inflammation. They also have a stimulatory effect on neutrophils [30,31].

In the current study, there was an increase in pro-inflammatory cytokine levels after I/R. TNF-α, IL-1, and IL-6 levels were decreased in response to the anti-inflammatory effects of

cordycepin. We believe that these effects are associated with the anti-inflammatory effects of the drug. Additionally, we observed a significant increase in TAS in the cordycepin versus I/R-untreated group and was also a significant decrease in the TOS level. In the present study, we showed that the activity of MDA increased in the hepatic tissue after I/R-induced injury. Conversely, cordycepine treatment decreased MDA levels.

Conclusion

For the first time, cordycepin has demonstrated significant hepatoprotective effects against I/R injury induced in rats through TAS elevation and reduction of TOS, MDA and proinflammatory cytokines. Treatment with cordycepine in patients with liver I / R injury in different clinical situations may be a promising strategy. However, newer and more extensive experimental studies may be indicative

Compliance with ethical statements

Conflicts of Interest: None.

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