Preventive role of gallic acid on hepatic ischemia and reperfusion injury in rats

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Abstract There is little information about the hepatoprotective effects of gallic acid against ischemia–reperfusion (I/R) damage. Animals were subjected to I/R. Gallic acid at doses of 50 and 100 mg/kg body weight (bw) were injected as a single dose prior to ischemia. Liver tissue homogenates were used for the measurement of malondialdehyde (MDA), catalase (CAT) and glutathione peroxidase (GPx) levels. At the same time alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) were assayed in serum samples and compared statistically. While the ALT, AST, LDH activities and MDA levels were significantly increased, CAT and GPx activities significantly decreased in only I/R-induced control rats compared to normal control rats ($P < 0.05$). Treatment with gallic acid at a dose of 100 mg/kg bw significantly decreased the ALT, AST, LDH activities and MDA levels, and markedly increased activities of CAT and GPx in tissue homogenates compared to I/R-induced rats with no treatment group ($P < 0.05$). In oxidative stress generated by hepatic ischemia–reperfusion, gallic acid contributes partially an alteration in the delicate

balance between the scavenging capacity of antioxidant defense systems and free radicals in favour of the antioxidant defense systems in the body.

Keywords Gallic acid · Ischemia–reperfusion · Liver · Oxidative stress · Rat

Introduction

Hepatic ischemia/reperfusion (I/R) injury is an important pathological process leading to systemic and hepatic damage after circulatory shock, hepatic trauma, transplantation or hepatic surgery (Daglar et al. 2009). I/R injury is a result of a series of complex mechanisms including free oxygen radicals activated by energy depletion and failure of oxygen delivery to the vital tissues in the ischemic period (Yaylak et al. 2008).

The increased production of reactive oxygen species (ROS) during I/R injury results in consumption and depletion of endogenous antioxidants. In this situation, the cells require exogenous antioxidant to protect them from ROS-induced injury (Korkmaz and Kolankaya 2010).

Plant derived polyphenolic compounds possess a wide range of pharmacological properties and the study of their mechanism of action has been the subject of considerable interest in recent years (Senapathy et al. 2011). Phenolic compounds have been reported to have a capacity to scavenge free radicals

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Fig. 1 Structural formula of gallic acid

and their antioxidant activities are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Padma et al. 2011).

Gallic acid (GA) (3,4,5-trihydroxybenzoic acid) is a polyphenol from plants (Senapathy et al. 2011; Stanely et al. 2009) and a natural product of tannins hydrolysis found abundantly in grapes, different berries, and other fruits as well as in wine (Fig. 1). Especially, tea is an important source of GA and contains up to 4.5 g/kg of fresh weight (Reckziegel et al. 2011).

Gallic acid (GA) has a biological activities such as antibacterial, antiviral, anti-inflammatory, antioxidant, antitumor (Liao et al. 2012) and antidiabetic effects and reduces heart infarction incidence and oxidative liver damage. It received much attention because of its potent ability to scavenge ROS, such as superoxide anions, hydrogen peroxide, hydroxyl radicals and hypochlorous acid (Mansouri et al. 2013). This polyphenol is even more effective than ascorbic acid to prevent lipid peroxidation (Reckziegel et al. 2011).

In the present study, GA as a strong antioxidant agent was investigated with respect to its hepatoprotective effects in hepatic I/R injury in rats. Hence, MDA levels, CAT and GPx activity in the liver tissue homogenates and ALT, AST and LDH activities in the serum were measured and then compared statistically.

Materials and methods

Chemicals

Gallic acid (GA) (3,4,5-trihydroxybenzoic acid) was purchased from Sigma (St. Louis, MO, USA).

Animals

Adult Sprague–Dawley albino rats were utilized in the present study. They were housed in polycarbonate cages in an air-conditioned room (lights on

7 a.m.–7 p.m., 22 ± 2 °C and 45–50 % humidity) and were fed with laboratory pellet chow and water was given ad libitum during the experimental period. All procedures were conducted in conformity with the Institutional Ethical Committee for Animal Care and Use at the Eskisehir Osmangazi University (Protocol no.: 121/2009) and the international guidelines on the ethical use of animals (NIH publications no.: 80–23).

Experimental protocols

The rats were randomly divided into four groups (each containing eight animals);

Group 1 (Normal control or NC) were made up of non-operated rats that received no treatment.

Group 2 (I/R-control (I/R-C) or I/R + saline) were operated rats with no treatment.

Group 3 (I/R + 50) were operated rats that received single dose 50 mg/kg bw gallic acid.

Group 4 (I/R + 100) were operated rats that received single dose 100 mg/kg bw gallic acid.

Gallic acid (GA) solutions used for the treatment were intraperitoneally (ip) injected as a single dose (dissolved in saline, 4 ml/kg bw volume), 15 min before ischemia operation.

In all surgical operations; the rats were anesthetized with intramuscular injection of ketamine hydrochloride at the dose of 70 mg/kg bw (Ketalar, Eczacibasi, Istanbul, Turkey) and xylazine hydrochloride at the dose of 10 mg/kg bw (Rompun, Bayer, Istanbul, Turkey).

Under anesthesia, a midline laparotomy was made using minimal dissection. Total hepatic ischemia was induced for 45 min by clamping the hepatic artery, the portal vein and the bile duct using a vascular clamp. During the period of ischemia 0.5 ml of saline was given ip. Albino rats were subjected to 45 min of hepatic ischemia followed by 60 min of reperfusion period (Sener et al. 2003).

Biochemical analyses

In the present study, biochemical investigations were made in serum and liver tissue. At the end of the experimental periods, the rats were sacrificed by ether anaesthesia and then whole blood samples from rats were collected in polystyrene tubes without anticoagulant. The serum was separated by centrifugation at 1,000 rpm at 4 °C for 15 min using cooling. The ALT,

Table 1 The ALT, AST and LDH activities in serum of rats treated with different doses of gallic acid (GA; 50 and 100 mg/kg ip) or saline

Experimental groups*	ALT (U/L)	AST (U/L)	LDH (U/L)
NC	66.26 ± 5.90	95.62 ± 3.50	227.03 ± 27.44
I/R-C or I/R + saline	821.10 ± 99.76 ^a	492.55 ± 53.96 ^a	2825.25 ± 204.95 ^a
I/R + 50	745.80 ± 64.22 ^a	440.90 ± 28.09 ^a	2440.62 ± 352.64 ^a
I/R + 100	455.60 ± 47.64 ^{a, b}	252.87 ± 61.51 ^{a, b}	1591.50 ± 280.43 ^{a, b}

* For details, see “Materials and methods” section. Values are mean ± SE (n = 8), one-way ANOVA. $P < 0.05$, significantly different from ^aNC group and ^bI/R-C groups by Tukey’s multiple range tests

AST and LDH levels in serum were immediately measured with a commercial kit (Biolabo, Maizy, France) using an auto analyzer (Crony Instruments, Airone 200 RA, Rome, Italy). The serum ALT, AST and LDH were expressed “U/L”.

Briefly, MDA was measured by thiobarbituric acid reaction as a lipid peroxidation product according to the method of Mihara and Uchiyama (1978). CAT activity was determined using ammonium molybdate–hydrogen peroxide reaction as described previously by Góth (1991). The hepatic MDA level and CAT activity were expressed nmol/g protein and kU/g, respectively.

Glutathione peroxidase (GPx) activity was determined using a cellular assay kit (Calbiochem®, Darmstadt, Germany and Cat. no.: 354104). The principle of this kit presented in short: spectrophotometric assay kit where glutathione peroxidase activity is quantitated by measuring the change in absorbance at 340 nm caused by the oxidation of NADPH. GPx was expressed as mU/ml.

Statistical analysis

The results were expressed as the mean ± SE of eight animals per group. One-way analysis of variance (ANOVA) and Tukey test were used for the analysis and comparison of data within and between groups. Differences were considered significant at $P < 0.05$.

Results

The results of this study of the protective effect of gallic acid against hepatic ischemia–reperfusion injury in rats are presented in the Tables 1 and 2.

Changes in serum the ALT, AST and LDH levels

As shown in Table 1, in ischemia–reperfusion groups, only treatment with GA at the dose of 100 mg/kg bw significantly decreased the ALT, AST and LDH levels in the serum ($P < 0.05$). Although administration of GA at the dose of 100 mg/kg bw significantly reduced the serum ALT (44.51 %), AST (48.66 %) and LDH (43.67 %) levels, the values were not restored to the same levels as those of the NC group.

Lipid peroxidation

Exclusively, hepatic MDA levels in the I/R + 100 group were significantly lower than in the I/R-C group ($P < 0.05$). Although administration of GA at the dose of 100 mg/kg bw significantly reduced the tissue MDA levels (27.39 %), the values were not restored to the same levels as those of the NC group (Table 2).

Changes in liver tissue: the CAT and GPx activities

In the I/R groups, solely GA at the dose of 100 mg/kg bw significantly increased ($P < 0.05$) CAT and GPx activities in the liver. Not only administration of GA at the dose of 100 mg/kg bw significantly reduced the tissue CAT (288.76 %) and GPx (217.68 %) activities in the liver, but also the values were restored to the same levels as those of the NC group (Table 2).

Discussion

In particular, oxygen-derived free radicals induced by ischemia–reperfusion have been studied as a contribution to cellular injury in the lung, intestine, liver etc.

Table 2 The CAT, GPx activities and MDA levels in liver tissue of rats treated with different doses of gallic acid (GA, 50 and 100 mg/kg ip) or saline

Experimental groups*	MDA (nmol/g protein)	CAT (KU/g)	GPx (mU/ml)
NC	16.93 ± 3.43	7.61 ± 1.08	32.81 ± 4.33
I/R-C or I/R + saline	37.53 ± 2.92 ^a	3.38 ± 1.19 ^a	17.99 ± 6.25 ^a
I/R + 50	35.71 ± 3.35 ^a	4.67 ± 1.03 ^a	24.64 ± 3.47 ^a
I/R + 100	24.54 ± 2.13 ^{a, b}	9.76 ± 1.15 ^b	39.16 ± 3.832 ^b

* For details, see “[Materials and methods](#)” section. Values are mean ± SE (n = 8), one-way ANOVA. *P* < 0.05, significantly different from ^aNC group and ^bI/R-C groups by Tukey’s multiple range tests

(Iwamoto et al. 2002). On the other hand, oxidative stress means an alteration in the delicate balance between free radicals and the scavenging capacity of antioxidant enzymes in favour of free radicals in the body systems (Avci et al. 2012).

Ischemia–reperfusion (I/R) frequently is encountered during liver transplantation and hepatectomies performed under vascular exclusion. Restoration of blood flow after a period of liver ischemia is associated with a series of events that aggravate the ischemic injury (Smyrniotis et al. 2005).

The implicated factors include free oxygen radicals, leukocyte migration and activation, microcirculatory abnormalities, sinusoidal endothelial cell damage, activation of the coagulation cascade, Kupffer cell activation due to the release of inflammatory cytokines, and proteolytic enzymes (Kucuk et al. 2009). Due to the large amount of oxygen influx during reperfusion, xanthine oxidase catalyzes the conversion of hypoxanthine to xanthine, and simultaneously generates superoxide (Iwamoto et al. 2002).

To control the detrimental effects of ROS (especially superoxide), besides inhibiting its production, organisms have developed a variety of antioxidant defense systems, especially the endogenous antioxidant enzymes (Seth et al. 2000; Wu et al. 2011; Wang et al. 2011), such as SOD (which dismutates superoxide to hydrogen peroxide, which represents the first step of the antioxidant pathway), hemecontaining CAT and/or the selenoenzyme GPx (which catalyses hydrogen peroxide conversion to water, which is the second step of the antioxidant pathway) (Jihen et al. 2009; Kim et al. 2009). In general, while GPx is more important than CAT in removing hydrogen peroxide, CAT has a predominant role at least in peroxisomes where it is concentrated (Kim et al. 2009). Also, overexpression of

GPx has been shown to be protective against oxidative stress in cultured cells and whole animals (Day 2009).

In I/R injury, ROS exert a central role in the injury of cellular membranes leading to lipid peroxidation in ischemic organs (Daglar et al. 2009). Endothelial cells, as well as Kupffer cells, primarily generate oxygen radicals by NADPH oxidase after hepatic I/R. MDA, which is one of the stable end-products of lipid peroxides, is produced from cell membrane destruction by oxygen radicals. Therefore, MDA is considered as a marker of oxygen radicals and lipid peroxidation of endothelial cells (Nakano et al. 2009) or a sensitive index to assess lipid peroxidation (Daglar et al. 2009).

In the present study, ischemia–reperfusion resulted in a significantly elevated MDA level and markedly decreased CAT and GPx activity. These results support the hypothesis that the lipid peroxidation process causes liver cell damage during I/R. Treatment with gallic acid at the dose of 100 mg/kg bw significantly attenuated the rise in tissue MDA, the decrease in CAT and GPx activity (Table 2). Impairment of liver functions indicates the suppression of oxygen radicals by treatment of gallic acid effectively inhibited the lipid peroxidation of endothelial cells and thereby preserved liver function.

Enzymes such as ALT, AST, and LDH are used as markers of cellular damage following hepatic I/R damage. In various studies, it has been shown that the serum concentrations of these enzymes increase in proportion with the duration of ischemia (Kucuk et al. 2009). In the present study, the serum AST, ALT and LDH levels in the I/R-100 group were significantly lower than in the I/R-C and I/R + 50 groups (*P* < 0.05) (Table 1). This suggests that gallic acid decreases hepatic cellular damage. These effects of gallic acid

after I/R injury may be due to protection of sinusoidal endothelial cells, which are the first target, and improvement of sinusoidal blood flow by gallic acid.

In conclusion, oxidative stress generated by hepatic ischemia–reperfusion, gallic acid at the dose of 100 mg/kg bw contributes to an alteration in the delicate balance between the scavenging capacity of antioxidant defense systems and free radicals in favour of the antioxidant defense systems in the body.

Conflict of interest We declare that we have no conflict of interest.

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