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Article

Beta-glucan attenuates cerebral ischemia/reperfusion-induced neuronal injury in a C57BL/J6 mouse model

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Beta-glucans (βg), that have many useful effects on human health, are natural polysaccharides. Our aim in this study was to determine useful effect of βg against oxidative and neuronal damage caused by global cerebral ischemia/reperfusion (IR) in stroke imitated mice via surgical operation. A total of 40 mice divided into four equal groups randomly. The group 1 (sham operated) was kept as control. Bilateral carotid arteries of subjects in group 2 (I/R) and group 4 (I/ R + βg) were clipped for 15 min, and the mice in group 4 (I/R + βg) were treated with βg (50 mg/kg/day), while the mice in group 2 (I/R) were treated with only vehicle for 10 days. The mice of group 3 (βg) were treated with βg for 10 days without carotid occlusion. Global cerebral I/R significantly increased oxidative stress and decreased members of anti-oxidant defense system. In addition, I/R caused histopathological damage in the brain tissue. However, βg treatment ameliorated both oxidative and histopathological effects of I/R. Our present study showed that βg treatment significantly ameliorated oxidative and histological damage in the brain tissue caused by cerebral I/R. Therefore, βg treatment can be used as supportive care for ischemic stroke patients.

Keywords: Global cerebral I/R. Oxidative stress. Neuronal damage. Beta-glucan. C57BL/J6 mice.

INTRODUCTION

The continuation of normal brain function depends on the provision of sufficient oxygen and glucose to the brain through blood flow, and a reduction or block in the brain blood flow can cause fatal brain damage (Ciftci, Oztanir, Cetin, 2014). A severe reduction or block in the brain blood flow occurs in several situations, such as cerebral ischemia, cardiac arrest, and cardiovascular surgery (Park *et al.*, 2016). Stroke is one of the most prevalent causes of death, adult long-term disability, and dementia, and approximately 80% of all stroke cases are ischemic stroke (Zhao *et al.*, 2017). After cerebral ischemia, restoration of perfusion to the ischemic area begins a cascade, causing secondary neuronal damage (Alawieh, Elvington, Tomlinson, 2015). Reperfusion causes an additional injury called ischemia/reperfusion (I/R) injury in the ischemic area. The I/R injury is responsible for cerebral microcirculatory disturbances, such as reactive oxygen species (ROS) outburst, inflammatory mediator overproduction, and leukocyte infiltration (Sun, Fan, Han, 2015). In fact, the pathological process of ischemic stroke has not been completely elucidated yet, but the responsibility of oxidative damage caused by ROS stands out in this process (Oztanir *et al.*, 2014a). Therefore, the ameliorating effects of natural antioxidants against brain damage caused by I/R have been extensively studied (Lalkovičová, Danielisová, 2016). Hesperidin (Oztanir *et al.*, 2014a), 18β-Glycyrrhetinic acid (Oztanir *et al.*, 2014b), and β-myrcene (Ciftci, Oztanir, Cetin, 2014) are some of the antioxidants shown to have beneficial effects against brain damage caused by I/R. However, to the best of our knowledge, beta-glucan (βg) was not tested on I/R-induced brain damage until now.

βgs are natural polysaccharides derived from many mushrooms, fungi, and cereal species, and they have many pharmacological effects, such as immunomodulation, antioxidant, free radical scavenging,

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antibacterial, antifungal, antitumoral, antilipid, antitoxic, and radioprotective activity (Gulmen *et al.*, 2010; Kaya *et al.*, 2016). The beneficial effects of βgs against brain damage induced by various causes have been shown in many previous studies (Alp *et al*., 2012; Kaya *et al.*, 2016; Selli *et al.*, 2015). For example, βg ameliorated both oxidative and histologic brain damage caused by cisplatin, a chemotherapeutic agent (Kaya *et al*., 2016). In addition, some previous studies showed that βgs have useful effects against damage to many organs other than the brain (Bolcal *et al.*, 2007; Gulmen *et al.*, 2010). In this context, the present study aimed to investigate the potential beneficial effect of βg against brain damage caused by I/R. For this purpose, the effect of βg on I/R-induced brain damage was evaluated biochemically and histologically.

MATERIAL AND METHODS

Chemicals

βg was purchased from a pharmacy as IMUNEKS (Mustafa Nevzat Drug Industry, İst, Turkey) in capsule form. Each capsule contains 50 mg of βg obtained from bread yeast. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) and were either analytical grade or the highest grade available.

Animals and experimental protocol

The current study was approved by the Ethical Committee on Animal Research of Inonu University (Date: 27/07/2015 and Protocol No: 2015/A-55) and performed appropriately according to the Guidelines for Animal Research of the National Institutes of Health (NIH). C57BL/6J male mice (clean grade) were provided from Inonu University Laboratory Animals Research Center (Malatya, Turkey). The mice weighing 18–22 g were accommodated in sterilized polypropylene cages and were nourished with standard commercial food pellets and water *ad libitum*. All mice were held under standard environmental conditions (12-h light/dark cycle, 21 ± 2 °C ambient temperatures, and 60 ± 5% humidity). In total, 40 mice were randomly partitioned into four groups (n = 10), and the groups were named group 1 (sham-operated [SH]), group 2 (global cerebral I/R), group 3 (βg), and group 4 (βg + I/R). Mice in the SH and I/R groups were given only an isotonic saline solution for 10 days orally by gavage. In the βg and βg + I/R groups, mice were treated with βg (50 mg/kg/day) orally for 10 days following the I/R procedure. At the end of 10 days, all mice were euthanized under ether anesthesia, and tissue samples were taken for biochemical analyses and histological examination.

Surgical procedure

For the purpose of creating global cerebral ischemia, the method of Yonekura *et al.* (2004) was used. The mice were anesthetized with xylazine (5 mg/kg, intraperitoneal [i.p.]) and ketamine (100 mg/kg, i.p.), and then the cervical midlines of the mice were incised. The bilateral common carotid arteries of the mice in the I/R and βg + I/R groups were separated and blocked simultaneously for 10 min using two vascular miniclips. The surgical procedure was repeated for mice in the SH and βg groups likewise, but their carotid arteries were not clipped. After the operation, all mice were kept in a thermal room to wake up from anesthesia.

Biochemical analyses

The brain tissue was homogenized as explained previously (Kaya *et al.*, 2016). Spectrophotometric methods were used to determine the levels of thiobarbituric acid reactive substances (TBARS) and total glutathione (GSH), along with the activities of catalase (CAT), CuZn-superoxide dismutase (SOD), and glutathione peroxidase (GPx). The TBARS level, an index for lipid peroxidation, was determined according to the method of Yagi (1998). SOD, CAT, GPx, and GSH are members of antioxidant defense systems that protect against oxidative damage (Kaya *et al*., 2016). The GSH level was measured using the method of Sedlak and Lindsay (1968) and expressed as nmol/ml. SOD activity was determined according to the method of Sun, Oberley, and Li (1988). In this method, the amount of protein that reduces the nitro blue tetrazolium reduction rate by 50% expresses one unit of SOD activity. CAT activity was evaluated using the method described by Aebi *et al*. (1974). The decrease in absorbance caused by the enzymatic decomposition of peroxide was measured at 220 nm. The difference in absorbance per unit time was considered a measure of CAT enzyme activity. GPx was measured according to the method of Paglia and Valentina (1967). GPx activity was expressed as IU/mg protein.

Histological assessment

Brain samples were fixed in 10% formalin and were embedded in paraffin. Tissue sections 5 μm in thickness were mounted on slides and stained with hematoxylin and eosin (H&E) for general cerebral structure. The severity of

the damage to the cerebral cortex was semi-quantitatively assessed according to the following: inflammatory cell infiltration, congestion, and eosinophilic degeneration of pyramidal cells.

Microscopic damage was identified as: (0) absent, (1) slight (0–<20% cerebral cortex injury, (2) moderate (21–50% cerebral cortex injury), and (3) severe (50–100% cerebral cortex injury) for each criterion. The sections were examined by a Leica DFC 280 light microscope by a histologist unaware of the status of the animals.

Statistical analysis

All statistical analyses were performed using the SPSS 13.0 computer program (SPSS Inc., Chicago, IL, USA). For the statistical analyses of biochemical values, one-way analysis of variance (ANOVA) and post-hoc Tukey's honestly significant differences test were used, and the degree of significance was designated as $p \leq 0.01$.

RESULTS

Biochemical results

The TBARS, GSH, CAT, GPx, and SOD levels in mice brain tissue are given in Table I. The results indicated that global cerebral I/R (I/R group) induced a significant increase in the TBARS level and a significant decrease in the GSH, CAT, GPx, and SOD levels compared with the SH and βg groups. For all parameters, there were no significant changes between the SH and βg groups. However, βg administration (βg + I/R group) significantly attenuated the TBARS level and significantly increased the GSH and GPx levels compared with the I/R group. However, βg treatment did not significantly improve the CAT and SOD levels. In addition, there was a significant difference between the SH and βg + I/R groups in terms of all parameters except CAT.

Histopathological results

The cerebral cortices of the mice in the sham group showed a normal cerebral structure and pyramidal cells (Figure 1A, B). The βg alone treated group was similar to that of the sham group (Figure 1C, D). However, in the I/R group, the cerebral cortices showed morphological damage. Inflammatory cell infiltration (Figure 2A) and congestion (Figure 2B) were seen in this group. In addition, some of the pyramidal cells showed eosinophilic degeneration. The cells appeared contracted and lost their processes with the eosinophilic cytoplasm with small and darkly stained nuclei (Figure 2C). In the βg + I/R group, although the cerebral tissue preserved its normal histological appearance, mild inflammatory cell infiltration (Figure 3A) and eosinophilic degeneration of the pyramidal cells (Figure 3B) were still marked in some areas.

The results of the histopathological score in all groups were reported in Table II.

DISCUSSION

Ischemic stroke is a global health problem that causes major complications, such as mortality, morbidity, and long-term disability (Oztanir *et al.*, 2014b; Zhao *et al.*, 2017). For this reason, many experimental models mimicking the ischemic stroke have been developed, and many therapeutic agents, such as β-myrcene and hesperidin, have been studied in these models (Ciftci, Oztanir, Cetin, 2014; Oztanir *et al.*, 2014a; Sommer, 2017). To our knowledge, the effects of βg have not been examined in I/R-induced neuronal damage. In this context, the present study investigated the ameliorating effects of βg in the neuronal damage caused by global cerebral I/R in a C57/BL6 mouse model.

Inflammatory reaction, blood-brain barrier disruption, oxidative stress, and neuronal apoptosis constitute major pathological mechanisms of ischemia-induced brain

TABLE I - The levels of SOD, CAT, GPx, GSH and TBARS in brain tissue of mice

	TBARS nmol/g tissue	Reduced GSH nmol/mL	CAT k/mg protein	SOD U/mg protein	GPx U/mg protein
Sham	7.21±0.85[a]	243.4±12.4[a]	0.034±0.001[a]	18.34±1.29[a]	198.1±14.8[a]
I/R	11.9±0.92[b]	184.3±14.1[b]	0.027±0.002[b]	14.60±2.81[b]	154.9±18.3[b]
Betaglucan	7.56±1.02[a]	236.9±10.6[a]	0.032±0.002[a]	19.36±2.06[a]	204.9±14.1[a]
I/R+betaglucan	9.32±0.91[c]	206.8±15.2[c]	0.030±0.004[ab]	15.23±1.38[b]	173.7±16.2[c]

Data were presented as mean± SD. Means bearing different superscripts within same column were significantly different (P<0.01)

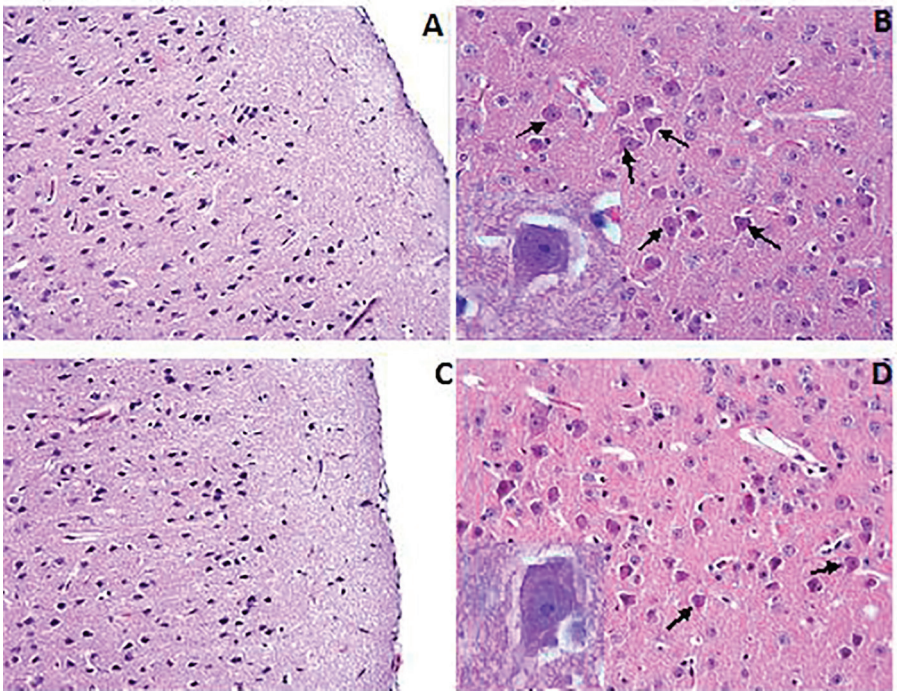

FIGURE 1 - Sham and β group showing normal cerebral cortex (A, C) H-E;X20 and pyramidal cell (*arrows*) (B, D). H-E;X40.

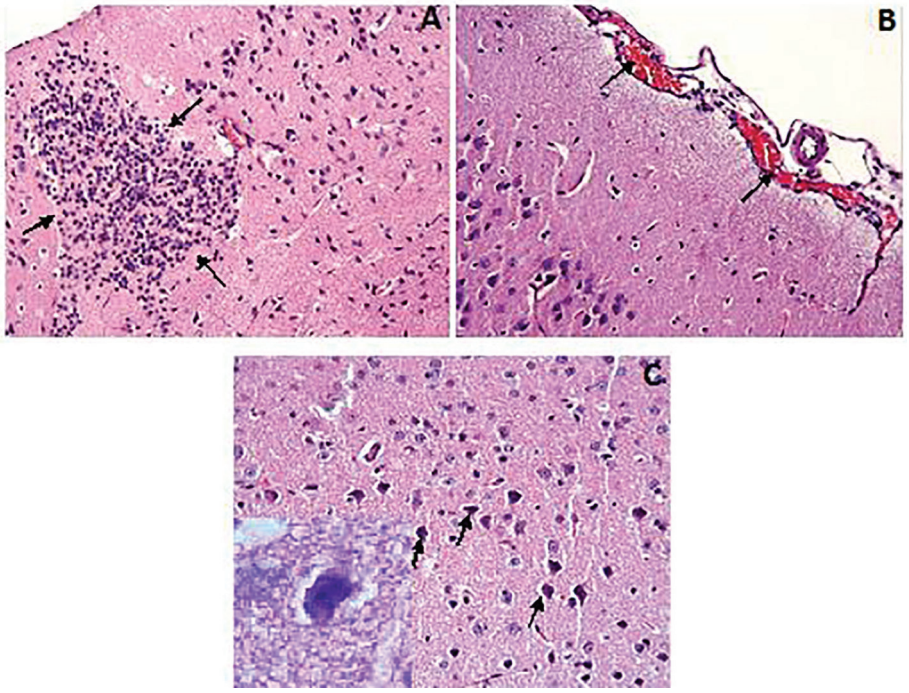

FIGURE 2 - I/R group. (A) notice inflammatuar cell infiltration (*arrows*) H-E;X20 (B) the appearance of congested blood vessels (*arrows*) H-E;X40 (C) the view of eosinophilic degeneration of pyramidal cells (*arrows*) H-E; X40.

damage. Therefore, treatment strategies target these four conditions, and especially antioxidants are successfully used in treatment (Lalkovičová, Danielisová, 2016). Oxidative stress is a cellular imbalance condition between ROS formation and the antioxidant defense system. The restoration of blood flow with reperfusion causes ROS accumulation in the ischemic tissue, and the mechanisms of cell death are triggered (Minutoli *et al.*, 2016). The brain tissue is more susceptible to lipid peroxidation than other tissues. The susceptibility of brain tissue to lipid peroxidation is probably related to its high oxygen demand, low antioxidant capacity, and high methyl ion content

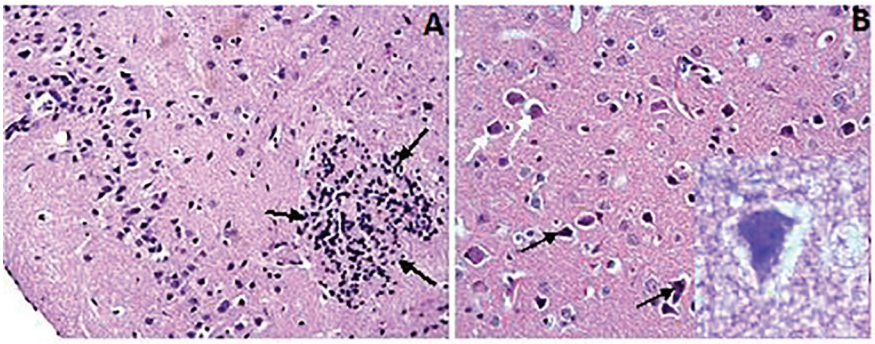

FIGURE 3 - I/R + β group (A) the appearance of mild inflammatuar cell infiltration (*arrows*) H-E;X20 (B) the view of intact pyramidal cells (*white arrows*) and eosinophilic degeneration of pyramidal cells (*black arrows*) H-E; X40.

TABLE II - The results of histopathological score in all groups

Groups	Histopathological score
Sham	0.14±0.14
Betaglucan	0.28±0.18
I/R	1.57±0.20[a]
I/R+Betaglucan	0.85±0.14[b]

Data were presented as mean± SEM. [a]Significant increase ($P < 0.05$), vs. Control group. [b]Significant decrease ($P = 0.05$), vs. I/R group

in some regions of the brain (Lalkovičová, Danielisová, 2016). Therefore, the mechanism of neuroprotection with antioxidants is based on the inhibition of neuronal apoptosis, a reduction in lipid peroxidation, and an increase in the enzymatic and non-enzymatic antioxidant defense systems (Oztanir *et al.*, 2014a).

In the current study, we first evaluated I/R-induced lipid peroxidation by measuring the TBARS level. We observed that global cerebral I/R significantly increased the TBARS level compared with the SH group. Many previous studies about the oxidative status of the brain tissue indicated that I/R caused significant lipid peroxidation (Ciftci, Oztanir, Cetin, 2014; Liang *et al.*, 2015; Oztanir *et al.*, 2014a; Oztanir *et al.*, 2014b; Yu *et al.*, 2016). However, βg treatment after I/R caused a significant decrease in the TBARS level. To our knowledge, the present study is the first to report the effects of βg on I/R-induced brain damage. Therefore, we are unable to compare our obtained results with any other work. Nevertheless, the results of some previous studies that have investigated the neuroprotective effects of βg on the brain tissue are consistent with our results. Alp *et al.* (2012) demonstrated that βg significantly ameliorated lipid peroxidation on the brain and sciatic nerve tissues of diabetic rats. In addition, the study of Şener *et al.* (2005) determined that βg decreased the malondialdehyde level in septic rat brain tissue. In addition, our previous study (Kaya *et al.*, 2016) indicated that βg treatment significantly decreased the TBARS level in cisplatin-damaged brain tissue.

At the same time, in this study, global cerebral I/R caused a significant reduction in the levels of GSH, CAT, SOD, and GPx, which are members of the antioxidant defense system. Similarly, Oztanir *et al.* (2014b) showed that the GSH, CAT, SOD, and GPx levels were significantly reduced due to experimental global cerebral I/R in C57 BL/6J mice. Furthermore, Yu *et al.* (2016) demonstrated that global cerebral I/R significantly decreases SOD and GPx activity in the four brain regions, including the cortex, hippocampus, hypothalamus, and striatum in rats. Our obtained data were consistent with these results and other similar work results (Ciftci, Oztanir, Cetin, 2014; Oztanir *et al.*, 2014a). However, βg treatment after cerebral I/R caused a significant increase in the GSH and GPx levels. The CAT and SOD levels were also increased, without statistical significance. A few previous studies sufficiently demonstrated the booster effects of βgs on the neuronal antioxidant defense system. For example, Şener *et al.* (2005) showed that βg administration significantly ameliorated the GSH level in the brain tissue of septic rats. Our previous study (Kaya *et al.*, 2016) indicated that 50 mg/kg/day of oral βg administration significantly increased the GSH, CAT, SOD, and GPx levels in cisplatin-damaged brain tissue. In addition, it was reported that βg treatment raised both the CAT level and total antioxidant status of the brain and sciatic nerve tissue of diabetic rats (Alp *et al.*, 2012). Furthermore, Kayali *et al.* (2005) indicated that βg did not affect the SOD level in rats with a damaged spinal cord. Moreover, the beneficial antioxidant effects of βg on many other organs and tissues outside the nervous system have been extensively demonstrated in past studies. Dietrich-Muszalska *et al*. (2011) showed that 1 h of incubation with βg diminished the haloperidol-induced TBARS

level by 25% in human plasma. In addition, Bolcal *et al*. (2007) indicated that 2 mg/kg i.p. βg treatment significant increased both the GPx and SOD levels in serum and muscle tissue in the limbs of ischemic rabbits. As well, Agostini *et al*. (2015) demonstrated that βg induced the SOD expression in a human vascular endothelial cell culture under an oxidative microenvironment. Our results are consistent with previous studies in general.

In the present study, we also evaluated the histopathological effects of global cerebral I/R in the brain tissue. Global cerebral I/R caused significant structural changes, such as inflammatory cell infiltration and congestion in the brain tissue. In addition, there was eosinophilic degeneration of some pyramidal cells (I/R group). These findings were consistent with the results of previous studies (Liang *et al.*, 2015; Naderi *et al.*, 2017; Oztanir *et al.*, 2014b; Sharifi *et al.*, 2015). For example, Liang *et al*. (2015) indicated that karyopyknosis caused by I/R decreased the number of neurons and increased the pyknosis ratio. Furthermore, in the study of Yan *et al*. (2017), it was shown that I/R induced atrophy and edema in both hippocampal and cortical brain cells. Moreover, Öztanır *et al*. (2014a) demonstrated that I/R created focal ischemic areas, vascular congestion, mononuclear cell infiltration, and the presence of cytoplasmic shrinkage and extensively dark picnotic neuronal nuclei in the brain tissue. On the other hand, the application of βg after I/R provided a regression in the histopathological damage largely in our present study (βg + I/R group). We could not find any study that histologically investigated the effects of βg on brain damage caused by I/R. However, the results of previous studies investigating the effects of βg against many pathological conditions in the nervous or other systems were largely compatible with our results. For example, Selli *et al.* (2015) showed that βg treatment reduced neural degeneration, the number of neurons with hyperchromatic nuclei, and 8-OHdG-positive cell numbers at the cerebral cortex tissue in post-menopausal rats. In addition, Gulmen *et al.* (2010) showed that βg significantly decreased aortic I/R-induced polymorphonuclear infiltration and intra-alveolar hemorrhage in lung tissue. Furthermore, βg administration caused improvement in histopathological negativities, such as vascular congestion, cell infiltration in the pia mater, and cell infiltration in the cerebral cortex in our previous study (Kaya *et al.*, 2016).

CONCLUSION

The present study clearly demonstrated that 50 mg/kg/day of oral βg administration ameliorated the oxidative and histopathological damage caused by global cerebral I/R in C57BL/J6 mice. It is likely that this effect is due to the antioxidant and radical scavenging properties of βg. For this reason, we claim that βg treatment diminishes global cerebral I/R-induced neuronal damage in the brain.

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