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EXPERIMENTAL STUDY

Resveratrol ameliorates hepatic injury and modulates hepatic biomarkers of regeneration, apoptosis and survival in a rat model of blunt hepatic trauma

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ABSTRACT

OBJECTIVE: To investigate the protective potential of resveratrol (RES) in blunt hepatic trauma (BHT) by exploring the anti-inflammatory and histopathologic effects as well as modulatory effects on hepatic biomarkers of acute injury, regeneration, apoptosis and survival in a rat model of BHT.

METHODS: A total of 21 Wistar Albino rats (weighing 120–250 g) were separated into 3 groups (n = 7 for each group), namely control group (CON; standard feeding), BHT group (BHT; blunt hepatic trauma plus observation) and trauma plus BES group (BHT-RES; blunt hepatic trauma plus intraperitoneal injection of 10 mg/kg RES). Serum levels for aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and bilirubin were measured on Day 7 and rats were sacrificed for histopathological (inflammation scores) and immunohistochemical [expression of proliferation marker (Ki-67) and apoptosis-related markers (Bcl-2, and Bax)] analyses in resected liver tissue.

RESULTS: The highest levels for serum AST (p = 0.002), ALT (p = 0.002) and LDH (p = 0.002) were obtained from BHT group of rats, as followed by BHT-RES and control groups, respectively. The highest scores for Ki 67 (p = 0.002 for each), Bcl 2 (p = 0.002 for each) and Bax (p = 0.002 for each) were obtained from BHT-RES group of rats, as followed by BHT and control groups, respectively. Inflammation scores were significantly higher in BHT vs BHT-RES group (2.42 ± 0.53 vs 1.42 ± 0.53; p = 0.001) and both groups had higher inflammation scores than the control group (0.0 ± 0.0; p = 0.001).

CONCLUSION: In conclusion, our findings revealed for the first time that RES exerts promising hepato-ameliorative effects against blunt hepatic injury as evidenced by decreased inflammation scores, parallel with improved hepatic histology, decreased serum transaminase activity as well as enhanced modulatory effect on regenerative and apoptotic processes in RES-treated rats subjected to experimentally-induced blunt hepatic trauma *(Tab. 1, Fig. 1, Ref. 37)*. Text in PDF *www.elis.sk*

KEY WORDS: blunt hepatic trauma; resveratrol; anti-inflammatory; histopathologic; transaminases; apoptosis; rats.

Introduction

Trauma is considered to be the third most common cause of death in all ages and the most common cause of death for individuals younger than 44 years of age (1). The management of blunt abdominal trauma is considered challenging due to a multifaceted picture, including head, thoracic and limb injuries besides the involvement of abdominal organs (2, 3).

The liver is the largest solid organ with highest injury rate in abdominal blunt trauma (4,5) while hepatic injuries occur in 35–45 % of patients with significant blunt abdominal trauma (6). Currently, the non-operative management (NOM), the standard of care in hemodynamically stable patients with blunt hepatic trauma (BHT), involves radiology with contrast-enhanced computerized tomography (CT) and hepatic arterial embolization, intensive care surveillance, and delayed surgery (7, 8). Hepatic traumas can be clearly identified due to technological advances in medical imaging, and together with improved intensive care, NOM has been associated with an estimated success rate exceeding 80–90 % (3, 9, 10).

Accordingly, aiming to reach the optimum recovery time for liver, the substances that offer hepatic healing after blunt abdominal trauma have become increasingly addressed in recent studies (10, 11). Given their potential protective effect against morbid changes related to BHT, the compounds that could be useful as antioxidants and anti-inflammatory agents such as phenolic compounds are gaining more interest in this regard (12). Resveratrol (RES; 3,45 trihydroxystilbene), a phytotoxic polyphenol derivative from plants, is considered to have a significant hepatoprotective potential through antioxidant, anti-inflammatory, and regenerative properties (11–15), alongside cardioprotective, neuroprotective, antidiabetic, and anti-asthmatic activities (16–18).

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RES has been documented to exert a hepatoprotective activity in several types of hepatic injuries such as ethanol-, thioacetamide-, paraquat- and ischemia reperfusion-induced liver damage (19–21), while the potential effects on the modulation of hepatic biomarkers of apoptosis and survival, p53-Bax axis, and B-cell lymphoma 2 (Bcl-2) were reported in animal models of paracetamol-induced (14) and paraquat-induced (22) acute liver injury. Although these findings suggest RES to have a potential to therapeutically intervene with liver injury (18), to the best of our knowledge, the protective effects of RES in an animal model of BHT have not been investigated before.

This study was therefore designed to investigate the protective potential of RES in BHT by exploring anti-inflammatory and histopathologic effects as well as modulatory effects on hepatic biomarkers of acute injury, regeneration, apoptosis and survival in a rat model of BHT.

Fig 1. The trauma platform specifically designed for induction of blunt hepatic trauma.

Methods

Animals

A total of 21 Wistar Albino rats (weighing 120–250 g) were kept in a light- and temperature-controlled room with a 12-h light-dark cycle, temperature of 22 °C and relative humidity of 30–70 %. The animals were fed standard rat pellets and provided with water *ad libitum*. The approval for the study was granted by Firat University Faculty of Medicine Animal Care and Use Committee (Date of Approval/ protocol no: 2010/1657).

Study protocol

The rats were separated into 3 groups (n = 7 for each group), namely control group (CON; standard feeding), BHT group (BHT; blunt hepatic trauma plus observation) and trauma plus BES group (BHT-RES; blunt hepatic trauma plus intraperitoneal injection of 10 mg/kg RES). A 6-h fasting period was applied prior to trauma and surgery.

Blunt hepatic trauma was induced under 40 mg/mL ketamine HCL (Ketalar® Flakon, Eczacibaşi Pharmaceutical Co., Istanbul, Turkey) and 20 mg/mL xylazine HCL (Rhompon® Flakon, Bayer Istanbul, Turkey) anesthesia via a 100-g constant weight, which was dropped from a 40-cm height with 0.784 J of kinetic energy onto the right lateral abdominal wall of the rats fixed on a table by a custom-manufactured platform (Fig. 1).

The rats in each group were followed for 7 days after trauma in separate cages for each group. At the end of the seventh day, the rats were fixed on a table in a supine position under general anesthesia, and laparotomy through a midline incision of 2.5 cm was applied following abdominal shaving and sterilization. After collecting 2-mL blood sample from the *vena cava* for the measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and bilirubin levels with commercial kits, the rats were sacrificed, their livers were totally resected and fixed in 10 % formaldehyde till pathological analysis.

To determine the expression of proliferation marker (Ki-67) and apoptosis-related markers (Bcl-2, and Bax), sections (5 μm) prepared from paraffin blocks of the tissue samples were mounted on poly-l-lysine-coated slides and then stained for Ki-67, Bcl-2 and Bax using automated method (Ventana, USA) to detect Ki-67 expression by immunohistochemistry (SP6; NeoMarkers, Thermo Fisher Scientific Anatomical Pathology, Fremont, USA) with ready-to-use rabbit monoclonal antibody, and to detect Bcl-2 and Bax expression by 100/D5 mouse monoclonal antibody Bcl-2 alpha Ab-1 (1:40, Novocastra, UK) and 2D2 mouse monoclonal antibody Bax Ab1 (1:75, Neomarkers, CA, USA), respectively. The staining pattern was evaluated by the method of Wintzer et al. (23). One hundred and fifty to 500 cells were counted on the slides at ×400 magnification, and the percentage of cells with positive nuclear staining were measured. Sections (5 to 6 μm) were stained with hematoxylin and eosin and the degree of inflammation was graded on a scale from 0 (no inflammation) to 3 (extensive inflammation) by the same pathology expert who was unaware of the experimental groups (10).

Statistical analysis

Statistical analysis was made using IBM SPSS Statistics for Windows, version 11.0 software (IBM Corp., Armonk, NY, USA).

Tab. 1. Hepatic injury and hepatic biomarkers of regeneration, apoptosis and survival in study groups

	CON (n=7)	BHT (n=7)	BHT-RES (n=7)	p value		
				CON vs. BHT	CON vs. BHT-R	BHT vs. BHT-RES
AST (U/L)	59.57±5.96	539.00±58.11	318.57 ±43.66	0.002	0.002	0.002
ALT (U/L)	31.42±6.05	313.57±10.67	113.85±6.12	0.002	0.002	0.002
LDH (U/L)	216.14±22.25	1776.85±23.91	1032.42±60.62	0.002	0.002	0.002
Bilirubin (mg/L)	2.04±0.15	2.24±0.18	2.21±0.13	0.07	0.051	0.745
Inflammation score	0.00	2.42±0.53	1.42±0.53	0.001	0.001	0.001
Ki 67	0.87±0.19	3.78±0.69	6.22±0.74	0.002	0.002	0.002
Bcl 2	0.80±0.20	3.34±0.43	5.84 ±0.25	0.002	0.002	0.002
Bax	0.62±0.12	3.97±0.26	5.71±0.38	0.002	0.002	0.002

Mann–Whitney U test was used to analyze inter-group differences in blood biochemistry values and histopathological scores. Data were expressed as mean± standard deviation (SD) and median (minimum-maximum) where appropriate; $p < 0.05$ was considered statistically significant.

Results

Hepatic injury and hepatic biomarkers of apoptosis and survival in study groups

The highest levels for serum AST ($p = 0.002$), ALT ($p = 0.002$) and LDH ($p = 0.002$) were obtained from BHT group of rats, as followed by BHT-RES and control groups, respectively (Tab. 1).

The highest scores for Ki 67 ($p = 0.002$), Bcl 2 ($p = 0.002$ for each) and Bax ($p = 0.002$) were obtained from BHT-RES group of rats, as followed by BHT and control groups, respectively (Tab. 1).

Inflammation scores were significantly higher in BHT group than in BHT-RES group of rats (2.42 ± 0.53 vs 1.42 ± 0.53; $p = 0.001$) and both groups had higher inflammation scores than the control group (0.0 ± 0.0, $p = 0.001$ for each) (Tab. 1).

Discussion

This study is the first to report that RES ameliorates hepatic injury (lower inflammation scores, decrease in serum transaminase levels) and modulates hepatic biomarkers of regeneration/healing (Ki-67), apoptosis (Bax) and anti-apoptosis/survival (Bcl-2) in an experimental model of BHT.

Higher levels of serum transaminases in BHT and BHT-RES groups than in the control group in our study support the consideration of serum hepatic transaminase concentration as a marker of blunt liver injuries (10). The potential role of RES treatment in amelioration of hepatic damage after blunt trauma was evidenced by significantly improved histological observations, parallel to the diminished serum ALT and AST activities in BHT-RES when compared to BHT group. Similarly, the administration of RES was reported to reduce liver enzymes and to prevent hepatic injury in different models of hepatic injury including diabetic rats (24), PQ-induced hepatic injury (22), paracetamol-induced acute liver injury (14), heat-stress- induced hepatic oxidative damage (25, 26), fructose-induced hepatic injury (27) and ethanol-, thioacetamide-, and ischemia reperfusion-induced liver damage (12, 19–21).

Monoclonal antibodies against Ki-67 antigen are used to assess hepatic healing and degree of hepatic regeneration, while the increase in Ki-67 expression is considered directly proportional to hepatic healing (10, 28). Bax and Bcl-2 are apoptosis-related genes, while Bcl-2 prevents apoptosis by removal of ROS and prevention of calcium release from the endoplasmic reticulum, and the pro-apoptotic gene Bax is considered a dominant inhibitor of Bcl-2 (22, 29).

In this regard, along with lower inflammation scores, the higher Ki-67 and Bcl 2 expression levels in the BHT-RES when compared to BHT group in our study seem to indicate an improved status of hepatic markers of regeneration and anti-apoptosis in RES-treated rats, and thus denote a marked healing potential of RES treatment in experimentally induced BHT model in rats.

RES was reported to be associated with a reduction in oxidative stress via reducing lipid peroxidation (decreased malondialdehyde), increase in antioxidant capacity (increased ferric-reducing ability of plasma) of liver tissue and inhibited production of ROS (15, 30–32). Indeed, agents with antioxidant and anti-inflammatory properties are considered likely to provide protective and antiapoptotic effects against tissue damage (22). Hence, the association of RES with hepatic biomarkers of apoptosis (p53-Bax axis) and survival (Bcl-2) was also reported in an animal model of paracetamol-induced acute liver injury (14). The authors reported a correlation between apoptosis or anti-apoptosis and liver injury biomarkers along with upregulation of the survival protein Bcl-2 and downregulation of the apoptosis regulator Bax gene expression by RES treatment (14). The antioxidant, anti-inflammatory, and anti-apoptotic properties of RES were also reported in the PQ-induced hepatic injury by PQ (22) as well as in heat-stress-induced, paracetamol- induced, CCl4-induced and paraquat-induced acute liver injuries (13, 22, 25, 26, 33).

RES has also been reported to be associated with modulation of apoptosis by enhancing the expression of anti-apoptotic Bcl-2 and suppressing the expression of pro-apoptotic Bax in other organs (34–36).

Notably, in a study of thioacetamide (TAA)-induced hepatic injury in rats, RES was reported to inhibit nuclear factor-kappa B and cytochrome 2E1, whereas it was shown to enhance the apoptosis of necrotic hepatocytes via increasing caspase-3 activity (12). Authors also emphasized that the potential hepatoprotective mechanisms of RES were associated with an inhibition of inflammation, enhancement of apoptosis of necrotic hepatocytes, and suppression of oxidative stress (12). In addition, the anti-apoptotic effect of RES was demonstrated to occur in a dose-dependent manner in an *in vitro* study, indicating an inhibition of apoptosis of the mouse primary hepatocytes and increase in cell viability by relatively higher concentrations of RES (≥ 1 mM) (18, 37). Accordingly, given that not only the expression of Bcl-2 but also that of Bax was significantly increased in RES-treated rats as compared with untreated rats in the current study, the exact impact of RES on apoptotic process needs to be justified in further studies, at least in terms of blunt hepatic injury.

Conclusion

In conclusion, our findings revealed for the first time that RES exerts promising hepato-ameliorative effects against blunt hepatic injury as evidenced by decreased inflammation scores, parallel with improved hepatic histology, decreased serum transaminase activity as well as enhanced modulatory effect on regenerative and apoptotic processes in RES-treated rats subjected to experimentally-induced BHT.

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