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Protective Effects of Resveratrol on Spleen and Ileum in Rats Subjected to Ischemia-Reperfusion

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ABSTRACT

Resveratrol is as an antioxidant with free radical-scavenging activity and finds its clinical application in the prevention of postischemic tissue injury following solid organ transplantation. This study investigates the effect of Resveratrol on spleen and ileum tissues subjected to hepatic ischemia-reperfusion (I/R) in rats. Twenty-four rats were recruited in the study as follows: group A: I/R ($n = 8$), group B: I/R + Resveratrol ($n = 8$), and group C: sham operation ($n = 8$). After intraperitonealy pretreatment of eight rats with resveratrol (15 mg/kg/d) for 5 days, 16 rats were subjected to 45 minutes of hepatic ischemia followed by 30 minutes reperfusion period. Resveratrol was given 15 minutes prior to ischemia and just before the reperfusion in rats. After reperfusion period all rats were sacrified. Spleen and ileum tissues were examined spectrophotometrically to measure malondialdehyde (MDA), glutathione (GSH), and total nitrite, nitrate as an end product of nitric oxide (NO) levels. Concerning the spleen, statistically significant decrease of GSH and increase of MDA and NO levels were found group A when compared to groups B and C ($P = .040$, $P = .004$, and $P = .001$ group A vs group B; $P = .05$, $P = .003$, and $P = .001$ group A vs group C, respectively). Parallel results were obtained in ileum. A statistically significant decrease in GSH and an increase in MDA and NO levels in group A in respect to group B and group C was obtained ($P = .048$, $P = .034$, and $P = .001$ group A vs group B; $P = .004$, $P = .001$, and $P = .003$ group A vs group C, respectively). The result of this study shows that resveratrol has a protective effect on spleen and ileal mitochondrial oxidative stress in rats subjected to I/R.

HEPATIC ISCHEMIA-REPERFUSION (I/R) is a common problem encountered in variety of clinical conditions such as liver transplantation, hepatic failure after shock, and liver surgery. During the reperfusion process a large amount of molecular oxygen is supplied to the tissues and abundant amounts of reactive oxygen species (ROS), which are responsible for reperfusion injury, are produced.[1] The organ dysfunction that accompanies this condition is generally associated with increased microvascular permeability, interstitial edema, impaired vasoregulation, inflammatory cell infiltration, and parenchymal cell dysfunction.[2] Free radical ablation for the treatment of reperfusion injury has found its first clinical application in the prevention of postischemic tissue injury following organ transplantation.[3] These agents proposed to be useful in the clinical settings of hepatic I/R damage include free radical scavengers.

Resveratrol (3, 5, 4′-trans-trihydroxystilbene) is a natural phytoalexin present in grapes and red wine, which possesses a variety of biological activities including anti-inflammatory, anticarcinogenic, and antioxidative activities.[4] Although Resveratrol has antioxidant features, its effect on spleen and ileum injury in rats after experimental hepatic I/R-induced tissue damage has not yet been investigated. The aim of this study is to evaluate whether Resveratrol administration can protect spleen and ileum tissue against oxidative stress in rats after experimental hepatic I/R-induced tissue damage. To asses the ability of protective effect of Resveratrol in hepatic I/R-performed rats, we measured the activities of malondialdehyde (MDA), glutathione (GSH), and total nitrite and nitrate as an end product of nitric oxide (NO) in the spleen and ileum tissues.

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MATERIALS AND METHODS

Experimental Conditions

A total of 24 Wistar albino rats weighing 200 to 250 g were included in the study. Rats were obtained from Firat University, Animal Laboratory, Elazığ, Turkey. The study was approved by Inonu University Ethics Committee. Rats were kept in stainless steel cages, allowed free access to food and water ad libitum, and quarantined 14 days before surgery. Food was withheld 8 hours prior to surgery, but free access to water was allowed. They were subjected to controlled conditions of temperature and humidity and housed in controlled animal quarters with a 12-hour light-dark cycles. All surgical procedures were performed while the rats were under intraperitoneal ketamine (50 mg/kg) and xylazine HCl (10 mg/kg) anesthesia. A total of 24 rats were divided into three groups: group A: I/R ($n = 8$); group B: I/R plus Resveratrol ($n = 8$); group C: sham ($n = 8$). Only laparatomy was performed on the sham group. Total hepatic ischemia was induced for 45 minutes by clamping the hepatic artery, the portal vein, and the bile duct using a vascular clamp. Thereafter, the clamp was removed and blood was reperfused for 30 minutes. During the period of ischemia 0.5 mL saline was given intraperitoneally. Animals in the Resveratrol-treated group were treated with 15 mg/kg Resveratrol (intraperitoneally) once a day throughout 5 days before surgery, 15 minutes before ischemia, and immediately before the reperfusion period. The spleen and ileum tissues were stored at −80°C for the analyses of MDA, nitric oxide NO, and reduced glutathione GSH levels.

Biochemical Analyses

The spleen and ileum tissues were homogenized and the MDA contents of homogenates were determined spectrophotometrically.[5] The amounts of lipid peroxides were calculated as thiobarbituric acid reactive substances of lipid peroxidation and are given as nmol/g tissue. As tissue nitrite (NO_2^-) and nitrate (NO_3^-) levels can be used to estimate NO production, we measured the concentration of these stable NO oxidative metabolites. Quantification of NO_2^- and NO_3^- was based on the Griess reaction, in which a chromophore with a strong absorbance at 545 nm is formed by reaction of NO_2^- with a mixture of naphthlethylenediamine and sulfanilamide.[6] Results were expressed as umol/g tissue. GSH was determined by the spectrophotometric method, which was based on the use of Ellman's reagent.[7] Results were expressed as nmol/g tissue.

Statistical Analysis

The statistical package for social sciences (SPSS) version 10.0 was used for statistical analysis. Individual group parameters were assessed using the Mann-Whitney U test. The results are given in the text as means ± standard deviations for all comparisons; statistical significance was defined as $P < .05$.

RESULTS

Concerning the spleen, a statistically significant decrease of GSH and an increase of MDA and NO levels were found in group A when compared to groups B and C ($P = .040$, $P = .004$, and $P = .001$ group A vs group B; $P = .05$, $P = .003$, and $P = .001$ group A vs group C, respectively). Parallel results were obtained in ileum. Statistically significant decrease in GSH and an increase in MDA and NO levels in group A compared to group B and group C was obtained ($P = .048$, $P = .034$, and $P = .001$ group A vs group B; $P = .004$, $P = .001$, and $P = .003$ group A vs group C, respectively; Table 1).

DISCUSSION

Several reports showed that oxidative stress associated with lipid peroxidation is involved in the ileum injury, but few cases report about spleen injury in rats subjected to I/R.[8,9] Despite studies concerning the prevention of I/R injury in rat ileum and spleen, to our knowledge this is the first study evaluating both the effect of hepatic I/R injury on ileum and spleen and the effect of Resveratrol as an antioxidant agent on these tissues in rats subjected to hepatic I/R.

MDA is a secondary product of lipid peroxidation and is released as a result of the toxic effect of ROS in rats subjected to hepatic I/R.[10] Increased concentrations of MDA reflect the level of lipid peroxidation in tissues and it is considered as a marker of tissue injury.[11] In the present study, increased levels of MDA in I/R group were more significant than those of I/R + Resveratrol and sham group both in spleen and ileum tissues. Although the mechanism is not exactly known, the decreased levels of MDA in the I/R + Resveratrol group may probably be due to Resveratrol's antioxidant and free-radical-scavenging effect.[12] GSH is an essential component of the cellular defence mechanism against oxidative stress-induced ROS in rats with hepatic I/R.[10] GSH plays a pivotal role in the defence mechanism against oxidative stress, as a cofactor of glutathione peroxidases (GPx), and participates in the elimination of hydrogen peroxide and lipid hydroperoxides.[13] Different mechanisms may contribute to a reduced activity of antioxidant enzyme in rats subjected to hepatic I/R. GSH is inhibited by superoxide anion[14] and lipid hydroperoxides may inactivate GPx, presumably by binding to the active site of the enzyme.[15] In our study, the increased spleen and

Table 1. NO, GSH, and MDA Levels in Spleen and Ileum Tissues

	Spleen ($n = 8$)	Ileum ($n = 8$)
NO (μmol/g tissue)		
Group A	371.9 ± 30.0*,**	311.8 ± 45.1*,**
Group B	117.1 ± 13.2	101.1 ± 25.1
Group C	118.70 ± 14.5	79.4 ± 25.9
GSH (nmol/g tissue)		
Group A	2.5 ± 0.4***,****	1.0 ± 0.3***,****
Group B	10.6 ± 0.8	7.1 ± 1.0
Group C	10.9 ± 1.0	7.3 ± 1.48
MDA (nmol/g tissue)		
Group A	396.6 ± 45.0***,****	203.3 ± 70.1***,****
Group B	110.3 ± 16.6	66.5 ± 8.1
Group C	117.07 ± 15.3	53.7 ± 7.4

All data are given as means ± SD. Group A: I/R; group B: I/R + Resveratrol; group C: sham-operated rats.
*$P < .001$ vs group B.
**$P < .001$ vs group C.
***$P < .05$ vs group B.
****$P < .05$ vs group C.

ileum tissue GSH levels in the I/R + Resveratrol group compared to I/R and sham group may be related to its antioxidant and free-radical scavenging effect. Another explanation of the significant increases in GSH levels seen in the I/R + Resveratrol group is the effect of Resveratrol upon the enzymes involved in glutathione synthesis.

NO is believed to be an important mediator of hepatic vascular tone under normal and diseased conditions. NO is produced in small amount in the liver by the constitutive form of NO synthase.[16] In the present study, levels of NO were significantly higher in I/R group than those of I/R + Resveratrol and sham groups both in spleen and ileum tissues. Possible explanations of statistically decreased NO levels in I/R + Resveratrol group include reduced excessive NO formatione, and/or neutralizing NO, once formed; this would lower oxidative damage and therefore allow Resveratrol to reduce spleen and ileum oxidative stress.

In conclusion, these results show that spleen and ileum tissue can be affected by hepatic I/R injury and intraperitoneal administration of Resveratrol reduces spleen and ileum oxidative damage in rats subjected to hepatic I/R. However, more investigations are required to evaluate the antioxidant effect of Resveratrol on spleen and ileum tissue damage in clinical and experimental models.

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