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Effects of Intracisternal and Intravenous Dexmedetomidine on Ischemia-Induced Brain Injury in Rat: A Comparative Study

Sığanlarda İskemiyle İndüklenen Beyin Hasarı Üzerine İntravenöz ve İntrasisternal Deksmomedetomidinin Etkileri: Karşılaştırmalı Bir Çalışma

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ABSTRACT

AIM: To compare the effect of dexmedetomidine administered by intracisternal route with by intravenous route on brain tissue of rat after incomplete cerebral ischemia.

MATERIAL and METHODS: Cerebral ischemia was produced by the combination of right common carotid artery occlusion and hemorrhagic hypotension during 30 minutes. Thirty minutes before the ischemia, 0.1 ml 0.9% NaCl (Group SIC, n=6) or 9 µg/kg dexmedetomidine (Group DIC, n=6) was administered into the cisterna magna. For the intravenous groups, 9 µg/kg dexmedetomidine (Group DIV, n=6) or 0.9% NaCl (Group CONTROL, n=6) 5 ml/kg/h was given in 2 hours. After 24 hours, the lipid peroxidation levels were measured in the brain tissue and plasma. Hippocampal formations were used for histopathological examination.

RESULTS: Intravenous dexmedetomidine produced a decrease in baseline mean arterial blood pressure and plasma glucose concentrations. There was a significant difference between the DIV group and DIC, SIC, CONTROL groups regarding the brain lipid peroxidation levels ($p<0.001$, $p<0.001$, $p=0.001$, respectively), and regarding the picnotic neuronal cell count ($p<0.001$, $p=0.01$, $p=0.009$, respectively). Mean plasma lipid peroxidation levels of the DIV group was different from the DIC group ($p=0.003$).

CONCLUSION: Systemically administered dexmedetomidine had neuroprotective effect in ischemia-induced neuronal damage, but centrally administered dexmedetomidine did not.

KEYWORDS: Dexmedetomidine, Intracisternal, Intravenous, Ischemia, Brain injury

ÖZ

AMAÇ: Sığanlarda inkomplet serebral iskemisi üzerine intrasisternal yolla verilen deksmedetomidinin etkilerinin intravenöz yolla verilen deksmedetomidinin etkileriyle karşılaştırılması amaçlandı.

YÖNTEM ve GEREÇLER: Ortak sağ karotid arter oklüzyonu ve hemorajik hipotansiyon ile 30 dakika süreyle serebral iskemi oluşturuldu. İntrasisternal grupta, iskemiden 30 dakika önce sisterna magna içine 0,1 ml % 0,9 NaCl (Grup SIC, n=6) veya 9 µg/kg deksmedetomidin (Grup DIC, n=6) verildi. İntravenöz grupta ise 9 µg/kg deksmedetomidin (Grup DIV, n=6) veya % 0,9 NaCl (Grup CONTROL, n=6) 5 ml/kg/h hızla 2 saat sürede infüze edildi. Yirmi dört saat sonra beyin dokusunda ve plazmada lipid peroksidasyon seviyeleri ölçüldü. Histopatolojik incelemeler için hipokampal yapı kullanıldı.

BULGULAR: İntravenöz deksmedetomidin ortalama arteriyel basınç ve plazma glukoz seviyelerinde bazale göre bir düşüşe neden oldu. Grup DIV ile Grup DIC, Grup SIC ve CONTROL grupları arasında beyin dokusu lipid peroksidasyon seviyeleri ve piknotik hücre sayıları bakımından anlamlı farklılık vardı (sırasıyla; $p<0,001$, $p<0,001$, $p=0,001$ ve $p<0,001$, $p=0,01$, $p=0,009$). Grup DIV için ortalama plazma lipid peroksidasyon seviyeleri Grup DIC'inkinden farklı bulundu ($p=0,003$).

SONUÇ: İskememin indüklediği nöronal hasarda sistemik yolla verilen deksmedetomidinin nöroprotektif etkisi varken santral yolla verilen deksmedetomidinin nöroprotektif etkisi yoktur.

ANAHTAR SÖZCÜKLER: Deksmedetomidin, İntrasisternal, İntravenöz, İskemi, Beyin hasarı

INTRODUCTION

Dexmedetomidine, the dextro enantiomer of medetomidine, is a potent and highly selective α_2 -adrenoreceptor agonist (24). There is evidence that dexmedetomidine may partially prevent neuronal damage in some models of brain ischemia (9, 11, 16, 17, 22). Although the mechanism of neuroprotection of α_2 -agonist has not been fully elucidated, putative mechanisms include decrease of ischemia-induced adrenaline over secretion (8, 23), an increase in expression of focal adhesion kinase (4), modulation of central glutamate release, decrease of Ca^{+2} entry, and apoptosis (20). Additionally, a decrease in sympathetic tone with dexmedetomidine may be protective by down regulation of inflammatory neurodegenerative processes (33). On the other hand, it has been demonstrated that dexmedetomidine administered via epidural route produced moderate or severe demyelination of myelin sheets in the white matter of spinal cord in rabbit (15) and this effect has been explained by its vasoconstrictive effect on medullar spinal vessels (12). It has been shown in an in vivo experimental study that topical application of dexmedetomidine had vasoconstrictor effects both on cerebral pial arterial and venous vessels (16). Additionally, it has been reported in another experimental study that, centrally administered dexmedetomidine reduced cerebral blood flow during normoxia and prevented adequate oxygen delivery during hypoxia (26).

On the basis of the literature knowledge, it can be thought that the effects of dexmedetomidine on brain and vascular structures can be different regarding to the administration route and maintained by different mechanisms. The purpose of this prospective, randomized, double-blind, controlled study was to investigate and to compare the effect of dexmedetomidine administered by intracisternal route with intravenous route on brain tissue of rat after incomplete cerebral ischemia through histopathological and biochemical evaluations.

MATERIAL and METHODS

This experiment was performed in accordance with the guidelines for the use of laboratory animal subjects in research set by the Ethical Committee of Kirikkale University. Twenty four male Wistar rats of 250-300 gram weight were used. The rats were placed in a temperature (21 ± 2 °C) and humidity (60 ± 5 %) controlled room for one week before the experiment, 12 h light/12 h dark cycle was maintained, and was allowed free access to food and water before and after surgery. The animals were divided into four groups randomly which were named as follows:

- DIC (Ischemic cerebral injury + 9 μ g/kg dexmedetomidine which was diluted with 0.9% saline infused in a volume of 0.1 ml via intracisternal route) (n=6),
- DIV (Ischemic cerebral injury + 3 μ g/kg dexmedetomidine intravenously (bolus in 5 minutes) and followed by a 120 minutes infusion at a dose of 3 μ g/kg/h (total dose 9 μ g/kg) (n=6),

- SIC (Ischemic cerebral injury + 0.9% NaCl in a volume of 0.1 ml via intracisternal route) (n=6),
- CONTROL (Ischemic cerebral injury + 0.9% NaCl 5 ml/kg/h intravenously for 120 minutes) (n=6).

Anesthesia was performed with intramuscular administration of 40 mg/kg ketamine HCl (Ketalar®; Pfizer Inc, USA) and 5 mg/kg xylazine HCl (Rompun® 2%; Bayer HealthCare AG, Germany). The rats were kept normothermic with thermoregulatory heating unit (Harvard Apparatus) connected the rectal probe. An additional probe was inserted into the temporalis muscle in order to monitor skull temperature (Omega, Model 680). The right femoral artery was cannulated for continuous blood pressure monitoring (CardioCap II, Datex, Finland), and to obtain arterial blood samples for blood gas analysis, arterial carbon dioxide tension ($PaCO_2$), arterial oxygen tension (PaO_2), blood pH, (ABL5, Radiometer, Denmark), and for measurement of the blood glucose concentrations (OneTouch II, Lifescan, USA). The left femoral vein was cannulated for blood withdrawal during ischemia. Drug infusions were obtained from cannulated tail veins. Under a stereomicroscope the right common carotid artery and the bifurcation area were exposed through a midline anterior cervical incision, and carefully separated from the surrounding vagus nerve fibers (Figure 1A). A temporary aneurysm clip (Sugita® Aneurysm Clip; Mizuho America, Inc.) was attempted to the right internal carotid artery for duration of 30 minutes (Figure 1B). Cerebral ischemia was produced by the combination of right common carotid artery occlusion and hemorrhagic hypotension with mean arterial blood pressure (MAP) at 35 ± 2 mmHg for 30 minutes (11). After 30 minutes of ischemia, the right carotid artery was unclamped and withdrawn blood slowly reinfused in 10 minutes. Skull temperature was maintained at 37 °C, $PaCO_2$ was maintained between 35-40 mmHg. Arterial blood pH, blood glucose concentrations, MAP, $PaCO_2$ and PaO_2 were measured at before ischemia (baseline), during ischemia, and 20 minutes after reinfusion of blood during recovery. Thirty minutes before the arterial occlusion, 0.1 ml normal saline (Group SIC, n=6) or 9 μ g/kg diluted dexmedetomidine in 0.1 ml volume (Group DIC, n=6) was administered within one minute from the posterior craniocervical junction of the rats using a 26G needle when the free cerebrospinal fluid flow was seen. For the rats in the Group DIV and group CONTROL cisterna magna was punctured and free cerebrospinal fluid flow was seen but nothing was injected into the subarachnoid space. The rats of Group DIV received 3 μ g/kg dexmedetomidine intravenously (bolus in 5 minutes) and followed by a 120 minutes infusion at a dose of 3 μ g/kg/h (total dose 9 μ g/kg) (14). The rats in Group CONTROL (n=6) received 0.9% NaCl 5 ml/kg/h intravenously for 2 hours. During the recovery period catheters were removed, the incisions were closed. Seventy two hours later, they were re-anaesthetized. For sacrifice, the whole body blood was collected from the vena cava inferior, and then the rats were decapitated (Figure 1C). The hippocampal formations of right hemisphere was dissected and stored in 10% buffered formaldehyde solution at room temperature

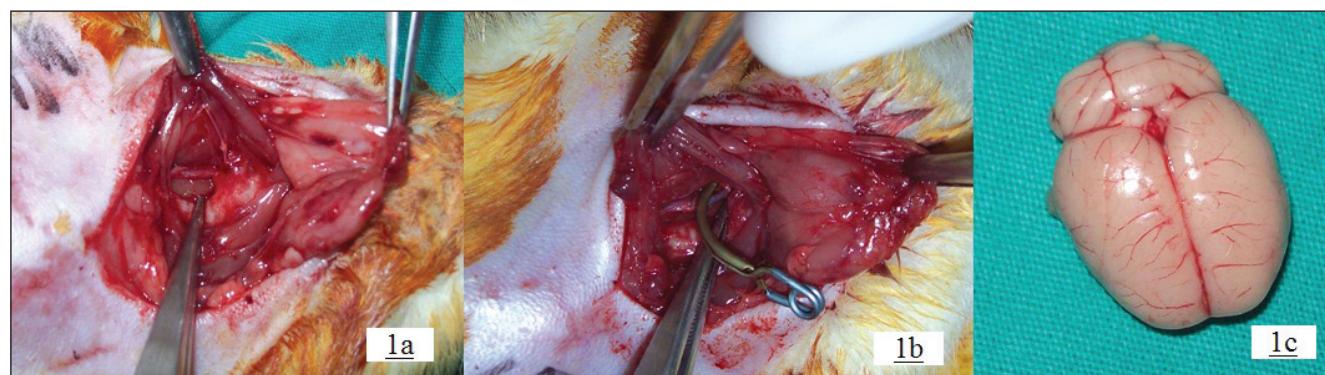


Figure 1: **A)** the right common carotid artery which was exposed through a midline anterior cervical incision; **B)** temporary aneurysm clip attempted to the right internal carotid artery; **C)** the brains harvested for future biochemical and histopathological examinations.

for future histopathological examination. For biochemical examination, remaining part of the right hemisphere and the plasma of the collected whole body blood were immediately stored at -30 °C in dry air.

Biochemical Analysis

Biochemical determinations were carried out by a biochemist blinded to the animal groups, and test materials. Frozen tissue samples were weighted and homogenized in 1:10 (w:v) potassium phosphate buffer (50 mM, pH: 7.4) by using a dounce homogenizer. Thiobarbituric acid reactive substances (TBARS) were measured as an index of the lipid peroxidation (LPO) by the method of Mihara et al (27, 29). Serum levels of lipid peroxides were determined by thiobarbituric acid (TBA) assay as described by Wade and van Rij (34).

Histopathological Analysis

For histological examination, all tissue samples were fixed at 10% buffered formaldehyde and processed according to routine light microscopic tissue processing technique. Serial coronal sections of 5 µm at the level of the hippocampus formation stained with hematoxylin-eosin were examined and photographed by an Olympus BH-2 microscope. Three random regions were examined at X 200 magnification and the number of picnotic neurons in three areas per section of hippocampal dentate gyrus (DG) region were identified and counted on the basis of the presence of picnotic nuclei and shrunken cytoplasm. Then the number of the picnotic neuronal cells was calculated as an average per rat.

Statistical Analysis

Data were analyzed using the SPSS 11.5 (SPSS Inc. Software, Chicago, Illinois, USA) statistical software. All data were presented as mean \pm SD. Because tissue LPO levels, plasma LPO levels, histopathological data and the data of physiological parameters were normally distributed and the variations were homogenous among all groups, analyzed by using one-way analysis of variance (ANOVA) and with Tukey test as to determine comparison among groups, and differences among groups, respectively. The repeated

measures of physiological parameters within different times were analyzed using general linear model analysis of variance for repeated measures. $p<0.05$ was considered statistically significant.

RESULTS

Physiological Parameters

Blood gas tensions, pH, plasma glucose concentrations, MAP, rectal and head temperatures were shown in Table I. Rectal and head temperatures, blood gas tensions, pH did not vary significantly during and after ischemia. There was a statistically significant difference among all groups regarding baseline MAP values ($F=198.702, p<0.001$). Dexmedetomidine produced a significant decrease in baseline MAP when administered intravenously compared to the CONTROL, DIC and SCI groups ($p<0.001, p<0.001, p<0.001$, respectively). There was a statistically significant difference among all groups regarding baseline plasma glucose concentrations ($F=46.096, p<0.001$). Plasma glucose concentrations were significantly more in the DIV group than in the DIC, SCI and CONTROL groups in the pre-ischemic period ($p<0.001, p<0.001, p=0.001$, respectively). During cerebral ischemia, plasma glucose levels increased in all study groups when compared to baseline values within each group (Table I). However there was no statistically significant difference between the groups at the ischemic period and recovery period.

Biochemical Analysis

There was a statistically significant difference among all groups regarding the brain LPO levels ($F=17.425, p<0.001$). When posthoc comparisons were made, statistically differences were observed between the DIV/DIC, DIV/SIC and DIV/CONTROL groups ($p<0.001, p<0.001, p=0.001$, respectively) (Table II, Figure 2). There was also statistically significant difference among all groups regarding the plasma LPO levels ($F=5.762, p<0.001$). The mean plasma LPO levels of the DIV group was statistically different from the DIC group ($p=0.003$) (Table II, Figure 3).

Table I: Physiological Parameters in the Study Groups

Group (n)	MAP (mmHg)	PaCO ₂ (mmHg)	PaO ₂ (mmHg)	pH	Plasma Glucose (mg/dL)	Rectal Temp. (°C)	Head Temp. (°C)
DIV (6)							
Baseline	91±7*	43.0±6.2	142.6±14.7	7.38±0.05	220±18 *	37.5±0.4	36.7±0.3
Ischemia	35±1 **	37.9±0.5	155.0±2.6	7.37±0.01	340±17**	37.4±0.4	36.6±0.3
Recovery	114±4	39.1±0.3	138.3±0.8	7.38±0.01	201±7	37.5±0.3	36.7±0.2
DIC (6)							
Baseline	107±3	45.0±4.4	134.0±16.9	7.37±0.02	183±5	37.4±0.3	36.6±0.3
Ischemia	35±1**	37.8±0.5	153.0±2.3	7.38±0.01	334±12**	37.4±0.5	36.7±0.3
Recovery	117±5	39.5±0.3	139.6±1.3	7.37±0.03	193±6	37.1±0.2	36.9±0.4
SIC (6)							
Baseline	139±11	40.0±5.3	125.0±22.3	7.40±0.02	165±10	37.0±0.1	36.6±0.2
Ischemia	35±1**	37.3±0.8	154.0±2.7	7.37±0.01	315±21**	37.0±0.2	36.7±0.1
Recovery	122±7	39.2±0.2	138.5±2.1	7.39±0.04	188±3	37.1±0.3	36.8±0.3
CONTROL(6)							
Baseline	135±6	44.0±5.8	125.3±21.4	7.40±0.02	171±4	37.1±0.2	36.8±0.2
Ischemia	35±1**	37.6±0.6	152.6±2.2	7.37±0.02	324±24**	37.0±0.1	36.8±0.2
Recovery	119±5	39.1±0.2	141±3.2	7.39±0.04	193±5	37.2±0.3	36.9±0.3

Data are expressed as mean ± SD, * p<0.001 compared to CONTROL group and SIC group, ** p<0.05 compared to baseline within each group, n = number of animal, MAP = Mean arterial blood pressure, PaCO₂ = arterial carbon dioxide pressure, PaO₂ = arterial oxygen pressure, Temp = temperature, DIV = dexmedetomidine intravenous, DIC = dexmedetomidine intracisternal, SIC = Saline intracisternal.

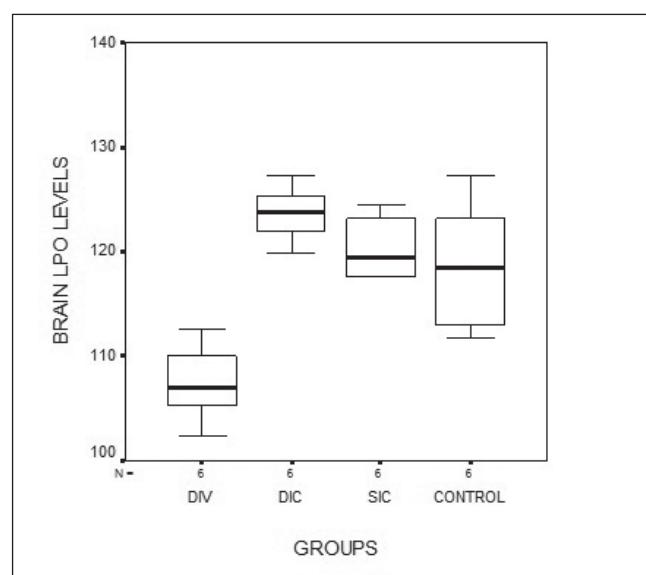


Figure 2: The mean values of the lipid peroxidation levels of the brain tissue of the groups. LPO = Lipid peroxidation; DIV = dexmedetomidine intravenous; DIC = dexmedetomidine intracisternal; SIC = Saline intracisternal, a = significant difference from DIV group.

Histopathological Analysis

There was a statistically significant difference among all groups regarding the picnotic neuronal cell counts in hippocampal DG region ($F=10.556$, $p<0.001$). When posthoc comparisons were made, statistically differences were observed between the DIV/DIC, DIV/SIC and DIV/CONTROL groups ($p<0.001$, $p=0.01$, and $p=0.009$, respectively) (Table II, Figure 4).

Histopathological examination of the DIC, SCI and CONTROL groups demonstrated severe edema both at neuropil and perivascular area. Disappearance of typical cellular arrangement, irregularities of intercellular organization, cellular swelling, intracellular vacuoles, shrinkage of nuclei and vascular stasis were observed (Figure 5 A,B,C, Figure 6 A,B,C). The number of excessively degenerated picnotic neurons in the DIC group was as more as in the SCI and CONTROL groups and the beginning of nuclear degenerative changes were observed nearly in all of the hippocampal neuronal cells in all hippocampal subfields (Figure 7 A,B,C). In contrast to the DIC group, the distribution of the picnotic neuronal cells was inhomogeneous in the SIC and CONTROL groups and the number of picnotic cells were especially more in the DG of the hippocampus than the CA1, CA2 and CA3 hippocampal

Table II: The Mean Values of the Pictonic Neuronal Cell Counts, Lipid Peroxidation Levels of Brain Tissue, Plasma Lipid Peroxidation Levels of the Study Groups

Group		n	Minimum	Maximum	Mean	SD
CONTROL	HPNC	6	7.333	65.000	42.000	22.752
	PLPO	6	3.538	4.692	4.025	0.437
	BLPO	6	111.692	127.346	118.672	6.313
SIC	HPNC	6	23.667	59.667	41.055	14.624
	PLPO	6	3.192	4.692	4.051	0.647
	BLPO	6	117.615	124.384	120.223	2.874
DIC	HPNC	6	44.333	65.000	57.555	7.197
	PLPO	6	4.192	4.807	4.563	0.223
	BLPO	6	119.730	127.376	123.613	2.676
DIV	HPNC	6	8.333	17.667	12.500	4.267
	PLPO	6	3.269	3.961	3.531	0.281
	BLPO	6	102.432	112.538	107.398	3.570

n = number of animal, **SD** = standard deviation, **HPNC** = Hippocampal picnotic neuronal cell count, **PLPO** = plasma lipid peroxidation level, **BLPO** = lipid peroxidation level of the brain tissue; **DIV** = dexmedetomidine intravenous, **DIC** = dexmedetomidine intracisternal, **SIC** = Saline intracisternal,

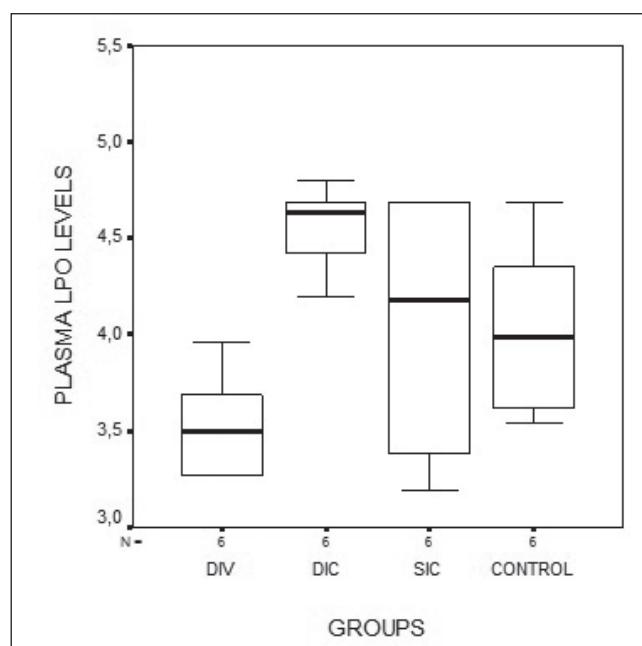


Figure 3: The mean values of the plasma lipid peroxidation levels of the groups. **LPO** = Lipid peroxidation; **DIV** = dexmedetomidine intravenous; **DIC** = dexmedetomidine intracisternal; **SIC** = Saline intracisternal; **a** = significant difference from DIC group.

regions. The histopathological findings were less severe in the DIV group. There was minimal edema at neuropil and perivascular area. The degenerated neuronal cell counts were significantly less than the other study groups and the degree of degeneration, irregularities of intercellular organizations were less severe than the other groups (Figure 8 A,B,C).

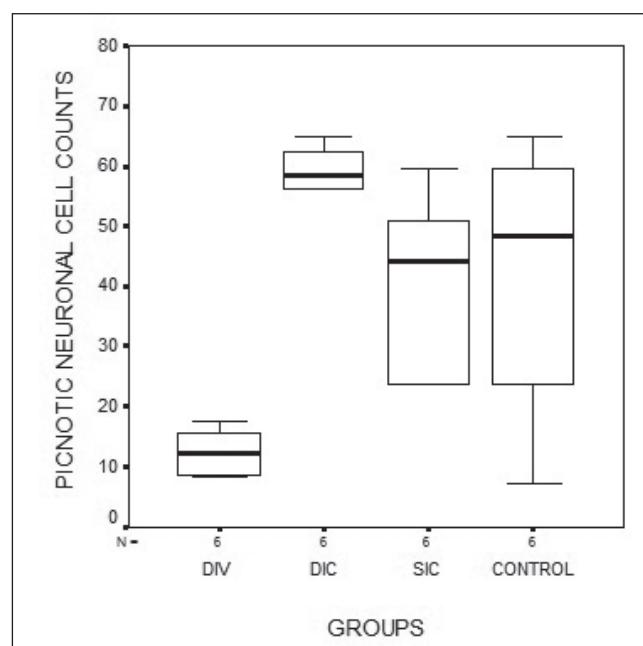


Figure 4: Hippocampal picnotic neuronal cell counts of groups. **DIV** = dexmedetomidine intravenous; **DIC** = dexmedetomidine intracisternal; **SIC** = Saline intracisternal; **a** = significant difference from the DIV group.

Additionally, although the count of degenerated neurons was inconsiderable in the DG region of hippocampus, it was increasing toward to CA2 and CA1 regions in the DIV group. The DIV group was distinguished as a more protected group against ischemia.

DISCUSSION

Dexmedetomidine is a selective α_2 agonist for which labeled indication is intensive care unit sedation. It can produce cooperative sedation associated with minimal respiratory depression and some analgesia (24). Those properties make it useful in many clinical situations, including the sedation of intensive care unit patients in whom the ability to perform frequent neurologic examination is of clinical importance. Additionally, because of the same properties, using this agent may be advantageous in central nervous

system related procedures such as awake craniotomy, carotid endarterectomy with regional anesthesia, carotid angioplasty and stenting. Many experimental studies have reported that dexmedetomidine provides neuronal protection against cell death by preventing ischemia when administered systemic circulation by intravenous or intraperitoneal routes (9, 11, 14,, 16, 17, 22). Decreased sympathetic tonus due to inhibition of noradrenalin release via presynaptic α_2 -adrenoreceptors or N-methyl-D-aspartate (NMDA) antagonism has been classically attributed as the neuroprotective effects of dexmedetomidine (9, 11, 14, 16, 17, 23). Decreased release of noradrenalin can alleviate potential detrimental effects of metabolizing excessive noradrenalin which can lead to the

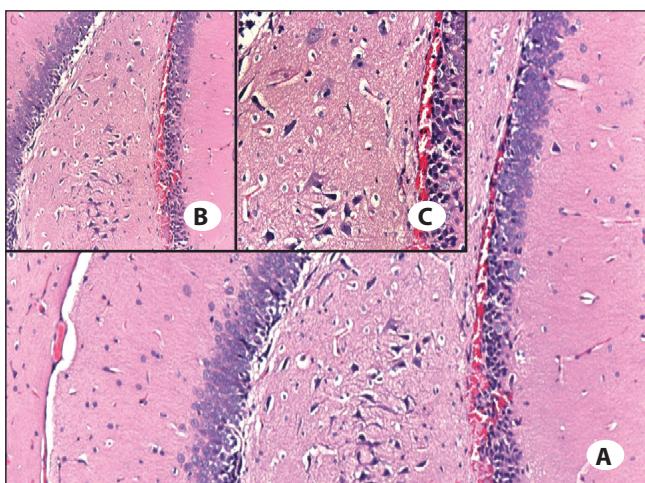


Figure 5: Histopathological specimen of the CONTROL group demonstrating severe degeneration and homogenizing cell changes in the dentate gyrus sector of the hippocampus and disappearance of typical cellular arrangement. (A, H&E X 200); (B, H&E X 100); (C, H&E X 400).

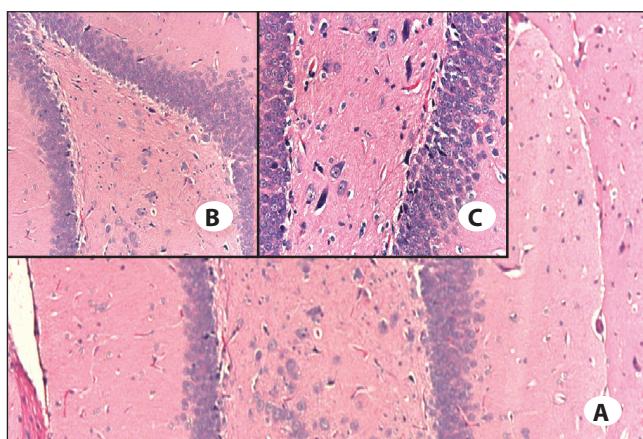


Figure 6: Histopathological specimen of the SIC group demonstrating homogenizing cell changes of neurons in the dentate gyrus sector of the hippocampus, with a slightly more organized cellular arrangement but there is still degeneration in many cells. (A, H&E X 200); (B, H&E X 100); (C, H&E X 400).

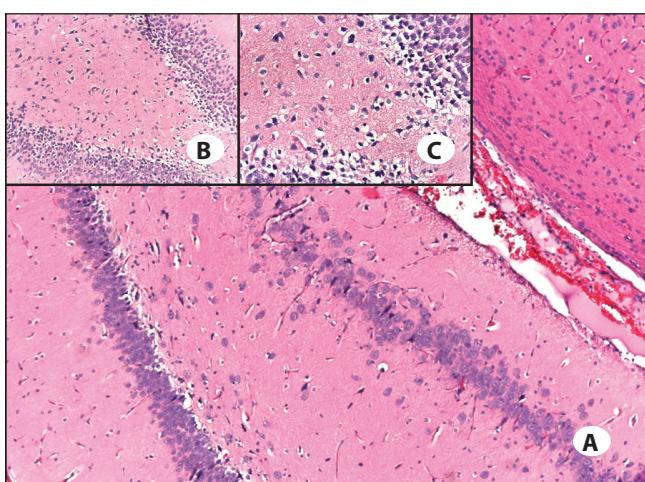


Figure 7: Histopathological specimen of the DIC group demonstrating severe degeneration and homogenizing cell changes in the dentate gyrus sector of the hippocampus. (A, H&E X 200); (B, H&E X 100); (C, irregularities in the borders of nuclei and homogenization of cytoplasm, beginning of nuclear degenerative changes were observed nearly in all of the hippocampal neuronal cells, H&E X 400).

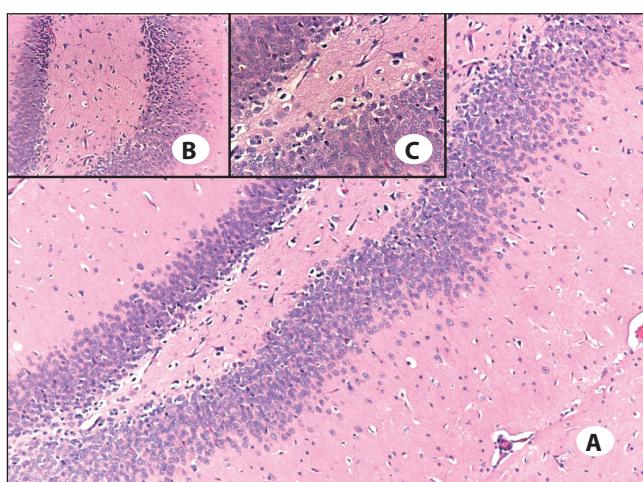


Figure 8. Histopathological specimen of the DIV group demonstrating preservation of intercellular arrangements of neurons in the dentate gyrus sector of the hippocampus. (A, H&E X 200); (B, H&E X 100); (C, a decreased number of degenerating cells, H&E X 400).

formation of free radicals (14). In addition, neuroprotective effect of dexmedetomidine may be due to a postsynaptic reduction in neuronal excitability and/or a possible presynaptic decrease in glutamate release (2, 32). Engelhard et al. proposed that dexmedetomidine prevented apoptotic processes induced by incomplete cerebral ischemia/reperfusion and upregulated anti-apoptotic proteins Bcl-2 and Mdm-2 (5). On the other hand, it is known that α_2 -adrenoceptors on presynaptic membranes of serotonergic and dopaminergic nerve terminals modulate the release of serotonin (5-HT) and dopamine (3, 30). It was reported that dexmedetomidine caused a dose-dependent decrease in the turnover of noradrenalin, serotonin and dopamine in mice when used at doses of more than 30 $\mu\text{g}/\text{kg}$ (18). Despite of all experimental studies, the neuroprotective effect mechanism of systemically administered dexmedetomidine and whether this effect is produced by presynaptic or postsynaptic action is still not clear. To the best of our knowledge, there is no study in the medical literature about the effect of centrally administered dexmedetomidine on incomplete cerebral ischemia. In previous studies, the anti-ischemic effects of dexmedetomidine have been shown at much higher doses (up to 100 $\mu\text{g}/\text{kg}$) (6, 11, 22). On the other hand, there are experimental studies which were reported the neuroprotective effect of dexmedetomidine when used in small doses (7, 9, 17). Kuhmonen et al. have reported in their study that 3 $\mu\text{g}/\text{kg}$ dose of dexmedetomidine was more effective than 30 $\mu\text{g}/\text{kg}$ dose and this result was compatible with a previous study, which showed a U-shaped dose-response curve against kainic acid-induced convulsions (10, 17). Nakano et al. have reported in their dose ranging study that, although following the administration of dexmedetomidine at doses of 0.01 $\mu\text{g}/\text{kg}/\text{min}$, 0.1 $\mu\text{g}/\text{kg}/\text{min}$ and 1 $\mu\text{g}/\text{kg}/\text{min}$ had neuroprotective effect; hypertension following the administration of high-dose (10 $\mu\text{g}/\text{kg}/\text{min}$) dexmedetomidine was associated with cerebral hypoperfusion and exacerbation of ischemic brain injury (28). They have explained these dose-depending different effects of dexmedetomidine by the stimulation of α_{2B} adrenoceptor activation which overcomes the effect of α_{2A} adrenoceptors with the high doses (28). Likewise, Lahdesmaki et al. have reported that dexmedetomidine at doses of more than 30 $\mu\text{g}/\text{kg}$ caused a dose-dependent decrease in the levels of the principal noradrenalin metabolite and an increase in the level of noradrenalin (18). In this study, we used the lowest dose of dexmedetomidine (total dose 9 $\mu\text{g}/\text{kg}$) which is known for its neuroprotective effects by previous studies (7, 14) without any serious systemic adverse side effects. The same doses of dexmedetomidine were used for systemic administration via intravenous route and for central administration directly into cerebrospinal fluid in cisterna magna. So, it is possible that the brain concentrations of the drug were different in the DIC and DIV groups. But the dose used in this study, $8.4 \times 10^{-5} \text{ M}$, was lower than 10^{-3} M which was known its vasoconstrictive effect was counterbalanced by the activation of adenosine triphosphate sensitive K^+ channels (13) and the only study investigated the centrally administered 100 $\mu\text{g}/\text{ml}$ ($4.2 \times 10^{-4} \text{ M}$) concentration of

dexmedetomidine in dogs reported by McPherson et al (26). In order to, it is possible to see the free cerebrospinal fluid flow and the easiness of technique; we preferred to administer the agents used in the study into cisterna magna by an injection from posterior craniocervical junction of rats. Due to the fact that, cerebrospinal fluid pressure of rat is very low and aspiration with using negative pressure might be harmful, we did not aspirate any cerebrospinal fluid before the administration of intracisternal drug. However, it was thought that the administered 0.1 ml additional volume within one minute might produce additional harmful effect to ischemic neuronal injury by increasing cerebrospinal fluid pressure. Therefore, the SCI group was added to the study for the observation of this possible harmful effect of 0.1 ml additional volume to ischemic neuronal injury and 0.9% NaCl which was used as solvent of dexmedetomidine was given within one minute by intracisternal route.

Kuhmonen et al. reported in their study that there were differences in the number of surviving neurons between the various hippocampal subfields after the dexmedetomidine treatment and this condition might be related to differences in their anatomic connections (17). They explained these regional differences by the relative distribution and terminal fields of noradrenergic fibers within the different subfields of the hippocampus (17). The noradrenergic afferents arising from the locus ceruleus form a dense network within the hilus of the DG and in the stratum lucidum of the CA3 area (31). However, the noradrenergic input to the CA1 subfield is less extensive and terminates largely in the stratum lacunosum moleculare. Therefore, they concluded that any therapeutic treatment that targets noradrenergic systems would be more potent in the hilus and in the CA3 subfield than in the CA1 region of the hippocampus. Hence, the picnotic neuronal cell counts were made in the hippocampal DG region in this study.

The results of this study demonstrated that in contrast to given systemically via intravenous route; the intracisternal administration of dexmedetomidine had no neuroprotective effect in this model of incomplete cerebral ischemia in rat. The number of excessively degenerated picnotic neurons in the DIC group was as more as the SCI and CONTROL groups and the beginning of nuclear degenerative changes were observed nearly in all of the neuronal cells (Figure 7). The histopathological findings were less severe in the DIV group. The DIV group was distinguished as a more protected group against ischemia. This result may explain with the increase in cerebrovascular tonus and the reduction in cerebral blood flow. McPherson et al. demonstrated in their study that ventricular-cisternal perfusion of dexmedetomidine resulted in widespread cerebral blood flow reduction either normoxic or hypoxic conditions (26). They concluded that dexmedetomidine-induced vasoconstriction is sufficiently robust to limit cerebral oxygen delivery during hypoxia and resulted in reduced cerebral oxygen consumption. Another explanation can be centrally administered dexmedetomidine did not reduce the circulating catecholamines. Engelhard et al. showed that the increase of circulating catecholamine

concentrations during cerebral ischemia was suppressed with systemically administered dexmedetomidine (6). In contrast, dexmedetomidine did not suppress elevation in brain noradrenalin and glutamate concentration associated with cerebral ischemia. And they suggested that the neuroprotective effect of dexmedetomidine was not related to inhibition of presynaptic noradrenalin or glutamate release in the brain (6). In addition, Hoffman et al reported that systemically administered α_2 -agonist dexmedetomidine improved outcome from incomplete ischemia by an action correlated with plasma catecholamines and this improved outcome abolished by the α_2 -antagonist atipamezole during ischemia (11). Experiments using isolated canine stellate ganglia have shown that dexmedetomidine inhibited the synaptic conduction by decreasing the postsynaptic response to a late, slow excitatory presynaptic stimulation through an α_2 -receptor-mediated process (25). Additionally, studies in isolated primary cortical neurons demonstrated that dexmedetomidine had neuroprotective effect which could be reversed by the α_2 -receptor antagonist yohimbine (19). Therefore, it is possible that dexmedetomidine is neuroprotective by postsynaptic action with consecutive reduction in Ca^{+2} currents. It is also known that high catecholamine concentrations increase the sensitivity of pyramidal neurons to the excitatory neurotransmitters such as glutamate which result in elevated intracellular Ca^{+2} concentrations with consecutive activation of intracellular catabolic enzymes (21). Also, we can not rule out the possibility that postsynaptically located α_{2A} -adrenoceptor subtypes may contribute to the neuroprotective effect of dexmedetomidine. At this site, activation of α_{2A} -adrenoceptor subtype can result in hyperpolarization via K^+ channel activation (24). Under these conditions, the NMDA receptor may be vigorously blocked by endogenous Mg^{+2} through a voltage dependent mechanism (24).

It is known that ischemia enhances the formation of reactive oxygen species (ROS) in brain tissue (36). It also enhances the activation of brain resident cells such as microglia localized within the ischemic region, and rapid synthesis of cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), and chemokines (1). Reactive oxygen species can cause cellular damage by oxidizing membrane lipids, essential cellular proteins and DNA. The impairment in membrane permeability can cause a decrease in membrane-bound Na^+K^+ ATPase enzyme activity. As a result, The K^+ and Mg^{+2} concentrations that are vitally important for protein synthesis are altered, and protein synthesis is inhibited. Increased lipid peroxidation also result in the release of proteolytic lysosomal enzymes and mitochondrial matrix enzymes into cytoplasm, which give rise to intracellular proteolysis and cellular destruction (35). It is reported in several studies that systemically administered dexmedetomidine could alleviate this ischemia-induced reactions through different mechanisms: Decreases systemic catecholamine concentrations (6, 11, 14), and decreases the level of pro-inflammatory cytokines such as TNF- α and IL-6 (7). In this study,

LPO levels were lower in both brain tissue and plasma in the DIV group than the other study groups and these biochemical data were compatible with the histopathologic data of the study. Although we can not explain the exact mechanism, it can be said that systemically administered dexmedetomidine could inhibit the lipid peroxidation and related neuronal damage in this incomplete brain ischemia model in rat, but intracisternal administered dexmedetomidine could not.

We did not observe any significant decrease in MAP in the DIC group as similar to the study reported by McPherson et al (26). Also any significant increase in blood glucose concentration was not observed in the DIC group. So, we can strongly suggest that there was not a significant absorption into the systemic circulation. Despite of the observed significant decrease in baseline MAP and significant increase in glucose concentration mediated with α_2 -adrenergic inhibition of insulin release, the histopathological findings were less severe in the DIV group and the DIV group was distinguished as a more protected group against ischemia. Our results were compatible with the previous studies reported that neuroprotective effect of systemically administered dexmedetomidine was not related to the differences in blood pressure or plasma glucose concentration (11, 14).

This prospective experimental study has some pitfalls. Firstly, this study could not be supported by immunohistochemical, and electron microscopic findings. Secondly, because the aim of the study was to investigate the early histopathological and biochemical changes in brain tissue after ischemia-induced neuronal damage, neurologic outcome was not evaluate and this study did not contain observations of functional outcome data and histopathological and biochemical evaluations occurring in the long run.

In conclusion, despite causing an increase in plasma glucose level and hypotension, systemically administered dexmedetomidine had neuroprotective effect in ischemia-induced neuronal damage in this incomplete cerebral ischemia model in rat. But centrally administered dexmedetomidine had no neuroprotective effect in ischemia-induced neuronal damage. So, although the effect mechanism and/or mechanisms is not clear, it can be suggested that the beneficial effect of dexmedetomidine was related with absorption into systemic circulation and may be at least partly related with its postsynaptic action.

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