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The Effects of Dimethyl Sulfoxide on Liver Damage Caused by Ischemia-Reperfusion

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ABSTRACT

Introduction. The aim of this study was to investigate the effects of dimethyl sulfoxide on liver damage caused by ischemia-reperfusion after portal vein clamping.

Material and methods. Forty New Zealand rabbits were divided into three groups with the portal veins of all the rabbits except the sham group clamped for 30 minutes: group I, sham procedure; group II, control group; and group III, 500 mg/kg DMSO. The drug was administered IM in the left inguinal region 30 minutes before the operation. Blood samples (5 mL) were taken from the animals at 15, 30, and 45 minutes. At the end of the experiment 1 g of liver tissue samples were obtained. Malondialdhyde (MDA), nitric oxide (NO), AST, ALT, and LDH plasma levels were measured in the blood samples. Liver tissue samples stained with hematoxylin eosin were examined under light microscopy for histopathological changes.

Finding. The liver enzymes in both clamping groups increased significantly compared with the sham group ($P < .01$). Enzyme levels of the DMSO group decreased significantly compared to the control clamping group ($P < .05$). Similar to the enzyme changes, MDA and NO levels increased in the portal vein clamping versus the sham group and decreased in the drug-administered group versus the control clamped group ($P < .03$). The severity of histopathological changes was less in the DMSO group than in the clamped controls.

Conclusion. DMSO decreased the severity of liver damage after portal vein clamping.

ISCHEMIA-REPERFUSION (I-R) damage develops in the liver generally as a result of hemorrhagic shock, ongoing sepsis, surgical intervention for massive trauma, resection of large tumor masses, or liver transplantation. Many agents have been used to treat I-R damage based upon various hypotheses. The aim of this study was to investigate the effects of dimethylsulfoxide (DMSO), a nonenzymatic, free-oxygen radical scavenger[1,2] in portal I-R injury.

MATERIALS AND METHODS

Forty New Zealand Albino rabbits of weights ranging between 2350 and 2550 g were were divided into three groups ($n = 10$ for sham, $n = 15$ for the other two groups). All surgical procedures were performed under ketamine HCl anesthesia. Only a laparotomy was performed in group I (sham group). Animals in group II were exposed to portal triad clamping (control group). Animals in both groups were treated before clamping with 500 mg/kg 0.9% NaCl intramuscularly. Before clamping of the portal triad, animals in group III received intramuscular 500 mg/kg DMSO (Merck Sharp & Dohme, UK). The animals were allowed to breathe spontaneously during the experiment. A midline laparotomy was performed on all groups under aseptic conditions. The portal veins of all groups except the sham group were clamped from time 0 to 30 minutes. Blood samples (5 mL) were obtained from the inferior vena cava at 15, 30, and 45 minutes. Tissue samples (1 g) were procured from the liver after the rabbits were sacrificed at 45 minutes.

The blood samples for biochemical analyses were centrifuged for separation of the plasma and kept in the deep freeze at −80°C until

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the measurement day. MDA and NO levels as well as AST, ALT, and LDH levels were measured in the serum. Plasma MDA levels were measured using thiobarbituric acid reactivity as defined by Hunter et al.[3] Plasma NO levels were determined using the method defined by Smarson et al.[4] Measurements for AST, ALT, and LDH were made with ILAB kits on an ILAB-1800 autoanalyzer. Liver tissue samples were preserved in formalin solution until the measurement day when they were stained with hematoxylin-eosin for histopathological changes by light microscopy.

Statistics

Repeated measurements were examined by variance analysis. When a spherical approach was not ensured, a Greenhouse-Geisser correction was applied. When the differences among the groups seemed significant, a Tukey test was used to determined the differences between 15 to 30, 30 to 45, and 15 to 45 minutes.

RESULTS

No animal died during the experiment. AST, ALT, and LDH levels in groups with clamping were significantly higher than those of the sham group ($P < .01$). Enzyme levels in the DMSO administered group were significantly lower compared with the control group ($P < .05$). NO and MDA levels showed significant increases in all groups consistent with the enzyme levels in the clamping groups ($P < .01$), whereas levels in DMSO administered animals were significantly lower compared with the control group ($P < .03$). Time-dependent changes in ALT and LDH levels were statistically significant in all groups, whereas no significant time-dependent change was found for AST, MDA, and NO levels. The group-time interaction was observed to be statistically significant for AST, ALT, and LDH levels. However, there was no significant group-time interaction for MDA and NO levels (Table 1). Hydrophic degeneration in perivascular regions was seen especially in the control group. The least microscopic damage was observed in DMSO-administered animals.

DISCUSSION

Reperfusion after liver ischemia paradoxically causes more damage to liver tissue.[5] Free oxygen radicals, which are toxic metabolites, are released following the ischemia-reperfusion sequence, inducing cell destruction. The increased free oxygen radicals stimulate lipid peroxidation, thereby, damaging cell membranes and producing deterioration in cell functions. The body has several endogenous antioxidant defense systems, such as *SOD, GSH*-patient, *GSH*-rd, and catalase, which suppress free oxygen radical generation. In addition, various endogenous agents suppress lipid peroxidation, such as α-tacopherol.[1,6,7]

Nevertheless, the causes of this damage cannot be only explained by biochemical reactions. Mediators and inflammatory events participate in ischemia and I-R damage, which are extremely complex and coassociated. As a consequence of the formation of free oxygen radicals, arachidonic acid metabolites, platelet activating factor, activated polymorphonuclear leukocytes, and xanthine oxidase cause tissue damage.[8–10] Several studies have been conducted to prevent occurrence of these mediators or neutralize the released substances.

The leading mechanism for DMSO to improve effects in I-R injury is minimizing the effects on free radical production. DMSO removes hydroxyl radical (OH), which is one of the most toxic products of superoxide radical formation.[11] It has been reported that reduction of free oxygen radical and exogenous NO production has protective effects on the body.[12] Stein and coworkers reported improvement in both biochemical and histological markers of liver injury by DMSO in a rat model.[13] Recent experimental studies in rabbit models support the ameliorative effects of DMSO on I-R injury of the liver.[14,15] Instead of survival analysis, these experiments measured biochemical markers of oxidative injury, scintigraphy, and histopathology of liver. DMSO was intravenously applied prior to ischemia at up to 1 g/kg dose

Table 1. The Biochemical Parameters of Liver Injury According to Experimental Groups.

Parameters	Time (min)	Group I (sham)	Group II (control)	Group III (DMSO)
AST (U/L)	15	28.1 ± 7.6	127.7 ± 51.6	85.1 ± 59.1
	30	44.3 ± 13.4	244.6 ± 75.9	157.5 ± 82.9
	45	51.9 ± 10.7	308.8 ± 90.7	208.1 ± 63.4
ALT (U/L)	15	28.8 ± 5.7	81.6 ± 21.4	45.6 ± 16.9
	30	50.8 ± 12.7	173.0 ± 44.2	89.9 ± 42.5
	45	32.4 ± 9.0	282.9 ± 39.4	113.6 ± 70.3
LDH (U/L)	15	211.1 ± 58.8	1570.3 ± 236.1	1004.6 ± 312.4
	30	237.2 ± 64.2	1622.3 ± 386.0	1243.5 ± 350
	45	161.2 ± 59.5	2225.1 ± 223.1	1370.1 ± 341.2
MDA (nmol/L)	15	4.54 ± 0.91	14.41 ± 2.47	8.11 ± 2.52
	30	4.67 ± 0.81	15.17 ± 3.05	8.75 ± 3.58
	45	4.52 ± 0.66	13.21 ± 3.42	8.22 ± 2.86
NO (μmol/L)	15	26.5 ± 6.0	52.4 ± 2	35.8 ± 7.7
	30	23.7 ± 7.8	58.1 ± 12.8	33.1 ± 3.1
	45	23.0 ± 5.9	53.5 ± 2.5	32.4 ± 2.6

All values are presented as mean and standard deviations.

range with sampling performed up to 30 minutes after injury.[14,15] Improved effects of DMSO were observed in a dose-dependent manner in the study by Hatipoglu et al.[15] Despite the relatively nontoxic dose and the slower distribution of the agent in our study, DMSO treated the oxidative injury of the liver seen after I-R. However, previously reported nephrotoxic side effects of DMSO[16] may limit the clinical application of this agent.

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