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Effects of putrescine on oxidative stress, spermidine/spermine-N(1)-acetyltransferase, inflammation and energy levels in liver and serum in rats with brain ischemia-reperfusion

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ABSTRACT

We aimed to examine the effects of brain ischemia-reperfusion (IR) especially on serum parameters or liver enzymes, free radicals, cytokines, oxidatively damaged DNA, spermidine/spermine N-1-acetyltransferase (SSAT). The effects of addition of putrescine on IR will be evaluated in terms of inflammation and oxidant-antioxidant balance in liver.

The study was conducted on 46 male Albino Wistar rats weighing 200–250 g. The rats were grouped into: 1-Sham group ($n = 6$). 2-IR group ($n = 8$): The carotid arteries were ligated for 30-min and reperfusion was achieved for 30-min under general anesthesia. 3-Ischemia + putrescine + reperfusion group (IPR) ($n = 8$): Unlike the IR group, a single dose of 250 μmol kg^{-1} putrescine was given by gavage at the beginning of reperfusion. In putrescine treatment groups in addition to the procedures performed in the IR group a total of 4 doses of 250 μmol kg^{-1} putrescine were given at 12-h intervals, with the first dose immediately after 30-min reperfusion (4-IR+putrescine group (IR+P1) ($n = 8$)); 3 h after the 30-min

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reperfusion (5-IR+putrescine group (IR+P2) (n = 8)); 6 h after the 30-min reperfusion (6-IR+putrescine group (IR+P3) (n = 8)). ALT, AST, ATP, NO, SSAT, 8-OHdG levels were analyzed in the serum, and liver samples. NF-κB and IL-6 levels were analyzed in the liver samples.

Brain IR causes inflammatory, oxidative and DNA damage in the liver, and putrescine supplementation through gavage reduces liver damage by showing anti-inflammatory and antioxidant effects.

KEYWORDS

ATP, Brain Ischemia-Reperfusion, liver, IL-6, NF-κB, NO, putrescine, rat, SSAT, 8-OHdG

INTRODUCTION

Ischemia is a condition that occurs with insufficient blood supply to the organs [1]. Reperfusion occurs when the ischemia is removed spontaneously or with the treatment. It is well known that ischemia-reperfusion (IR) causes serious damage to the brain, and causes changes such as status of apoptosis, oxidative stress, and inflammation in the brain [2].

Wesley et al. [3] indicated that major metabolic changes are seen during the early acute phase of injury following an ischemic stroke in the brain (at day 2 of IR). These changing occur sequentially during extended reperfusion time of the late repair phase in a tissue-specific manner. Then, alterations in circulating metabolites in plasma occur in the brain due to local injury during acute-repair phase transition time (from day 2 until 1 weeks of IR). Finally, the delayed systemic effect is seen in the peripheral organ, such as liver that exhibits significant and persistent changes in these metabolites during later reperfusion time (from day 3 until 2 weeks of IR) [3].

Brain IR duration has different effects on the liver. Whereas 2 h middle cerebral artery occlusion (MCAO)-24 h reperfusion did not cause a significant change in alanine transaminase (ALT) and aspartate transaminase (AST) levels, it caused a decrease in the levels of superoxide dismutase (SOD) [4]. Yang et al. [5] determined that 2 h MCAO-3 hours reperfusion increased serum ALT and AST, and did not cause a significant changes in SOD.

In a study, performed 24 h or 5 days after the temporary brain ischemia stimulated by 4-vessel occlusion, SOD levels of liver were lower both at 24 h, and 5 days after ischemia than the control group. Furthermore, serum AST levels were higher only at 24 h after ischemia [6].

Other than the brain, studies have been carried out on the different organs. The effects of related organ IR on liver has been indicated in most of the studies [7–10] that IR causes an increase in serum ALT and AST levels.

The studies showed that abdominal aorta ligation-reperfusion increased portal inflammation, periportal and intralobular necrosis [7], intestinal IR reduced liver SOD levels [9]. Furthermore, bilateral renal ischemia for 30 min-4 hours of reperfusion caused a decrease in liver SOD levels, and an increase in serum ALT levels [11].

The effects of IR on inflammation markers were found as follows: 30 min of intestinal ischemia-15 min of reperfusion increased liver interleukin-6 (IL-6) and nuclear factor kappa B (NF-κB) levels [9], 30 min infrarenal aortic occlusion-90 min reperfusion caused an increase in serum interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) levels [8]. Various time of application of renal IR caused an increase in liver IL-10 and TNF-α levels [10].

In addition, reperfusion of the femoral arteries caused an increase in plasma IL-6 levels [12]. 2 h of MCAO-24 h of reperfusion caused an increase in serum TNF-α and IL-1 levels [4].

Putrescine as a polyamines, plays a role in cell growth and development, cell differentiation and proliferation, and regulation of transcription and translation [13]. Spermidine/Spermin N1-acetyl transferase (SSAT) is an important enzyme in polyamine metabolism [14] which transfers the acetyl group to the aminopropyl groups of spermidine and spermine [15]. Zoli et al. [16] determined that increased SSAT expression may contribute to the increases in putrescine and decreases in spermidine levels after ischemia. SSAT is an important mediator that causes tissue damage in kidney and brain IR [16–18]. Hypoxia in the heart can indirectly stimulate SSAT expression by decreasing adenosine triphosphate (ATP) levels [19]. A significant increase in liver SSAT mRNA levels was observed in rat bilateral kidney IR [11]. Renal IR increased significantly the liver SSAT mRNA expression [10].

High SSAT levels are strongly related with low ATP levels [15]. It is known that SSAT induces polyamine oxidation which contributes to neuronal damage [14]. Starting 3 days before liver IR and up to 6 days after reperfusion, 250 μmol kg^{-1} day gavage of polyamines reduces the damage through reducing the serum IL-1β, IL-6, TNF-α levels and apoptosis [20].

Seen from this aspect, the number of studies investigating the effect of cerebral IR on the liver is limited so that the underlying mechanisms are still not fully explained. Therefore, the aim of this study is to examine the effects of brain IR especially on the liver and the changing in SSAT, ATP, 8-hydroxy deoksiguanosine (8-OHdG), ALT, AST, and total nitrate/nitrite (NO) levels in the serum, and liver; and NF-κB, and IL-6 levels in the liver. The effects of putrescine supplementation on brain IR will be evaluated in terms of inflammation and oxidant-antioxidant balance in the liver. We aimed to investigate the positive-negative effects of putrescine supplementation in the liver as a distant organ in brain IR-model. The other focus of the study was to determine the potential effects of putrescine supplemented at different time interval following the brain IR.

MATERIALS AND METHODS

This study supported by the Selcuk University Scientific Research Projects Coordinatorship with the project number 20401001, was conducted on 46 male Albino Wistar rats (200–250 g), with approvement Ethics Committee of Selcuk University Experimental Medicine Application and Research Center (no: 2019-27).

Surgical procedure

Intraperitoneal injection of ketamine HCl (60 mg kg^{-1}) and xylazine (5 mg kg^{-1}) was used to induce general anesthesia in all surgical procedure. A ventral incision was made in the midline of the neck using the method of Oz et al. [21]. The carotid arteries carefully separated from the peripheral tissues, and vagus nerves, were ligated to induce ischemia. The threads were untied to create reperfusion. The ischemia/reperfusion were visually confirmed.

Putrescine supplementation

Putrescine dihydrochloride (catalog no: Sigma P7505, Saint Louis, MO 63103, USA) was dissolved in distilled water, and applicated by intragastric gavage with stainless steel gavage applicator at a dose of 250 μmol kg^{-1} with adding saline (V/V:1/1) [1, 20].

Design of groups

Sham group ($n = 6$): The carotid arteries were isolated. No other action was taken.

IR group ($n = 8$): Carotid arteries were ligated for 30 min, and reperfusion was achieved for 30 min.

Ischemia+putrescine+reperfusion group (IPR) ($n = 8$): Unlike the IR group, a single dose of 250 µmol kg^{-1} putrescine was given by gavage at the beginning of reperfusion. The other procedures were the same.

In putrescine treatment groups, in addition to the procedures performed in the IR group, a total of 4 doses of 250 µmol kg^{-1} putrescine were given at 12-h intervals, with the first dose immediately after 30-min reperfusion (IR+putrescine group (IR+P1) ($n = 8$)); 3 h after the 30-min reperfusion (IR+putrescine group (IR+P2) ($n = 8$)); 6 h after the 30-min reperfusion (IR+putrescine group (IR+P3) ($n = 8$)) (Fig. 1).

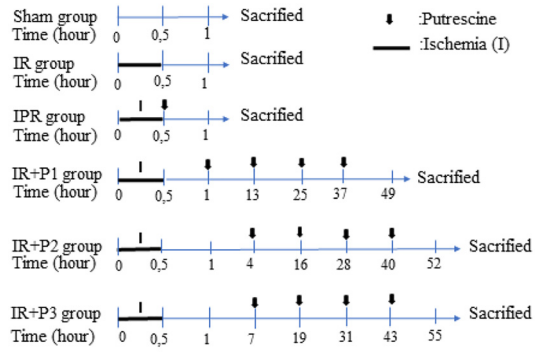
Then after the 30-min reperfusion in the IR, and IPR groups, and 12 h after the final dose of putrescine in the IR+P1, IR+P2, and IR+P3 groups, the rats were sacrificed by cervical dislocation under general anesthesia and blood samples were taken via intracardiac route.

Sample preparation

The weight of the liver tissues were recorded. Then they were placed in a homogenizer (Misonix Microscan ultrasonic tissue shredder) and homogenized at 4 °C with 1/10 PBS (0.01M, pH = 7.4, Sigma, P-4417, Germany). The obtained homogenates were centrifuged at 3,000 rpm for 15 min (Allegra X-30, Beckman Coulter, Turkey). After centrifugation the supernatant was obtained for the analysis. Blood samples were centrifuged at 3,000 rpm for 10 min. Serum samples were obtained. All the serum and liver supernatants were stored at −80 °C until analysis.

Biochemical analysis

ALT, AST, ATP, NO, SSAT, 8-OHdG levels were analyzed in serum, and liver samples. NF-κB and IL-6 levels were analyzed in liver samples. Measurements of ALT, AST, ATP, SSAT, 8-OHdG, NF-κB and IL-6 were performed with ELISA methods by Rayto Microplate Elisa



The timeline of experimental procedures and intraperitoneal injection of putrescine

Fig. 1. The timeline of experimental procedures and intraperitoneal injection of putrescine

washer (RT-2600; Shenzhen, China) and the ELISA reader BMG Labtech (Germany). NO levels were analyzed by colorimetric method with the device BMG Labtech (Germany). We calculated the liver tissue's values as per gram tissue in the study. Therefore, we divided the final values to the weight's of the liver.

The commercial ELISA test kits (BT-Lab, Shanghai China) used in the experiments as follows; ALT (catalog no:E0155Ra) both in serum (U/L) and liver (U/g tissue), AST (catalog no:E0594Ra) both in serum (U/mL) and liver (U/g tissue), ATP (catalog no:E0920Ra) in serum (ng mL^{-1}) and liver (ng g^{-1} tissue), IL-6 (catalog no:E0135Ra) in liver (ng g^{-1} tissue), NF-κB (catalog no:E0287Ra) in liver (ng g^{-1} tissue), the SSAT (catalog no:E2456Ra) in serum (ng mL^{-1}) and liver (ng g^{-1} tissue), 8-OHdG (catalog no:E0031Ra) in serum (ng mL^{-1}) and liver (ng g^{-1} tissue). The commercial colorimetric test kit was used to analyze NO (Cayman (catalog no:780001) Ann Arbor, MI, USA) in serum (μmol L^{-1}) and liver (μmol g^{-1} tissue).

Statistical analysis

The statistical analysis was performed using the SPSS 22.0 package program (Armonk, NY: IBM Corp.). To determine the homogeneity of the data, the "Shapiro-Wilk" test was used. One-way analysis of variance (ANOVA) test was used to determine the differences between the groups, and the 'Tukey' test which is one of the Post Hoc tests, was used to determine in which group the difference originated from. Difference at the $P < 0.05$ level was considered significant.

RESULTS

Result of ALT and AST measurement

There was a significant decrease in the serum AST levels of the IR+P2, and IR+P3 group when compared to the sham group. Serum ALT levels were significantly lower in the IR+P1 group than sham, IR, IR+P2, and IR+P3 group. In the liver, 4 doses of putrescine supplementation, significantly reduced liver ALT and AST levels compared to the sham group. But these levels did not differ in the IR group and putrescine supplemented groups except the IR+P3 group according to AST level. Furthermore, just only a single dose of putrescine before reperfusion significantly decreased liver AST levels when compared to the sham group (Table 1).

Table 1. AST, and ALT levels of liver and serum

Groups	*N*	Serum AST levels (U/mL)	Serum ALT levels (U/L)	Liver AST levels (U/g tissue)	Liver ALT levels (U/g tissue)
Sham	6	14.81 ± 5.26^{a}	7.40 ± 2.36^{a}	58.89 ± 6.31^{a}	23.96 ± 4.35^{a}
IR	8	11.98 ± 5.81^{ab}	7.14 ± 1.73^{a}	51.10 ± 13.42^{ab}	19.04 ± 4.29^{ab}
IPR	6	13.83 ± 3.94^{a}	6.42 ± 1.56^{ab}	44.91 ± 9.59^{b}	19.40 ± 3.89^{ab}
IR+P1	8	11.57 ± 4.12^{ab}	6.18 ± 2.08^{b}	47.15 ± 9.72^{b}	18.03 ± 4.15^{b}
IR+P2	8	8.07 ± 4.65^{b}	6.98 ± 2.23^{a}	37.14 ± 13.63^{bc}	16.50 ± 2.14^{b}
IR+P3	8	7.72 ± 3.48^{b}	7.45 ± 1.91^{a}	34.80 ± 10.03^{c}	17.82 ± 5.05^{b}

a > b > c: The difference between the means between groups with different letters in the same column is significant ($P < 0.05$).

Results of ATP measurement

While the liver ATP levels decreased due to cerebral IR, a single dose of putrescine supplementation at the beginning of the reperfusion increased this level, but it did not show significance. However, the initiation of 4 doses of putrescine supplementation immediately after reperfusion significantly increased ATP levels. Initiation of supplementation after 3 h or 6 h did not cause significant changes in ATP levels (Table 2).

A single dose of putrescine at the beginning of reperfusion significantly increased serum ATP levels compared to the IR group. When compared to the IR group, initiation of 4 doses of putrescine supplementation immediately after reperfusion or 3 h after reperfusion, increased the serum ATP levels. But, it was not a significant increase. Putrescine supplementation 6 h after reperfusion did not cause a significant change in the serum ATP levels (Table 2).

Results of SSAT measurement

While brain IR caused a significant increase in liver SSAT levels, it was decreased in the putrescine supplemented groups compared to the IR group. Serum SSAT levels did not show a significant increase in the IR group. It was decreased significantly in groups received 4 doses of putrescine compared to the IR group (Table 2).

Results of NO measurement

Brain IR significantly increased serum NO levels. Also IR led to an unsignificant increases in liver NO levels when compared to the sham group. However, a significant decrease was found in all putrescine treated groups except the IPR group compared to the IR group. Both NO levels of serum and liver did not differ in different time periods of putrescine treated group from each other (Table 3).

Results of 8-OHdG measurement

There was a significant increase in the level of 8-OHdG both in liver and serum of the IR group compared to the IR+P2, and IR+P3 group. Oxidative DNA damage was decreased in both serum and liver by the putrescine supplementation. The lowest levels were seen in the IR+P2 group both of liver and serum (Table 3).

Table 2. ATP, and SSAT levels of liver, and serum

Groups	*N*	Liver ATP levels (ng g^{-1} tissue)	Serum ATP levels (ng mL^{-1})	Liver SSAT levels (ng g^{-1} tissue)	Serum SSAT levels (ng mL^{-1})
Sham	6	168.43 ± 77.65^{a}	37.06 ± 5.96^{ab}	1.60 ± 0.34^{b}	0.59 ± 0.18^{ab}
IR	8	86.76 ± 6.24^{b}	31.31 ± 7.87^{b}	2.74 ± 0.83^{a}	0.73 ± 0.12^{a}
IPR	8	128.47 ± 34.96^{ab}	41.03 ± 9.16^{a}	1.71 ± 0.60^{b}	0.58 ± 0.12^{ab}
IR+P1	8	164.28 ± 23.20^{a}	38.02 ± 6.91^{ab}	1.69 ± 0.61^{b}	0.54 ± 0.11^{b}
IR+P2	8	96.72 ± 8.31^{b}	33.41 ± 7.03^{ab}	1.33 ± 0.24^{b}	0.43 ± 0.03^{bc}
IR+P3	8	155.19 ± 22.51^{ab}	28.88 ± 7.13^{b}	1.51 ± 0.39^{b}	0.49 ± 0.10^{bc}

a > b > c: The difference between the means between groups with different letters in the same column is significant ($P < 0.05$).

Table 3. NO, and 8-OHdG levels of liver, and serum

Groups	*N*	Liver NO levels (μmol g^{-1} tissue)	Serum NO levels (μmol L^{-1})	Liver 8-OHdG levels (ng g^{-1} tissue)	Serum 8-OHdG levels (ng mL^{-1})
Sham	6	18.98 ± 2.87^{ab}	0.24 ± 0.14^{b}	2.97 ± 0.47^{ab}	0.49 ± 0.10^{ab}
IR	8	21.17 ± 1.60^{a}	0.48 ± 0.09^{a}	3.10 ± 0.84^{a}	0.59 ± 0.10^{a}
IPR	8	16.07 ± 5.21^{ab}	0.39 ± 0.027^{ab}	2.20 ± 0.15^{b}	0.50 ± 0.07^{ab}
IR+P1	8	14.48 ± 2.61^{bc}	0.28 ± 0.17^{b}	2.60 ± 0.52^{ab}	0.39 ± 0.13^{b}
IR+P2	8	15.98 ± 2.65^{b}	0.31 ± 0.16^{b}	2.06 ± 0.49^{b}	0.29 ± 0.11^{b}
IR+P3	8	15.14 ± 2.61^{bc}	0.30 ± 0.14^{b}	2.23 ± 0.45^{b}	0.37 ± 0.20^{b}

a > b > c: The difference between the means between groups with different letters in the same column is significant ($P < 0.05$).

Results of IL-6 and NF-κB measurement

IL-6 and NF-κB levels of the liver were higher in IR group than the levels of sham group. While a single dose of putrescine at the beginning of the reperfusion did not cause a change in the level of IL-6, which is increased by IR, starting 4 doses of putrescine immediately after reperfusion and 3 h after the reperfusion caused a significant decrease in IL-6 levels (Table 4). A single dose of putrescine at the beginning of the reperfusion, and initiation of 4 doses of putrescine immediately after reperfusion or 3 h after reperfusion caused a significant decrease in the NF-κB levels (Table 4).

DISCUSSION

There is not enough knowledge about of putrescine treatment and its metabolic effects on SSAT levels in IR model. In the present study, the metabolic effects of putrescine supplementation in the IPR, IR+P1, IR+P2, IR+P3 groups have been tried to explain comprehensively.

A study determined that 2 h of MCAO-3 hours of reperfusion increased levels of ALT and AST in serum [5]. In another study, 2 h of MCAO-24 h reperfusion did not show any important changing in serum ALT and AST levels [4]. In our study by using bilateral common carotid artery occlusion model, we identified different response in liver, and serum and provided evidence for systemic effects of brain IR. The results of the study showed that the levels of AST in

Table 4. IL-6, and NF-κB levels of liver

Groups	*N*	IL-6 levels (ng g^{-1} tissue)	NF-κB levels (ng g^{-1} tissue)
Sham	6	2.79 ± 0.60^{b}	2.55 ± 0.29^{b}
IR	8	4.60 ± 1.30^{a}	3.45 ± 1.02^{a}
IPR	8	4.42 ± 1.22^{a}	2.41 ± 0.27^{b}
IR+P1	8	3.06 ± 0.70^{b}	2.51 ± 0.59^{b}
IR+P2	8	2.81 ± 0.70^{b}	2.30 ± 0.37^{b}
IR+P3	8	3.87 ± 0.87^{ab}	3.05 ± 0.73^{ab}

a > b: The difference between the means between groups with different letters in the same column is significant ($P < 0.05$).

both serum and liver of the IR+P2 and IR+P3 group were significantly lower than the sham group. In this case, we can emphasize that the addition of putrescine did not cause any damage to the liver in terms of any enzyme levels and did not cause toxicity by evaluating liver functional biochemical tests. We concluded that even in the treatment that started 3 and 6 h after IR, the AST levels of the liver functions were processing properly, and the functional losses caused by IR were straightened. The effect of putrescine on serum biochemical parameters was independent of the given time of the application. As it seen, putrescine applied in different time periods after IR did not show a time-dependent difference. AST levels in the liver were lower in the IR+P3 group compared to the IR group. The AST and ALT levels were not affected by time-dependent putrescine administration. Therefore, the putrescine supplementation did not cause a necrotic status in the liver when it was evaluated in general terms of biochemical aspects, which indicates that distant tissue damage is not in this context.

In our study, brain IR caused a significant increase in liver SSAT levels, while it caused a decrease in the putrescine supplemented groups compared to the IR group. These results are in agreement with previous studies. Moreover, it was determined that liver SSAT mRNA levels were increased in renal IR [10, 11]. It is also known that SSAT level increases in organs where IR occur. Kidney [11, 17], liver [18] and brain [16] IR causes an increase in SSAT expression. Oral supplementation of putrescine provides protection against liver damage [22]. In our study, the serum SSAT levels insignificantly increased in the IR group, however, a significant decrease was found in the putrescine supplementation groups compared to the IR group. Therefore, we conclude that the levels of SSAT in the liver change independently of the time of administration and that the polyamine metabolism in the early period after brain IR may be regulated according to the production of putrescine. Externally given putrescine also makes an important contribution to this metabolic process.

As it indicated that spermidine, one of the polyamines, plays a protective role against liver IR [23]. So, acetyl polyamines are crucial markers in determining SSAT activity, and their accumulation is related to their degradation which is done by acetyl polyamine oxidase [15]. In addition, high concentrations of polyamines and polyamine analogs increase transcription and translation, while the degradation of SSAT protein decreases [15]. When evaluated as a whole, it can be said that putrescine supplementation significantly reduces SSAT levels in serum and liver, which are increased by IR.

While liver tissue of ATP levels were decreased due to cerebral IR, a single dose of putrescine supplementation at the beginning of the reperfusion increased unsignificantly this level. However, initiation of putrescine supplementation immediately after reperfusion significantly increased the level of ATP. Serum ATP levels did not increase significantly in the IR group, but a single dose of putrescine at the beginning of the reperfusion significantly increased the ATP level compared to the IR group. Initiation of supplementation after 3 h or 6 h did not cause significant changes in ATP levels. Increased levels of ATP were found in the IPR group compared to the IR group and the difference was statistically significant. Likewise, the difference in the ATP levels between the IPR and IRP groups was statistically significant. Since serum ATP levels reflect the energy metabolism in all tissues, moreover, it is also seen in our findings that serum levels differed from the energy changes and supports our idea. Ischemia causes depletion of energy sources, especially ATP, which is essential for cellular homeostasis; and ultimately leads to ion imbalance on the cell membrane [24, 25]. In addition, Pegg [15] indicated that SSAT plays a key role in changes in ATP levels. Based on this information, low ATP levels in the IR

group are an indication of decreased ATP production which are the result of irregularity in the blood supply and oxygenation of the tissues. Ryu et al. [19] demonstrated that SSAT was up-regulated mainly by ATP depletion in hypoxia in the heart. In accordance with this knowledge our findings also support the relation between ATP and SSAT levels. Whereas both liver and serum SSAT levels were high in the IR group, ATP levels were low.

In the present study, significantly lower serum NO levels were found in the sham group compared to the IR group. The liver and serum NO levels were decreased in groups supplemented 4 doses of putrescine when compared to the IR group ($P \leq 0.05$). The NO levels of the liver were below the value measured in the sham group. These findings demonstrate that exogenous putrescine administration has a time-independent effect on brain IR regarding to liver NO levels, and there is no difference between supplementation immediately, 3 h, or 6 h after reperfusion. As a result, putrescine supplementation took an important role in decreases of total NO values. Therefore, the addition of putrescine did not cause an increases in the oxidant damage of the liver. This study indicated that forward reaction of superoxide oxygen radicals and ONO_2-(nitrite) decreased the levels of NO_3-(nitrate), and radical formation is reduced by the supplementation of putrescine.

8-OHdG is a biomarker of this oxidative DNA damage [26]. Hepatic IR increases 8-OHdG levels in the liver [27]. As it shown in Table 3, there was an insignificant increase in the level of 8-OHdG in the liver due to brain IR. A single dose of putrescine supplementation at the beginning of the reperfusion, and 4 doses of putrescine after 3 h or 6 h after reperfusion reduced 8-OHdG levels below the sham level ($P < 0.05$). Only the difference between the IR and IPR group was found to be insignificant among the putrescine supplemented groups in serum. Although 8-OHdG levels were not increased significantly in serum of the IR group, a significant decrease was found with putrescine supplementation compared to the IR group. Kawabori and Yenari [28] indicated that when reaction occurs between NO and superoxide, it forms peroxynitrite and furthermore directly causes DNA damage. Thus, pharmacological inhibition of iNOS leads to improved neurological outcomes. In a study it has been shown that 8-OHdG levels increased significantly in plasma in brain IR [2].

It is known that inflammation in the liver increases due to IR of the intestine [9] and kidney [10]. Parallel to these studies, in our study, brain IR caused an increase in inflammation in the liver as a distant organ. Supplementation of 4 doses of putrescine starting immediately or 3 h after reperfusion caused a significant decrease in the IL-6 and NF-κB levels compared to the IR group. Putrescine supplementation starting 6 h after reperfusion did not cause a significant decrease in the IL-6 and NF-κB levels compared to the sham group. Based on this, the importance of timing of putrescine supplementation is important. We concluded that the anti-inflammatory response decreases when the first dose administration time of putrescine is delayed. A single dose of putrescine supplementation at the beginning of reperfusion did not cause a change in the IL-6 level but decreased NF-κB levels significantly. That is, the sooner time dosing of putrescin is started from the onset of reperfusion, the more inflammation can be suppressed.

As a result, increased DNA damage, SSAT and NO levels were determined in the liver and serum in brain IR model. In the experimental groups in which putrescine was applied with constant doses at different time intervals, DNA damage and inflammation decreased, SSAT levels and the formation of superoxide radicals reduced. Our results show that putrescine supplementation has an independent effect from the time of application. Even in the group supplemented with putrescine 6 h after IR (IR+P3), putrescine reduced the tissue damage as like

the first group that exogenous putrescine was administered (IPR), prevented inflammation and protected the tissues from radical damages. In terms of the relationship with SSAT levels, a significant decrease was observed in the IPR and IR+P3 groups compared to the IR group. Brain IR increased inflammation and SSAT levels in the liver, caused a decrease in ATP levels, and increased serum NO levels. Besides, the putrescine supplementation by gavage reversed the effects of IR, reduced inflammation, SSAT, and NO levels, suppressed DNA damage, reduced liver damage and improved its functions, while increasing ATP levels. Additionally, we concluded that the changes in ALT and AST levels, which are a marker of liver functions, did not cause damage to the liver with the addition of putrescine and that there was no acute inflammatory condition when evaluated in terms of serum values.

CONCLUSION

Brain IR causes inflammatory, oxidative and DNA damage in the liver, and putrescine supplementation through gavage reduces the liver damage by exhibiting anti-inflammatory and antioxidant effects.

Conflicts of interest: The authors declare no conflict of interest.

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ABBREVIATIONS

ALT	Alanine transaminase
AST	Aspartate transaminase
ATP	Adenosine triphosphate
IL-1β	Interleukin-1β
IL-6	Interleukin-6
IR	Ischemia-reperfusion
IPR	Ischemia + putrescine + reperfusion group
IR+P1	IR+ putrescine group 1
IR+P2	IR + putrescine group 2
IR+P3	IR + putrescine group 3
MCAO	Middle cerebral artery occlusion
NF-κB	Nuclear factor kappa B
SOD	Superoxide dismutase
SSAT	Spermidine/spermin N1-acetyl transferase
TNF-α	Tumor necrosis factor-α
8-OHdG	8-hydroxy deoksiguanosine

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