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Different doses of dexmedetomidine reduce plasma cytokine production, brain oxidative injury, PARP and caspase expression levels but increase liver oxidative toxicity in cerebral ischemia-induced rats

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ABSTRACT

Cerebral ischemia-induced progression of brain, liver, and erythrocyte oxidative injuries might be modulated by dexmedetomidine (DEX) as a potent antioxidant and anti-inflammatory drug. The present study was conducted to explore whether two different doses of DEX protect against plasma cytokine and brain, liver and erythrocyte oxidative toxicity and apoptosis in cerebral ischemia-induced rats.

Forty-two rats were equally divided into 7 groups. The first and second groups were used as untreated and sham controls, respectively. The third (DEX4) and fourth (DEX40) groups received 4 mg/kg and 40 mg/kg DEX treatments. The fifth, sixth and seventh group were operated on to induce cerebral ischemia. The fifth, sixth and seventh groups are used to represent cerebral ischemia, cerebral ischemia + DEX4, and cerebral ischemia + DEX40, respectively. DEX was intraperitoneally given to the DEX groups at the 3rd, 24th and 48th hour.

Brain and erythrocyte lipid peroxidation (MDA) levels and plasma IL-1β and TNF-α levels were high in the cerebral ischemia group although they were low in the DEX4 and DEX40 groups. Decreased glutathione peroxidase (GSH-Px) and reduced glutathione (GSH) values in the brain and erythrocyte of the cerebral ischemia group were increased by the DEX treatments, although PARP, and the caspase 3 and 9 expressions in the brain were further decreased. Conversely, the cerebral ischemia-induced decrease in the liver vitamin A, vitamin E, GSH, and GSH-Px were further decreased by the DEX treatments, although MDA level, PARP, and caspase 3 and 9 expressions were further increased by the DEX treatments.

In conclusion, DEX induced protective effects against cerebral ischemia-induced brain and erythrocyte oxidative injuries through regulation of the antioxidant level and cytokine production. However, both doses of DEX induced oxidative toxicity and apoptotic effects in the rats' livers.

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1. Introduction

Cerebral ischemia-induced injury is a major cause of death and disability in adults and newborns. The long-term outcome for the brain in cerebral ischemia-induced injury includes neuronal diseases such as cerebral palsy, epilepsy, and mental retardation (Lynch and Nelson, 2001). Tissue damage occurs in ischemia through energy depletion, accumulation of toxic metabolic products and activation of phospholipase (Cai et al., 2014; Kumar et al., 2014). Reperfusion leads to further neuronal damage through generating reactive oxygen species (ROS). In addition to a poor enzymatic antioxidant defense system, brain and erythrocytes have a high rate of oxygen consumption and a rich content of polyunsaturated fatty acids (PUFAs). Because of the three reasons (their high rate of oxygen consumption, high content of PUFAs and poor enzymatic antioxidant scavenger system), the brain and erythrocytes exhibit increased vulnerability to ischemia-induced oxidative stress (Nazıroğlu, 2011; Yang et al., 2014). The liver is also essential for detoxification of ROS and cytokine productions. Hence, liver is also one of the main defense systems against ischemia-induced excessive production of ROS and inflammation in the body (Gong et al., 2012; Gul et al., 2015).

Abbreviations: DEX, dexmedetomidine; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; IL-1β, interleukin-1β; ISC, ischemia; MDA, malondialdehyde; NF-kB, nuclear factor kappa B; PARP, poly (ADP-ribose) polymerase; ROS, reactive oxygen species; TBAR, thiobarbituric-acid; TNF-α, tumor necrosis factor-alpha.

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Dexmedetomidine (DEX) as a selective agonist of α2-adrenoreceptor has analgesic, anxiolytic and sedative effects. Postsynaptic adrenergic activity is decreased by selective binding DEX to presynaptic α2 adrenergic receptors. In addition to its reducing postsynaptic activity, accumulating evidence from studies proved that DEX plays a key role in the ROS production through regulation of antioxidant redox and anti-inflammatory systems in the pathogenesis of oxidative brain injuries (Cai et al., 2014; Akpınar et al., 2016). Furthermore, recent experiments showed plasma cytokine and brain oxidative stress levels were diminished in spinal cord injury-induced rats after DEX treatment (Aslan et al., 2009). DEX is mainly metabolized in the liver and is induced in rat liver during the metabolize process (Gul et al., 2015). However, there is no report of DEX on plasma cytokine production, apoptosis and oxidative stress levels in the brain, erythrocyte and liver of cerebral ischemia-induced rats. The recommended safe and effective clinical dosage range of DEX is 0.5–1 μg/kg/hour and 1–25 μg/kg in humans (Li et al., 2015; Luo et al., 2016; Ren et al., 2016) and rats (Aslan et al., 2009; Cakir et al., 2015; Gul et al., 2015), respectively. It was recently reported that high doses of DEX have been used to achieve adequate sedation in patients (Mason et al., 2008; Chen et al., 2015). More recently, we observed neuroprotective action of a moderate dose of DEX on apoptosis and oxidative stress in dorsal root ganglion and hippocampus of cerebral ischemia-induced rats (Akpınar et al., 2016). In turn, there may be related to oxidative stress and cytokine production in the high doses of DEX. Further research is needed on this subject.

The generation of ROS induced by ischemia-reperfusion injury, plays a central role in the pathogenesis of oxidative brain injury (Engelhard et al., 2002) and these radicals can stimulate the cascade of cytokines through nuclear factor kappa B (NF-kB) activation (Sifringer et al., 2015). The release of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β) induces chemokine synthesis in ischemic tissues (Frangogiannis, 2007). IL-1β has been reported to contribute to brain failure and apoptosis through ischemia stimulation (Engelhard et al., 2002). Furthermore, IL-1β attracts macrophages which can act as an extra source of IL-1β and ROS (Frangogiannis, 2007). Results of limited reports have indicated that the antioxidant and anti-inflammatory roles of DEX may relate to its inhibiting effect on the augmentation of pro-inflammatory cytokines and ROS in the brain of ischemic rats (Engelhard et al., 2002). To our knowledge there are scarce reports on the effects of DEX in plasma TNF-α and IL-1β levels in cerebral-ischemia-induced rats, and this subject needs further studies.

Thus, the present study aimed to investigate whether or not two different dosses of DEX have protective effects on cerebral ischemia-induced brain, liver and erythrocyte oxidative damage and apoptosis in rats. We also investigated the role of cerebral ischemia, DEX4 and DEX40 on cytokine production, PARP, and caspase 3 and 9 expressions in the brain and liver of rats. The obtained results are discussed with the intent of explaining the mechanism of actions of DEX4 and DEX40 on ischemic brain, liver and blood injury.

2. Materials and methods

2.1. Animals

Forty-two male Wistar albino rats weighing 180–200 g were obtained from the Animal Research Laboratory, Suleyman Demirel University (SDU). The animals were housed in individual cages in a temperature- controlled room with alternating 12-h light-dark cycles and acclimatized for a week before the study. Food was removed 12 h before the cerebral ischemia induction, but all the animals were allowed free access to water and rat chow diet at all other times. The study was approved by the Local Experimental Animal Ethical Committee Neuroscience Research Center (NÖROBAM) of SDU (Protocol number: 15-04). The experiments were carried out over a 2 days period after cerebral ischemia induction.

2.2. Experimental design

The rats were equally divided into seven groups (n = 6), as follows:

Control Group. Non-ischemic, non-supplemented rats receiving 0.9% w/v saline via intraperitonel injection (IP) at the 3rd, 24th and 48th hour.

Sham Group. The group was operated on to expose the cerebral artery, and the incision was then closed without inducing cerebral ischemia induction. The rats in this group received physiologic saline solution (0.9% w/v) IP injection at the 3rd, 24th and 48th hour after the operation.

DEX4 Group. Non-ischemic rats but treated with DEX (4 μg/kg and DEX hydrochloride, Meditera Inc. İstanbul, Turkey) via IP at the 3rd, 24th and 48th hour.

DEX40 Group. Non-ischemic, non-supplemented rats receiving DEX (40 μg/kg) via IP injection at the 3rd, 24th and 48th hour.

Ischemic Group. Cerebral ischemia was subjected to 30 min of cerebral artery occlusion followed by IP injection of physiologic saline solution at the 3rd, 24th and 48th hour.

Ischemic + DEX4 Group. Ischemic rats treated with DEX (4 μg/kg) via IP injection at the 3rd, 24th and 48th hour after cerebral ischemia induction.

Ischemic + DEX40 Group. Ischemic rats treated with DEX (40 μg/kg) via IP injection at the 3rd, 24th and 48th hour after cerebral ischemia induction.

Twelve hours after the last DEX and physiological saline administration, all rats were sacrificed via cervical dislocation, and blood, liver and brain samples were taken in NÖROBAM of SDU.

2.3. Induction of cerebral ischemia

All surgical procedures were performed after the rats had been sufficiently anaesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) cocktail, which was determined by assessing the eye touch response. The lower neck was shaved and sterilized with antiseptic iodine solution and then the animals were placed in a supine position. A heat lamp was used to maintain the body temperature at 37 °C during the experiment.

The right middle cerebral artery was exposed through a ventral midline incision in the neck, was carefully isolated from the vago-sympathetic trunks, and was then loosely encircled with an atraumatic microvascular clamp. A 3–0 suture was positioned so that it encircled the middle cerebral artery for further occlusion. Cerebral ischemic surgery was performed by occlusion of the right middle cerebral artery and the right middle cerebral artery for 1 h. In the current work, the sham-operated rat had the same surgical operation performed on it without the occlusion of both arteries.

2.4. Preparation of blood, brain and liver samples

Rats were sacrificed by cervical dislocation. The blood (3–6 ml) was taken into anticoagulant coated tubes protected against light. Blood samples were separated into plasma and erythrocytes by centrifugation at 1500*g* for 10 min at +4 °C. The erythrocytes samples were washed three times in cold isotonic saline (0.9%, v/w), then hemolyzed with a nine-fold volume of phosphate buffer (50 mM, pH 7.4). The hemolyzed erythrocytes and plasma samples were stored in a deep freeze (−85 °C) until processing (maximum

Table 1
The effects of DEX4 (4 μg/kg) and DEX40 (40 μg/kg) treatments on lipid peroxidation (MDA), reduced glutathione (GSH), antioxidant vitamin concentrations and glutathione peroxidase (GSH-Px) activity in brain of control and cerebral ischemia (ISC)-induced rats (mean ± SD and n = 6).

Parameters	Control	Sham control	DEX4	DEX40	ISC	ISC + DEX4	ISC + DEX40
MDA (μmol/g protein)	18.40 ± 1.04	18.50 ± 1.57	18.00 ± 1.69	18.70 ± 1.54	21.10 ± 1.49[a]	18.30 ± 1.57[c]	17.60 ± 1.63[c]
GSH (μmol/g protein)	11.00 ± 0.42	10.80 ± 0.67	11.40 ± 0.43	11.70 ± 0.80	9.71 ± 0.59[a]	11.00 ± 1.16[c]	11.40 ± 0.65[c]
GSH-Px (IU/g protein)	28.90 ± 1.75	29.60 ± 2.15	31.70 ± 2.92	32.30 ± 2.12	19.00 ± 1.96[b]	29.40 ± 1.98[c]	30.50 ± 2.52[d]
Vitamin A (μmol/g brain)	2.40 ± 0.21	2.41 ± 0.19	2.44 ± 0.26	2.48 ± 0.31	2.36 ± 0.13	2.40 ± 0.34	2.41 ± 0.18
β-carotene (μmol/g brain)	1.80 ± 0.28	1.87 ± 0.19	1.88 ± 0.26	1.91 ± 0.22	1.78 ± 0.27	1.79 ± 0.23	1.83 ± 0.25
Vitamin E (μmol/g brain)	13.80 ± 1.27	13.40 ± 1.42	13.60 ± 1.16	14.00 ± 0.84	13.30 ± 1.27	13.20 ± 1.12	13.40 ± 0.79

[a] $p < 0.05$.
[b] $p < 0.01$ versus control, sham, DEX4 and DEX40 groups.
[c] $p < 0.05$.
[d] $p < 0.01$ versus ISC group.

2 weeks) antioxidant, lipid peroxidation (malondialdehyde, MDA) and cytokine assays.

The liver and brain was also taken as follows; the cortex was dissected out after the brain was split in the mid-sagittal plane as described in our previous studies (Kahya et al., 2015; Yazgan et al., 2016). The brain and liver samples were washed twice with cold saline solution, placed into glass bottles, labeled and stored in a deep freeze (−85 °C) until processing. After weighing, half of the brain and liver was placed on ice, cut into small pieces using scissors, and homogenized (2 min at 2500 g) in 5 vols (1:5, w/v) of ice-cold Tris–HCl buffer (50 mM, pH 7.4), by an ultrasonic homogenization (SONOPULS HD 2070, Bandelin Electronic, Berlin, Germany). All preparation procedures were performed on ice. The homogenate was used for determination of MDA and antioxidant levels. Remaining half of liver and brain samples were stored in the deep freeze (−85 °C) until processing the western blot analyses.

2.5. Malondialdehyde (MDA) and protein determinations

MDA levels in the hemolyzed erythrocytes, brain and liver homogenates were measured with the thiobarbituric-acid (TBAR) reaction by the method of Placer et al. (1966). The MDA levels were analyzed for products of lipid peroxidation by monitoring TBARs formation. MDA was expressed in the absorption to the standard curve of MDA equivalents generated by acid catalyzed hydrolysis of 1,1,3,3 tetramethoxypropane. The results were expressed as μmol/g protein in the erythrocytes, brain and liver samples. Lowry's method (Lowry et al., 1951) was used for the analyses of protein contents of the erythrocytes, brain and liver.

2.6. Reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) assay

The GSH contents in the erythrocytes, brain and liver samples were spectrophotometrically (UV1800, Shimadzu, Kyoto, Japan) measured by the method of Sedlak and Lindsay (1968). After centrifugation at +4 °C and 1000*g* for 5 min, the supernatant was removed. Then, DTNB solution was added and the color formed was read against at 412 nm. GSH-Px activities of erythrocytes were measured spectrophotometrically (UV1800, Shimadzu) at 37 °C and 412 nm according to method of the Lawrence and Burk (1976). GSH-Px catalyzes the oxidation of GSH by cumene hydroperoxide. The decrease of enzymatic reaction in absorbance at 412 nm against blank was measured spectrophotometrically. GSH-Px activity and GSH level in the erythrocytes, brain and liver were expressed as IU/g protein and μmol/g protein, respectively.

2.7. Antioxidant fat soluble vitamins analyses in brain and liver

Vitamin E (α-tocopherol) concentrations were determined in the brain and liver samples by a modification of the method of Desai (1984) although vitamins A (retinol) and β-carotene concentrations in the tissue samples were analyzed modification of Suzuki and Katoh (1990) method as described in previous studies (Senol et al., 2014; Yazgan et al., 2016). Vitamin A and β-carotene concentrations were measured at 325 and 453 nm, respectively. Then reactants were added for vitamin E analyses and the absorbance value of vitamin E in the hexane extract was measured in the spectrophotometer at 535 nm. Standard solutions of all-trans retinol, β-carotene and α-tocopherol in hexane were prepared in dark room and calibrations were performed using standard solutions in hexane.

2.8. Cytokine determinations in plasma

TNF-α and IL-1β are well-known two major proinflammatory cytokines and they are produced in the immune system and the central nervous system. Their productions are especially increased in plasma by cerebral ischemia conditions (Frangogiannis, 2007). Therefore, we investigated plasma TNF-α and IL-1β levels as indicators of proinflammatory cytokines of cerebral ischemia by using the commercial ELISA kits. The methods detailed described in previous studies (Senol et al., 2014; Kahya et al., 2015). Briefly, the methods employ the quantitative sandwich techniques. The analyses were performed in ELISA of a plate reader (Multimode microplate reader, Infinite® 200 PRO series, Männedorf Switzerland). Determinations were made in duplicate, and TNF-α and IL-1β results were expressed in nano gram (ng) per milliliter. Enzyme-linked immunosorbent assay (ELISA) cytokine (rat TNF-α and rat IL-1β) kits were purchased from DRG Inc. (Marburg, Germany).

2.9. Western blot analyses

Antibodies, chemical and solution in the Western Blotting analyses of current study were used as described in previous studies (Akpınar et al., 2016; Yazgan et al., 2016). To detect β-actin, Poly (ADP-ribose) Polymerase (PARP), procaspase 3 and procaspase 9 protein expressions, the frozen cells were homogenized in lysis buffer; the supernatant was removed and conserved after centrifuge at 16,000*g*, 20 min. Bradford reagent was used for the total protein was spectrophotometrically (UV1800, Shimadzu) assayed at 595 nm. Obtained bands were visualized by G:BOX F3 gel doc system (Syngene, Cambridge, UK) and the bands was normalized

Table 2
The effects of DEX4 (4 μg/kg) and DEX40 (40 μg/kg) treatments on lipid peroxidation (MDA), reduced glutathione (GSH) levels and glutathione peroxidase (GSH-Px) activity in erythrocyte of control and cerebral ischemia (ISC)-induced rats (mean ± SD and n = 6).

Parameters	Control	Sham control	DEX4	DEX40	ISC	ISC + DEX4	ISC + DEX40
MDA (μmol/g protein)	20.20 ± 1.38	20.10 ± 1.01	19.40 ± 1.58	18.40 ± 2.20	22.00 ± 1.14[a]	19.70 ± 1.98[c]	19.90 ± 1.43[c]
GSH (μmol/g protein)	10.70 ± 0.59	10.50 ± 1.08	10.08 ± 0.44	10.20 ± 1.08	9.91 ± 0.47	10.70 ± 0.28	10.90 ± 0.61
GSH-Px (IU/g protein)	23.90 ± 2.89	23.70 ± 1.93	25.30 ± 1.21	25.40 ± 1.56	20.30 ± 1.64[b]	26.60 ± 0.88[d]	27.00 ± 1.33[d]

[a] $p < 0.05$.
[b] $p < 0.01$ versus control, sham, DEX4, high DEX40 groups.
[c] $p < 0.05$.
[d] $p < 0.01$ versus ISC group.

against β-actin protein. The data are presented as relative density over the pretreatment level.

2.10. Statistical analysis

The results are presented as means ± standard deviation (SD). The SPSS Statistical program (17.0, SPSS Inc. Chicago, Illinois, USA) was used for the statistical treatment of the data. The Mann-Whitney *U* test was used to establish the significance of differences among the four groups. The significance level was set at $p < 0.05$.

3. Results

3.1. MDA results

Lipid peroxidation level as MDA is widely used as a biomarker of oxidative stress. The mean brain, erythrocytes and liver MDA in the four groups are shown in Tables 1–3 , respectively. The results showed that MDA levels in the brain, liver and erythrocyte samples in the cerebral ischemia group were significantly ($p < 0.05$) higher than in the control and sham control groups. Therefore, the oxidative stress level in the blood, liver and brain of the rats was increased by induction of cerebral ischemia. However, administrations of DEX4 and DEX40 a caused decrease in the MDA level of the brain and erythrocytes in the rats, although the liver MDA levels were further increased in the DEX4 and DEX40 treated groups ($p < 0.05$). In other words, the MDA levels in the liver were high in the DEX4 and DEX40 groups as compared to control and sham control groups ($p < 0.05$). The MDA levels were further increased in ischemia + DEX4 and ischemia + DEX40 groups compared to the DEX4 and DEX40 groups ($p < 0.05$). However, the MDA levels in the brain and erythrocyte in the ischemia + DEX4 and ischemia + DEX40 groups were significantly ($p < 0.05$) lower than in the DEX4 and DEX40.

3.2. Results of GSH-Px activity and GSH level

The mean GSH-Px activities and GSH results in the brain, erythrocytes and liver of the seven groups are presented in Tables 1–3, respectively. The results showed that GSH concentration in the brain and liver ($p < 0.05$), and GSH-Px activities in the brain ($p < 0.01$), erythrocyte ($p < 0.05$) and liver ($p < 0.05$) were significantly lower in the cerebral ischemia group compared to the control and sham control groups. The liver MDA levels were also high in the DEX4 and DEX40 groups compared to the control and sham control groups ($p < 0.05$). However, GSH concentration in the brain ($p < 0.05$) and GSH-Px activity in the brain ($p < 0.01$) and the erythrocytes ($p < 0.05$) were markedly increased by the two (DEX4 and DEX40) treatments although GSH concentration and GSH-Px activity were further decreased in the ischemia + DEX4 and ischemia + DEX40 groups compared to the DEX4 and DEX40 groups ($p < 0.05$).

3.3. Antioxidant vitamin concentrations in the brain and liver

The mean vitamin A, β-carotene, and vitamin E concentrations in the brain and liver of the 7 groups are shown in Tables 1 and 3, respectively. Vitamin A ($p < 0.05$) and vitamin E ($p < 0.01$) concentrations in the liver were significantly ($p < 0.01$) lower in the DEX4, DEX40 and ischemia groups compared to the control and sham control groups. The decreased liver vitamin A and E concentrations were not improved by the DEX4 and DEX40 administration

Table 3
The effects of DEX4 (4 μg/kg) and DEX40 (40 μg/kg) treatments on lipid peroxidation (MDA), reduced glutathione (GSH), antioxidant vitamin concentrations and glutathione peroxidase (GSH-Px) activity in liver of control and cerebral ischemia (ISC)-induced rats (mean ± SD and n = 6).

Parameters	Control	Sham control	DEX4	DEX40	ISC	ISC + DEX4	ISC + DEX40
MDA (μmol/g protein)	25.89 ± 1.46	25.62 ± 2.27	28.30 ± 3.09[a]	28.86 ± 1.35[a]	28.30 ± 1.87[a]	31.57 ± 1.52[b,d]	32.08 ± 1.55[b,d]
GSH (μmol/g protein)	15.70 ± 0.60	15.00 ± 0.68	13.40 ± 1.03[a]	12.40 ± 0.75[b]	14.10 ± 1.21[a]	12.60 ± 1.15[b,c,d]	11.30 ± 0.86[b,c,e]
GSH-Px (IU/g protein)	38.50 ± 2.34	38.90 ± 2.16	30.50 ± 2.32[a]	29.60 ± 2.20[a]	31.20 ± 2.66[a]	27.20 ± 1.43[b,e]	26.20 ± 2.15[b,e]
Vitamin A (μmol/g liver)	4.91 ± 0.15	4.86 ± 0.11	4.51 ± 0.22[a]	4.48 ± 0.23[a]	4.69 ± 0.27[a]	4.29 ± 0.20[b,c,e]	4.30 ± 0.22 [b,c,e]
β-carotene (μmol/g liver)	1.97 ± 0.23	1.92 ± 0.24	1.88 ± 0.18	1.85 ± 0.46	1.86 ± 0.27	1.88 ± 0.32	1.83 ± 0.17
Vitamin E (μmol/g liver)	25.90 ± 2.39	25.70 ± 1.65	22.70 ± 0.91[a]	22.20 ± 1.08[b]	23.30 ± 1.87[a]	21.30 ± 1.55[b,c,e]	20.50 ± 2.33[b,c,e]

[a] $p < 0.05$.
[b] $p < 0.01$ versus control and sham groups.
[c] $p < 0.05$ versus DEX4 and DEX40 groups.
[d] $p < 0.05$.
[e] $p < 0.01$ versus ISC group.

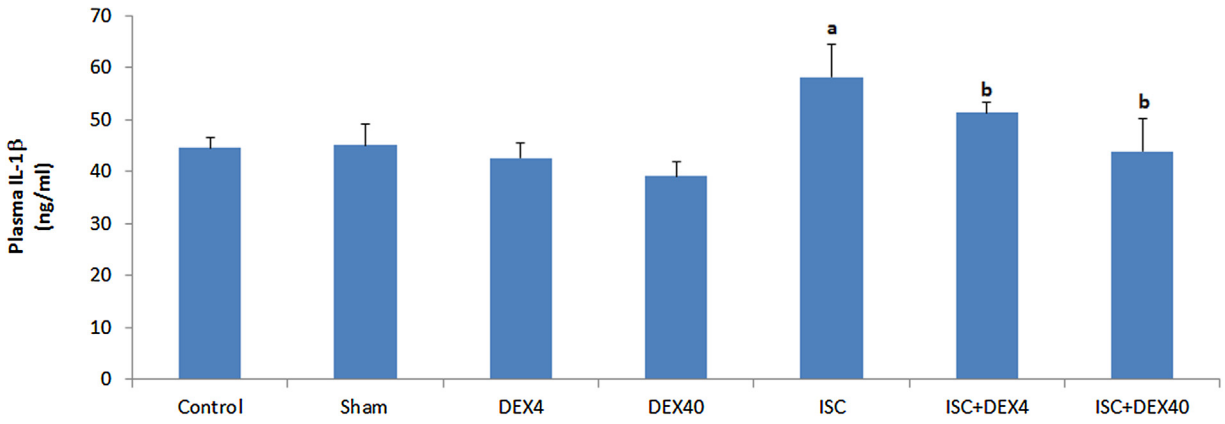


Fig. 1. The effects of DEX4 (4 μg/kg) and DEX40 (40 μg/kg) treatments on plasma IL-1β levels in control and cerebral ischemia (ISC)-induced rats (mean ± SD and n = 6). [a] $p < 0.05$ versus control, sham, DEX4 and DEX40 groups. [b] $p < 0.05$ versus ISC group.

($p < 0.001$). The liver vitamin A and E concentrations were further decreased in the ischemia + DEX4 and ischemia + DEX40 groups compared to the DEX4 and DEX40 groups ($p < 0.05$). However, there were no significant differences in the brain vitamin A, β-carotene and vitamin E concentrations in the seven groups.

3.4. Results of cytokine in plasma

The mean IL-1β and TNF-α levels in the plasma of seven groups are shown in Figs. 1 and 2, respectively. The plasma TNF-α and IL-1β levels are significantly ($p < 0.05$) increased by cerebral ischemia induction although, their level is markedly ($p < 0.05$) decreased by DEX4 and DEX40 treatments.

3.5. Results of procaspase 3, procaspase 9 and poly (ADP-ribose) polymerase (PARP)

Caspase 3 and 9 activities are apoptotic indicators in the executioner caspase-activated pathways and the mitochondrial apoptotic pathway, respectively (Li et al., 1997; Uğuz et al., 2009). We assayed caspase 3 and 9 expression levels as indicators of apoptosis in the brain (Fig. 3a) and liver (Fig. 4a). PARP is an abundant enzyme present in cells that indicates and signals damage to DNA repair mechanisms (Nazıroğlu, 2007). We also assayed PARP expression levels as indicators of oxidative DNA damage in brain (Fig. 3b) and liver (Fig. 4b). The expression levels of the PARP, and caspase 3 and 9 in the brain and liver of the rats were significantly ($p < 0.05$) higher in the ISC group compared to the controls, and the results indicated that the expression levels of PARP, and caspase 3 and 9 were increased in the brain and liver by induction of cerebral ischemia. However, their expression levels in the brain were decreased by the DEX4 ($p < 0.05$) and DEX40 ($p < 0.05$ and $p < 0.001$) treatments. The PARP, and caspase 3 and 9 expression levels in the liver were further increased in the ISC + DEX4 and ISC + DEX40 groups compared to the ISC, DEX4 and DEX40 groups ($p < 0.05$).

4. Discussion

We found that MDA level in the brain, liver and erythrocyte, and TNF-α and IL-1β levels in the plasma were increased by ischemia although the brain, liver and erythrocyte GSH-Px and GSH values and the erythrocyte GSH-Px activities decreased as results of the cerebral ischemia. Hence, ischemia induction in the animals is characterized by increased MDA and decreased GSH and GSH-Px values. Administration of DEX4 and DEX40 caused a decrease in brain MDA and plasma TNF-α and IL-1β levels, although GSH and GSH-Px values increased. Conversely, MDA level was further increased in the liver by the DEX treatments although vitamin A, vitamin E, GSH-Px and GSH values were further decreased by the treatments. Hence, we have shown that DEX-based treatments in the brain and blood decreased the balance of pro- and anti-inflammatory cytokines in rats by down-regulating the levels of pro-inflammatory cytokines namely TNF-α and IL-1β. However, the treatment induced liver oxidative toxicity. To the best of our knowledge, the current study is the first to compare the drug DEX with particular reference to its effects on oxidative stress, cytokine production and the antioxidant

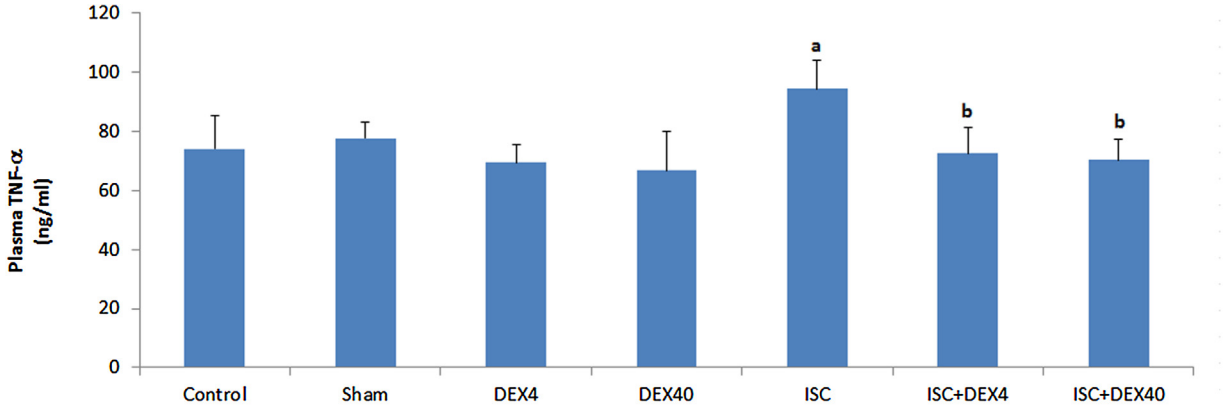


Fig. 2. The effects of DEX4 (4 μg/kg) and DEX40 (40 μg/kg) treatments on plasma TNF-α levels in control and cerebral ischemia (ISC)-induced rats (mean ± SD and n = 6). [a] $p < 0.05$ versus control, sham, DEX4 and DEX40 groups. [b] $p < 0.05$ versus ISC group.

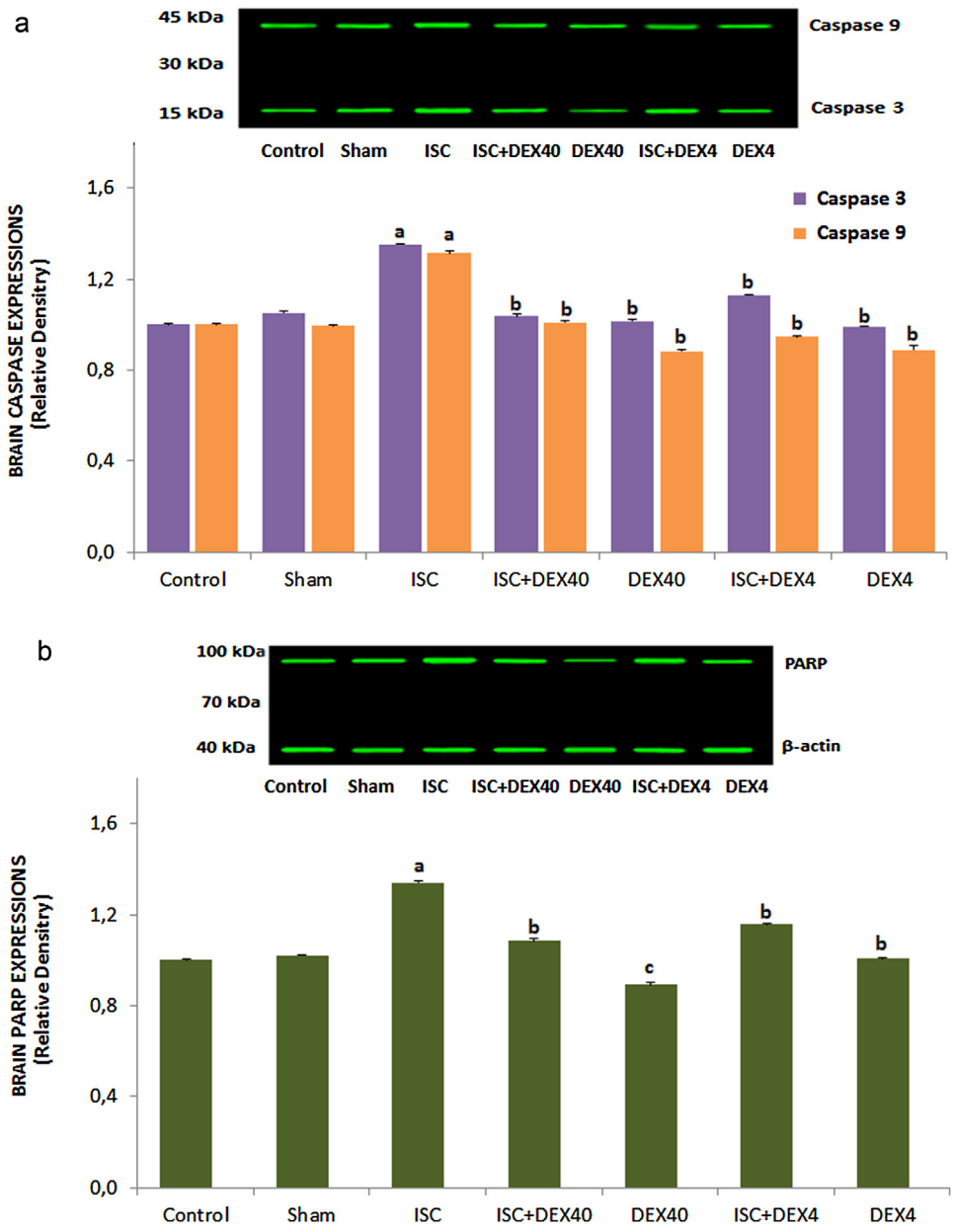


Fig. 3. The effects of DEX4 (4 μg/kg) and DEX40 (40 μg/kg) treatments on caspase 3, caspase 9 (a) and PARP (b) expression levels in brain of cerebral ischemia (ISC)-induced rats (mean ± SD and n = 3). [a]$p < 0.05$ versus control and sham groups. [b]$p < 0.05$ and [c]$p < 0.001$ versus ISC group.

redox system in experimental cerebral ischemia-induced oxidative injury in rats.

In the current study, we observed that MDA levels were decreased in the brain and erythrocytes of the experimental cerebral ischemia-induced rats after DEX4 and DEX40 treatment. This study also provides evidence that both doses of DEX are able to protect the brain and erythrocytes against ROS and cytokine productions by cerebral ischemia-induced oxidative toxicity and apoptosis. Treatments with DEX regulated the levels of MDA in ischemic brain tissue and erythrocyte, and the levels of TNF-α and IL-1β in plasma, suggesting that DEX plays an essential physiological function that retards the oxidative processes and it serves as a potential antioxidant and anti-inflammatory agent (Engelhard et al., 2002; Sifringer et al., 2015). In consequence, DEX administrations may enhance the antioxidant capacity of ischemic subjects or prevent cerebral ischemia-induced oxidative and cytokine damage directly through its antioxidant and anti-inflammatory actions (Engelhard et al., 2002; Sifringer et al., 2015). Similarly, Li et al. (2015) reported that MDA levels are decreased via DEX infusion (25 μg/kg) in human plasma after implant surgery. Aslan et al. (2009) reported that the MDA roxidation level is decreased in the whole blood of the spinal cord injury-induced rabbits by DEX (1 μg/kg) treatment. Recently, decreased MDA and cytokine production were reported in the serum of patients with malignant brain tumor after DEX (1 μg/kg) treatment (Luo et al., 2016). Decreased TNF-α and IL-1β levels through intraperitoneal treatment of 5, 15 and 25 μg/kg intraperitoneal DEX in the brain of rats were reported in brain ischemia hypoxia-induced neonatal rats (Ren et al., 2016).

The cytochrome P450 enzyme in the liver is an important and abundant physiological enzyme involved in the detoxification of ROS products in body. DEX is metabolized through the liver by the hepatic cytochrome P450 (Daskalopoulos et al., 2012). However, metabolization rate of DEX is decreased by the presence of liver

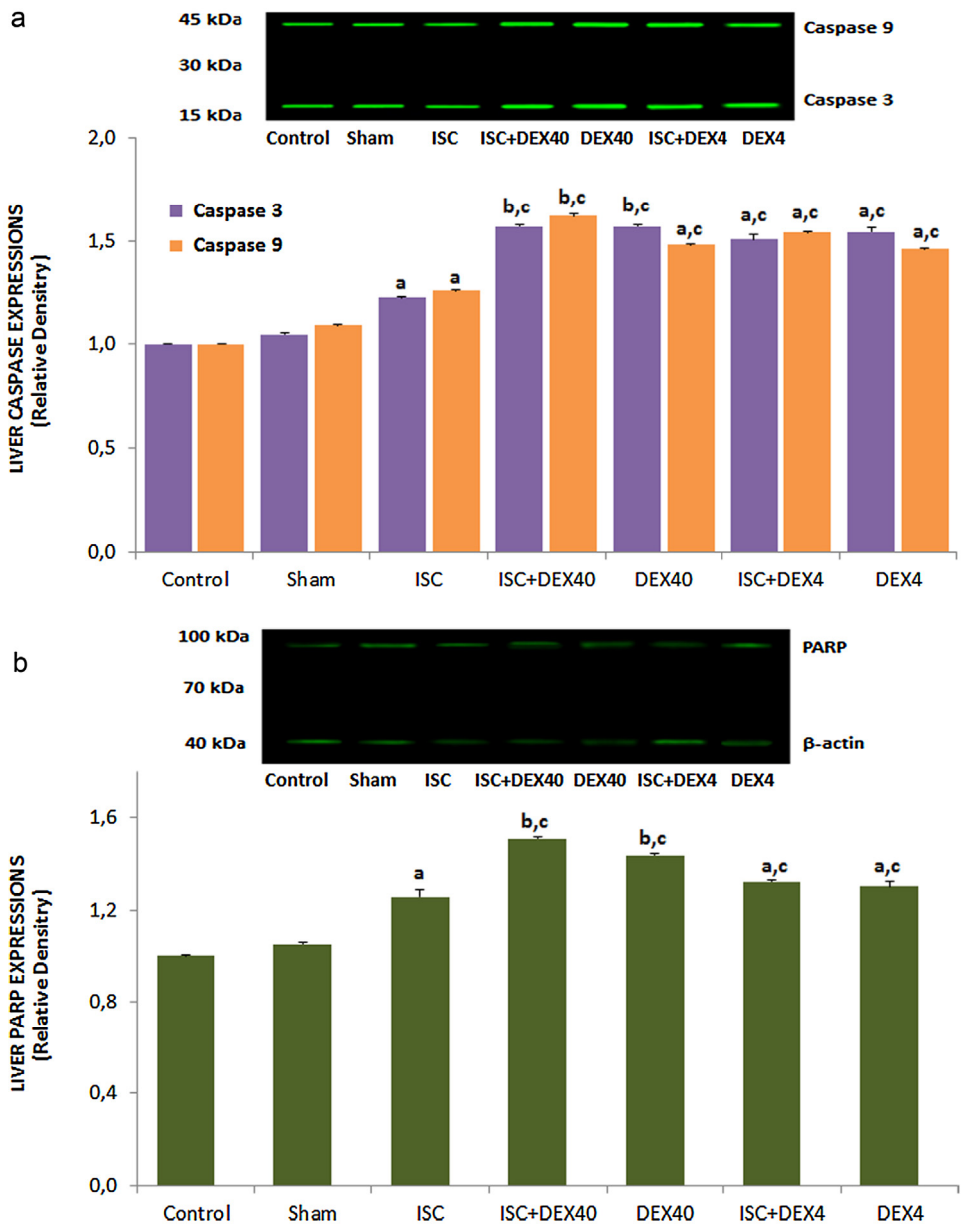


Fig. 4. The effects of DEX4 (4 μg/kg) and DEX40 (40 μg/kg) treatments on caspase 3, caspase 9 (a) and PARP (b) expression levels in liver of cerebral ischemia (ISC)-induced rats (mean ± SD and n = 3). [a]$p < 0.05$ and [b]$p < 0.01$ versus control and sham groups. [c]$p < 0.05$ versus ISC group.

injury and diseases. It has been reported that the detoxification of DEX decreased by up to 68% in the presence of severe liver disease (Panzer et al., 2009). Cerebral ischemia induces severe liver disease through excessive production of ROS (Gong et al., 2012). In the current study, MDA levels were increased in the cerebral ischemia-induced rats due to oxidative stress characterization of cerebral ischemia although vitamin A, GSH and GSH-Px were decreased in cerebral ischemia group. In addition, the MDA levels in the liver were further increased by the DEX treatments although, vitamin A, GSH and GSH values in the liver were further decreased by the DEX treatments. Similarly, decreased MDA level through DEX treatment was reported in rat liver with inflammatory bowel disease (Gul et al., 2015).

The GSH-Px enzyme as a co-factor contains selenium and is a member of the thiol group of antioxidant enzymes. It functions in the reductive detoxification of hydrogen peroxide and alkyl hydroperoxides (Nazıroglu, 2009). GSH is a non-enzymatic antioxidant and is most abundant as a non-protein thiol source in the cells. Therefore, GSH plays an important role in protecting cells against oxidative injury (Nazıroğlu et al., 2013). As such, GSH-Px is important to brain and erythrocyte functions because it regulates the thiol redox system homeostasis (Senol et al., 2014). Additionally, brain and erythrocyte antioxidant and anti-inflammatory protection by DEX and GSH-Px may also play a critical role in preventing brain and erythrocyte complications in ischemic diseases (Dong et al., 2014; Yang et al., 2014). Recently it was reported that DEX treatment restored brain, muscle and erythrocyte GSH-Px and mitochondrial ROS production in rats (Dong et al., 2014; Yang et al., 2014). In the current study, brain and erythrocyte GSH-Px and GSH values, and erythrocyte GSH-Px activity values decreased in ischemia-induced rats, although the same values were increased by DEX treatments. Hence, the results of the study support the idea that ischemia-induced mitochondrial oxidative stress in the brain and erythrocyte is reduced by DEX treatments.

Inflammatory reactions in phagocytic cells such as neutrophil and microglia are increased by reperfusion of ischemic tissues,

resulting in excessive ROS production (Frangogiannis, 2007). It is well known that an important feature of the ischemia-induced inflammatory response is the stimulation of cytokine production (Nazıroğlu, 2011; Kahya et al., 2015). ROS triggers the cascade of cytokines through NF-kB activation. The release of proinflammatory cytokines such as TNF-α and IL-1β induces chemokine synthesis in ischemic tissues (Frangogiannis, 2007). Increases of IL-1β levels are induced by clinical and experimental ischemic tissues (Sifringer et al., 2015; Luo et al., 2016; Wang et al., 2016). The generation of ROS induced by tissue ischemia plays a central role in the pathogenesis of ischemic tissue injury, and these radicals can stimulate the production of cytokines (Cakir et al., 2015; Sifringer et al., 2015) DEX in the neonatal rat brain has antioxidant activities, but it decreases pro-inflammatory cytokine expression and oxidative stress after experimental ischemia (Sifringer et al., 2015). In the current study, the DEX treatments in the blood and brain reduced these effects via antioxidant and anti-inflammatory properties. In other words, the increase in the plasma IL-1β and TNF-α levels of cerebral ischemia-induced groups was decreased by DEX4 and DEX40 treatments. Thus, the DEX4 and DEX40 treatments' attenuation of proinflammatory cytokines and MDA along with the diminution of ischemia and the improved antioxidants are clearly suggestive of the beneficial effects of these treatments in ischemic plasma. Similarly, expression levels of TNF-α and IL-1β in the intestines of ischemia-induced rats via cecum ligation have been reported after DEX (5 μg/kg/hour) treatment (Chen et al., 2015).

It is well known that excessive production of ROS is stimulated by ischemia-induced brain injury (Sifringer et al., 2015). The excessive ROS production, in turn, can further increase inflammation and cause further brain DNA damage (Frangogiannis, 2007). PARP activity in the nucleus is increased by the oxidative DNA damage (Nazıroğlu, 2007). The production of superoxide anion and MDA is increased by increased TNF-α and IL1-β, which then induces oxidative stress, DNA damage and apoptosis (Engelhard et al., 2002). In the current study, the PARP and caspase 3 and 9 expressions levels in the brain increased in the ischemia group, while their levels were low in the ischemia + DEX4 and ischmia + DEX40 groups. However, the PARP and procaspase 3 and 9 expression levels in the liver were further increased in the ischemia groups by the DEX4 and DEX40 treatments. The current study provides a mechanistic underpinning for the antioxidant and anti-inflammatory actions of DEX4 and DEX40. It does this by demonstrating that DEX reduces MDA production in the brain, reduces DNA damage due to excessive ROS production after ischemia induction, and increases pro-apoptotic caspase 3 and 9 activations. Conversely, the PARP and caspase 3 and 9 expression levels, through excessive oxidative stress and cytokine production in the liver, are further increased by the DEX treatments.

5. Conclusion

In conclusion, we demonstrated in an experimental animal model that DEX administrations preserved brain and erythrocyte functions and ameliorated oxidative, apoptotic and cytokine changes in the blood and brain of cerebral ischemia-induced rats, resulting in retardation of the progression of cerebral ischemia-induced brain injury. However, both doses of DEX induced liver oxidative toxicity and apoptotic effects in the cerebral ischemia-induced rats. The antioxidant, anti-inflammatory, anti-apoptotic, anti-DNA damage effects of both DEX doses on the brain and erythrocyte might be modulated by increases in the GSH redox system. Thus, the DEX treatments may have a beneficial effect in managing oxidative brain injuries alongside as their oxidant complications. However, long term administration of the treatments should be investigated by future studies.

Conflict of interest

The authors report no declarations of interest. We have not been funded by any individual or organization. The authors alone are responsible for the content and writing of the paper.

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