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SCIENTIFIC ARTICLE

Histopathologic comparison of dexmedetomidine's and thiopental's cerebral protective effects on focal cerebral ischemia in rats

Ebru Çanakçı[a,*], **Sevilay Akalp Özmen**[b], **Mustafa Ferhat Çolak**[c], **Hüsnü Kürşad**[d]

[a] *Ordu University, Training and Research Hospital, Clinic of Anesthesiology and Reanimation, Ordu, Turkey*
[b] *Erzurum Training and Research Hospital, Department of Pathology, Erzurum, Turkey*
[c] *Ordu State Hospital, Clinic of Anesthesiology and Reanimation, Ordu, Turkey*
[d] *Atatük University, Faculty of Medicine, Research Hospital, Clinic of Anesthesiology and Reanimation, Erzurum, Turkey*

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KEYWORDS
Brain ischemia;
Dexmedetomidine;
Thiopental;
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Abstract This study was designed to investigate whether dexmedetomidine and thiopental have cerebral protective effects after focal cerebral ischemia in rats. Thirty male Sprague Dawley rats were randomly assigned to three groups: control group (Group C, $n = 10$), dexmedetomidine group (Group D, $n = 10$), thiopental group (Group T, $n = 10$). After all rats were anesthetized, they were intubated, then mechanically ventilated. A catheter was inserted into the right femoral artery for continuous mean arterial pressure, physiological parameters and blood sampling at baseline, 5 min after occlusion and 20 min after reperfusion. A catheter was inserted into the left femoral vein for intravenous (IV) medication administration. Right common carotid artery of each rat was isolated and clamped for 45 min. At the end of the duration common carotid artery were unclamped and the brain reperfusion was achieved for 90 min. Dexmedetomidine was administered for Group D IV infusion, and Group T received thiopental IV. According to histopathologic scores cerebral ischemia was documented in all rats in Group C, but no ischemia was found in three rats in Group T and in four rats in Group D. Grade 3 cerebral ischemia was documented in three rats in Group C, and in only one rat in both groups T and D. For histopathologic grades the difference between Group T and Group D was not significant ($p > 0.05$). But the differences between Group C and Group T ($p < 0.05$) and Group C and Group D ($p < 0.01$) were statically significant. In conclusion, we demonstrated that dexmedetomidine and thiopental have experimental histopathologic cerebral protective effects on experimental focal cerebral ischemia in rats.

* Corresponding author.
E-mail: canakciebru@gmail.com (E. Çanakçı).

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PALAVRAS-CHAVE
Isquemia encefálica;
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Tiopental;
Neuroproteção

Comparação histopatológica dos efeitos protetores cerebrais de dexmedetomidina e tiopental sobre isquemia cerebral focal em ratos

Resumo Este estudo foi desenhado para investigar se dexmedetomidina e tiopental possuem efeitos protetores cerebrais após isquemia cerebral focal em ratos. Trinta ratos da linhagem *Sprague Dawley* foram randomicamente alocados em três grupos: controle (Grupo C, n = 10), dexmedetomidina (Grupo D, n = 10) e tiopental (Grupo T, n = 10). Após a anestesia, todos os ratos foram intubados e ventilados mecanicamente. Um cateter foi inserido na artéria femoral direita para monitoração contínua da pressão arterial média (PAM), dos parâmetros fisiológicos e para coleta de amostras de sangue na fase basal, 5 minutos após a oclusão e 20 minutos após a reperfusão. Um cateter foi inserido na veia femoral esquerda para administração intravenosa (IV) de medicamentos. A artéria carótida comum direita de cada rato foi isolada e pinçada durante 45 minutos. Ao final dos 45 minutos, o pinçamento foi removido e a reperfusão do cérebro foi obtida por 90 minutos. Dexmedetomidina foi administrada por infusão IV no Grupo D e thiopental no Grupo T. De acordo com as pontuações histopatológicas, isquemia cerebral foi observada em todos os ratos do Grupo C, mas não foi encontrada em três ratos do Grupo T e em quatro ratos do Grupo D. O grau 3 de isquemia cerebral foi observado em três ratos do grupo C e em apenas um rato de ambos os grupos T e D. Para os graus histopatológicos, a diferença entre o Grupo T e o Grupo D não foi significativa ($p > 0,05$). Porém, as diferenças entre o Grupo C e o Grupo T ($p < 0,05$) e entre o Grupo C e o Grupo D ($p < 0,01$) foram estatisticamente significativas. Em conclusão, demonstramos que dexmedetomidina e tiopental possuem efeitos histopatológicos protetores cerebrais sobre isquemia cerebral focal experimental em ratos.

Introduction

It has been demonstrated by prospective epidemiological studies conducted in developed western countries that cerebrovascular diseases (CVDs) are responsible for 10% of all deaths and are ranked third after heart diseases and cancer as a cause of mortality.[1–3] About 75% of CVDs result from thrombotic or embolic cerebral infarcts.[2,4]

The infarct model provided by occlusion of experimental middle cerebral artery (MCA) representing the focal cerebral infarction which is the most common type of CVD in clinical practice has been widely accepted.[2,4,5] Rats bear a very close resemblance to the human brain in terms of cerebrovascular anatomy and physiology and thus, they are widely used in cerebral ischemia studies.[3] The validity of this model of cerebral ischemia highly depends on strict control of physiological parameters which may cause fluctuations and changes in the results such as body temperature, blood pressure, blood gases, and glucose levels.[4]

In clinical use, there is still no ideal medication which bears all the desired characteristics and is effective enough in cerebral ischemia.[6,7] In order to respond to clinical needs, in animal models, a large number of agents from different chemical groups which is thought to have beneficial effects in cerebral ischemia are still being tested and their effects on cerebral ischemia are methodologically examined.[2,4] The novel molecular-level information on the pathophysiology of cerebral ischemia have led to the use of numerous potential therapeutic agents estimated or alleged to be effective on such pathophysiologic mechanisms in experimental studies.[8,9]

The effects of barbiturates as well as the thiopental, an agent mostly used in anesthesia practice in this class, on cerebral blood flow and cerebral metabolism were researched and found to cause a decrease in cerebral blood flow and cerebral metabolic rate depending on the dose. It has been observed that cerebral oxygen metabolism is reduced by 55–60% at a barbiturate dose causing isoelectricity in EEG; however, cerebral metabolism proceeded and the cerebral basal metabolism that is necessary to ensure physiological functions and cell integrity of neurons did not depressed through a further increase of the blood barbiturates dose.[10,11]

There are different views about whether or not barbiturates are neuroprotective. In post-traumatic focal cerebral ischemia; they are found to reduce hyperemia by making a deep depression in the cerebral metabolic rate, improve the harmony of flow & metabolism, increase perfusion in the low-flow region and have brain protective effect by minifying the infarcted area.[12,13] Thiopental and methoheksital were again studied at different doses in the models of focal cerebral ischemia in rats and were histopathologically shown to reduce infarct volume at doses causing burst suppression in EEG.[14]

Dexmedetomidine is a new medication gained popularity in anesthesia practice. A α-2 receptor agonist, this medication provides analgesia without cooperative sedation, anxiolysis and respiratory depression. Alpha 2 adrenoceptors show a broad dissemination in cerebral vascular bed and activation of these receptors causes a specific vasoconstrictive response. Although being very common in the cerebral vasculature, their effects on the control of

cerebral blood flow and the cerebrovascular reactivity is still unclear.

It has been observed that administering dexmedetomidine in focal cerebral ischemia reduces the infarct volume in the cortex by 40% and in addition to this, causes minimal hyperglycemia and hypotension.[15] It has been also observed that, through administration of dexmedetomidine in incomplete cerebral ischemia, the plasma catecholamine levels were decreased and the histopathological improvement became better than control, depending on the dose.[16] Besides, in terms of the infarct volume after temporary occlusion (15 mcg/kg), a 31% decrease in cortex and 20% decrease in striatum were reported in rats given the high dose of dexmedetomidine.[17] It has been also reported that, while circulating catecholamines were decreasing during cerebral ischemia, the noradrenaline and glutamate concentrations in the brain were not affected by dexmedetomidine.[18] The α-2 adrenoceptor subtypes causing neuroprotective effects were reported to be α-2A.[19]

Purpose

The purpose of this study is to histopathologically study whether or not thiopental and dexmedetomidine which are among the intravenous anesthetics have cerebral protective effects on focal cerebral ischemia in rats.

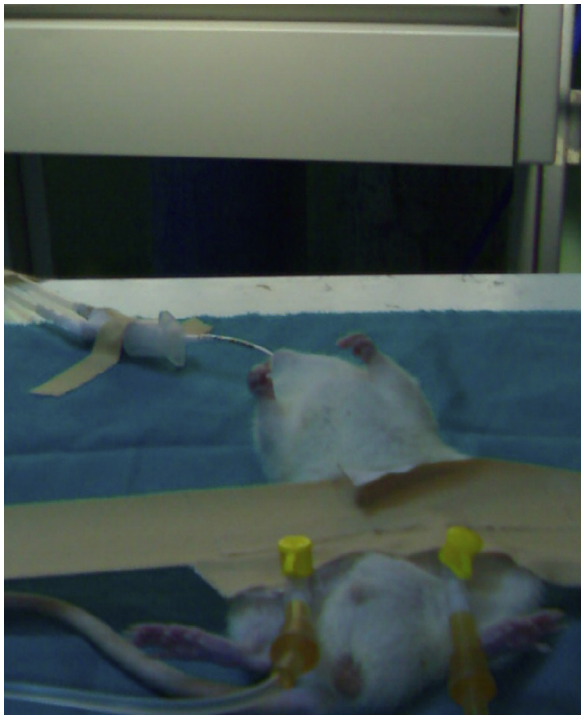

Figure 1 One of the rats used and intubated in the study.

Materials and methods

This study was carried out in Atatürk University, Experimental Medical Research and Application Center (ATADEM) with permission from the ethics committee established by Atatürk University, the Office of the Dean of the Faculty of Medicine. The five-month-old Sprague Dawley-type 30 male rats which are 250–300 g in weight were obtained from ATADEM.

For the sake of having safe and healthy research results, particular attention was paid to select subjects which were not used in a study before and were not exposed to any medication as well as which did not have any disease and were fully healthy during our experiments.

No subjects included in the study were fed 12–16 h before the study in order to keep them hungry. In the meantime, each subject's weight was determined and recorded. Then the rats were randomly divided into three groups: control group (Group C, $n = 10$); dexmedetomidine group (Group D, $n = 10$); thiopental group (Group T, $n = 10$).

All subjects were anesthetized with 5% isoflurane (ForanefJ, Abbott, Istanbul, Turkey) in the glass vase through the anesthesia machine in the research laboratory of ATADEM. Neck regions of the subjects in the supine position were shaved and the sterilization of the surgical site was achieved. Their heads were slightly extended and the midline pretracheal surgical incision was made. Subsequently, the trachea was intubated (Fig. 1) and their lungs began to be mechanically ventilated. Along with the ventilation, 2% isoflurane, 30% oxygen and 70% nitrous oxide was given to subjects. Ventilation was applied in a way to fix the number of breaths per minute to 60 and the tidal volume to 12 mL/kg. Ventilation was continued by achieving 35 mmHg of end-tidal carbon dioxide. A catheter was inserted into the right femoral artery in order to continuously monitor the mean arterial pressure and take blood sample and into the left femoral vein for medication administration. Immediately after arterial cannulation (basal values); all physiological parameters (MAP; body temperature) were recorded three times for three times including at the 5th minute of arterial occlusion and 20th minutes of reperfusion and, blood sample was obtained from the left femoral vein in order to determine pH, pO_2, pCO_2, glucose and hematocrit values in the arterial blood gas and sent to the laboratory of the Department of Biochemistry.[5,20] Their left femoral veins were, however, cannulated for the infusion of anesthetic medications and heparinization. For the motorization of mean arterial pressure (MAP), a radio-telemetry device probe was connected to the tails of rats. Rats were studied on blanket in order to keep them at normothermic level.[15] Subsequently, their internal carotid arteries were found through side neck dissection (under sterile conditions) (Fig. 2) and occlusion was achieved by a vascular clamp for a duration of 45 min (Figs. 3 and 4). After 45 min, the vascular clamp was opened and brain reperfusion was achieved for 90 min duration. During occlusion and reperfusion, dexmedetomidine infusion was administered to Group D at a rate of 9 mcg/kg/h and thiopental infusion was administered to Group T at a rate of 140 mcg/kg/h[20] while no intravenous anesthetic medication was administered to control group. At the end of reperfusion, 0.1 mg/kg vecuronium bromide (Norcuron, Roche, Turkey) was administered to rats in order to achieve ideal muscle relaxation and their brains were duly removed through temporal craniotomy. The brains were put into 10% formalin solution in order to be sent to the Department of Pathology and were evaluated for an appropriate histopathological analysis.

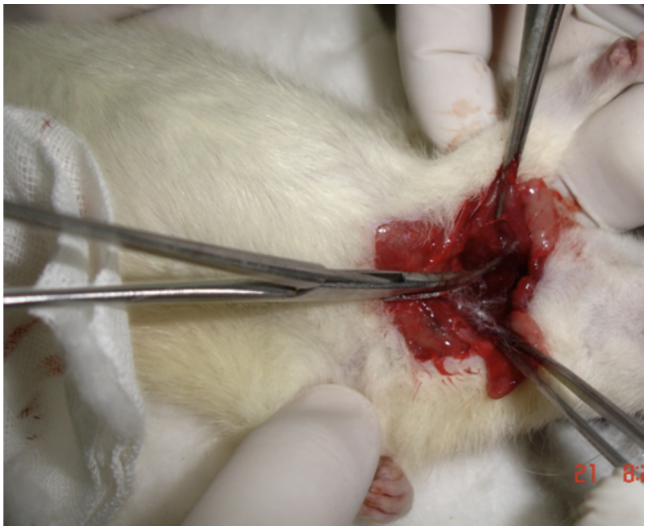

Figure 2 One of the rats to which a right carotid artery dissection was performed.

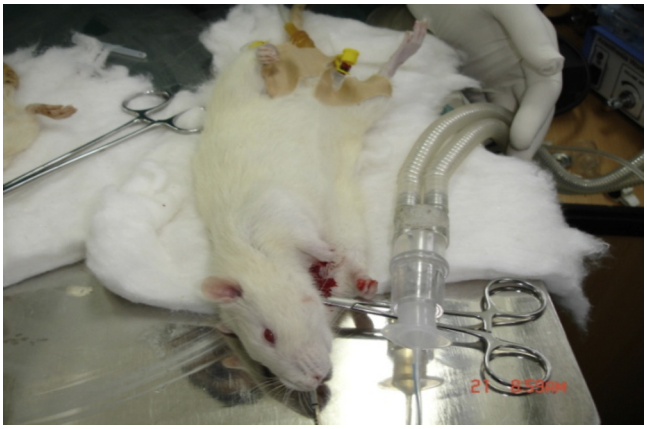

Figure 3 The rat to which a right carotid artery occlusion was performed.

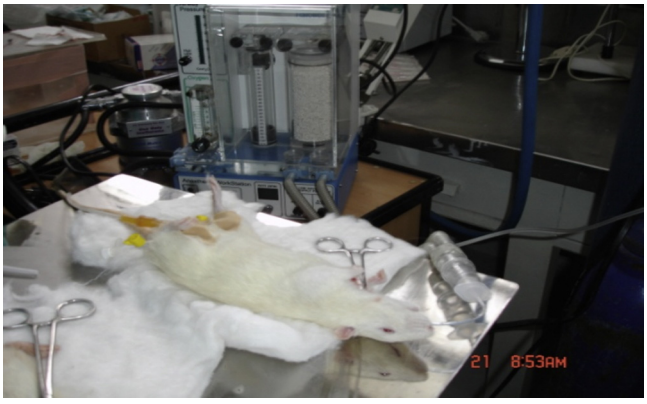

Figure 4 The intubated rat to which right carotid artery occlusion was performed and the anesthesia apparatus used.

Immediately after ischemia was developed, all subjects were killed by decapitation under anesthesia and were sacrificed. For histopathological examination, all the brains removed in whole were treated in the following sequence: The brains were put in 10% phosphate-buffered formalin for duration of 48 h. Hippocampus cornuammonis 1 (CA 1) zone was detected (in the coronal plane, 3300 μm posterior to Bregma). The tissue samples taken were dehydrated with ethanol and were embedded into paraffin blocks after passing through xylol; then, 4 mm thick coronal slices were collected; they were stained with hematoxylin–eosin and 5 mm coronal slices were collected, the analysis was evaluated, through 400× magnification, by a pathology specialist experienced in this field who had no knowledge of the groups. Based histopathologically on the Nissl substance loss, increase in cytoplasmic eosinophilia and the presence of pyknotic homogenous nucleus, the ischemic neurons were evaluated as Grade O = ischemic neurons, Grade 1 = presence of ischemic neurons less than 10%, Grade 2 = presence of ischemic neurons between 10% and 50% and Grade 3 = presence of ischemic neurons more than 50% according to the frequency of ischemic neurons in CA 1.

The brains from rats in all groups were given code numbers and put into bottles containing 4% formaldehyde in order to ensure fixation. Then the tissues were washed under streaming water and passed through respectively 70%, 80%, 96% and 100% alcohol series for dehydration. In the next step, the tissues were passed through xylene series for transparency. Finally, they were kept in paraffin at 60 °C heated oven and embedded into paraffin blocks.5 μm-thick slices were taken from the paraffin blocks obtained and kept in an oven at 60 °C for duration of 45 min. Then, they were passed through xylene for 2 times and graded alcohol series for 4 times (respectively 100%, 96%, 80%, 70%); washed in streaming water and plunged into acid alcohol after being stained with hematoxylin for 30 s. Then they were stained with eosin for 1 min, washed again and treated with xylene after passing through the series of alcohol. At the last stage, Entellan (Merc) was dropped on them and they were put under lamella. Then, stereological counting was started.

Based on the data obtained from two-dimensional projections of three-dimensional objects, stereology is a discipline that enables us to obtain information on real properties and numerical values of biological structures (size, number, surface area, volume, etc.).[20] There are several methods used in stereology. Among them is the fractionator method which we used in our study. Through this method, we can learn many details about the structure on hand (surface area, density, etc.). In this study, we calculated the numerical density of neurons for all groups.[20]

The slices ready for analysis were examined under the light microscope with Olympus BH 40 brand camera attachment and the photographs of all relevant groups were taken. In this study, we used fractionator method, which is one of stereological methods, as well as *unbiased counting frame* and *Stereo Investigator* (version 6.0, Micro Bright Field, Colchester, VT) with special software. The said apparatus consists of a microscope camera, a motorized systems moving the microscope table and a computer containing software to control their use. In the study, the slices obtained from each animal were placed in the microscope table and the contours of the area (cortex) on which we will make measurements were drawn with the help of a program through 2.5× magnification. After determining the areas to be measured, the systematic random sampling command was given to the apparatus at a magnification of 40. According to this command, the step intervals were determined as

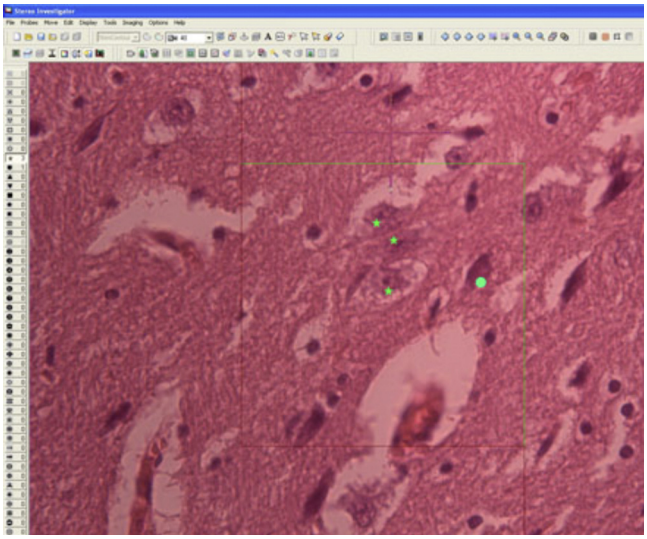

Figure 5 Counted neurons are seen in the unbiased counting frame at a magnification of 40 in the Stereo-Investigator (version 6.0, Micro Bright Field, Colchester, VT). Stars indicate intact neurons and circles indicate necrotic neurons.

150–150 μm in the *x*–*y* plane and 75 μm × 75 μm in the area of the unbiased counting frame. Then, the unbiased counting frame was placed onto the section (the software in the computer enables this) and the intact and damaged neurons in the cortex were counted by using different markers and in accordance with the stereological rules. Each animal's stereologically numerical density of neurons was calculated (Fig. 5).

Statistical analysis

For statistical analysis of the data between groups, Windows-compatible SPSS (version 10.0) program was used. Cerebral ischemia scores between groups and the weights of the subjects were statistically evaluated using respectively chi-square test in the 4 × 2 contingency table and independent sample *t*-test. ANOVA (Two Way Variance Analysis) was used for statistical evaluation of physiological parameters. While comparing the groups with each other, ''*p*-value'' was obtained and interpreted using the LSD (Least Square Difference) method. $p < 0.05$ was considered as significant.

The data obtained by stereological views were uploaded to the SSPS version 15.0. Data of the intact group and that of the necrotic group were compared with each other. Mann–Whitney *U* test was used for statistical analysis. In the comparison between the data of intact group and that of the necrotic group, it was found to be statistically significant in three groups.

Findings

Their physical data of the subjects are shown in Table 1. No statistical difference was found between the weights of all subjects ($p > 0.05$). When the table was examined in detail, no statistical difference was found between mean arterial pressure, heart rate, body temperature, hematocrit, blood glucose, HCT, PO_2, PCO_2 and pH at the stages of start before ischemia and occlusion and reperfusion after ischemia between the three groups ($p > 0.05$). For instance, initial values of mean arterial pressure were found to be 104.9 ± 6.7 in the control group (Group C), 105.1 ± 4.1 in the dexmedetomidine group (Group D) and 105.8 ± 5.5 in the thiopental group (Group T). When compared statistically, no differences were observed in these values. Again, the occlusion values of the mean arterial pressure were found to be 136.9 ± 3.9 in the control group (Group C), 37.1 ± 4.1 in the dexmedetomidine group (Group D) and 37.3 ± 3.9 in the thiopental group (Group T). In reperfusion, however, these values were found to be respectively 96.9 ± 4.4, 95.5 ± 5.2 and 96.1 ± 4.7. When compared statistically, no differences were observed in these values.

Table 1 also provides statistical comparison of subjects' physiological values between start, occlusion and reperfusion values within each group.

It was found out that while the initial value of the mean arterial pressure was found to be 104.9 ± 6.7 in the control group (Group C), it decreased to 36.9 ± 3.9 in occlusion and raised to 96.9 ± 4.4 again during the reperfusion. When the values for this group were statistically compared, a statistically significant difference was found between the initial value of the mean arterial pressure and that of the occlusion value as well as the value of occlusion and the value of reperfusion ($p < 0.001$). A significant difference was also found between the initial value and the value of reperfusion ($p < 0.05$). However, similar statistical differences were found to be between the initial, occlusion and reperfusion values of mean arterial pressure in the dexmedetomidine and thiopental groups.

While the initial value of heart rate was 329.3 ± 20.4 in the control group (Group C) 329.3 ± 20.4, they were found to have decreased to 304.9 ± 23.6 in occlusion and to 311.2 ± 27.3 in reperfusion. A statistically significant difference was detected between the initial, occlusion and reperfusion values of the heart rate ($p < 0.05$). Again in the dexmedetomidine and thiopental groups, similar statistical differences were found between the initial, occlusion and reperfusion values of the heart rate.

It was found out that while the initial value of the body temperature was found to be 37.89 ± 0.17 in the control group (Group C), it decreased to 36.92 ± 0.14 in occlusion and reached to 37.54 ± 0.10 again during the reperfusion. When the values for this group were statistically compared, a statistically significant difference was found between the initial value of the body temperature and that of the occlusion value as well as the value of occlusion and the value of reperfusion ($p < 0.05$). No significant difference was found between the initial value and the value of reperfusion. However, similar statistical differences were found to be between the initial, occlusion and reperfusion values of body temperature in the dexmedetomidine and thiopental groups.

It was found out that while the initial value of pO_2 was found to be 92.1 ± 3.3 in the control group (Group C), it decreased to 54.2 ± 3.8 in occlusion and reached to 77.2 ± 4.2 again during the reperfusion. When the values for this group were statistically compared, a statistically significant difference was found between the initial value of the pO_2 and that of the occlusion value as well as the value of occlusion and the value of reperfusion ($p < 0.001$).

A significant difference was also observed between the initial value and the value of reperfusion ($p < 0.001$). Again, similar statistical differences were found to be between the initial, occlusion and reperfusion values of pO_2 in the dexmedetomidine and thiopental groups.

It was found out that while the initial value of pCO_2 was found to be 35.7 ± 1.6 in the control group (Group C), it reached to 40.2 ± 1.2 in occlusion and decreased to 37.5 ± 1.7 again during the reperfusion. When the values for this group were statistically compared, a statistically significant difference was found between the initial value of the pCO_2 and that of the occlusion value as well as the value of occlusion and the value of reperfusion ($p < 0.01$). A significant difference was also observed between the initial value and the value of reperfusion ($p < 0.05$). Again, similar statistical differences were found to be between the initial, occlusion and reperfusion values of pCO_2 in the dexmedetomidine and thiopental groups.

It was found out that while the initial value of glucose was found to be 135.4 ± 2.7 in the control group (Group C), it reached to 142.9 ± 1.6 in occlusion and decreased to 139.7 ± 1.9 again during the reperfusion. When the values for this group were statistically compared, a statistically significant difference was found between the initial value of the glucose and that of the occlusion value as well as the value of occlusion and the value of reperfusion ($p < 0.01$). A significant difference was also observed between the initial value and the value of reperfusion ($p < 0.05$). Again, similar statistical differences were found to be between the initial, occlusion and reperfusion values of glucose in the dexmedetomidine and thiopental groups.

While the initial value of Hct was found to be 37.4 ± 2.1 in the control group (Group C), it was observed to be 37.5 ± 2.4 in occlusion and 38.6 ± 1.9 in reperfusion. No statistically significant difference was found between the initial, occlusion and reperfusion values of Hct ($p > 0.05$). Again, similar statistical differences were found to be between the initial, occlusion and reperfusion values of Hct in the dexmedetomidine and thiopental groups (Table 1).

Histopathological scores

Histopathological scores of all the three groups are shown in Fig. 6. According to the histopathological score, there was ischemia in all subjects in the Group C. There was no cerebral ischemia in 4 subjects in the Group D and in 3 subjects in the Group T. While Grade 1 cerebral ischemia was found to be in 2 subjects in the Group C and in 4 subjects in the Group D, it was observed in 5 subjects in Group T. Grade 2 cerebral ischemia was only found in 3 subjects in Group C and in one subject in Group D and Group T. Yet, Grade 3 cerebral ischemia was found in 3 subjects in Group C and only in one subject in Group D and Group T.

No statistically significant difference was observed between Group D and Group T in terms of histopathological scores ($p > 0.05$). However, a statistically significant difference was found between Group C and Group D ($p < 0.05$) and Group C and Group T ($p < 0.01$) in this respect.

The microscopic images of score obtained through histopathologic examination are shown in Fig. 6 (Grade 0, Grade 1, Grade 2 and Grade 3).

Stereological results

In Fig. 7, the numerical density of necrotic neurons in the control group as well as in the groups treated with dexmedetomidine and thiopental. In the study, it was observed that the average numerical density of necrotic neurons was very high in the control group ($0.00047\,n/\mu m^3$) while it was less in the groups treated with dexmedetomidine ($0.00044\,n/\mu m^3$) and thiopental ($0.00044\,n/\mu m^3$).

In Fig. 8, numerical density of intact neurons was given for the control group and the groups treated with dexmedetomidine and thiopental. The number of intact neurons was observed to be ($0.00055\,n/\mu m^3$) in the group treated with dexmedetomidine and higher ($0.00053\,n/\mu m^3$) in the group treated with thiopental while less ($0.00038\,n/\mu m^3$) in the control group.

Discussion

In order to create experimentally the most realistic cerebral ischemia model, various techniques[22,25] were created from various animal groups.[21–25] In our study, we preferred to use rats for obvious reasons such that their cerebral circulatory system is very similar to that of humans and they are readily available and inexpensive.

In this study, the neck region of the subjects in the supine position in supine position was shaved and the sterilization of the surgical site was achieved. Their heads were slightly extended and the midline pretracheal surgical incision was made. Reaching the trachea, the right carotid artery in the posterolateral of the trachea, vagus nerve and cervical sympathetic plexus were maintained and then explored and, they were fixed with a suture for being clamped subsequently. 5 min after administrating 30 units of heparin for the purpose of anticoagulation, about 3–5 mL of blood was collected in a controlled manner through injector from the femoral artery in order to produce hemorrhagic hypotension. Carotid arteries was occluded with vascular clamp for a duration of 45 min when mean arterial pressure (MAP) reached to 35 mmHg. During this period, MAP was kept fixed at 35–40 mmHg. The clamps were removed at the end of the period and brain reperfusion was achieved for 90 min.

In clinical practice, the importance of ischemia has led researchers to create and develop experimental models for cerebral ischemia. In various models applied, whole or regional, complete or incomplete and permanent or temporary ischemia were produced.

The models applied may cause a permanent or transient ischemia; among the methods applied in the model, intravascular embolization or extravascular ligation may be used and the occlusion of Arteria Cerebralis Media (ACM) may be produced through bipolar coagulation.[24–29] Through the intravascular embolization technique, focal, permanent ischemia can be obtained without having to craniectomy, however, It is impossible to determine the localization of embolism and generally, multifocal infarct areas are developed in different widths and locations.[27] In extravascular ligation techniques, obtaining a focal and permanent ischemia is relatively hard and often requires invasive procedures.[28,29]

Table 1 All subjects' weights, mean arterial pressures 5 min before ischemia and during occlusion and reperfusion, heart rate, body temperature, pH, PO_2, PCO_2, glucose and hematocrit values and their statistical comparison.

Variables	Group C ($n = 10$) (Control)	Group D ($n = 10$) (Dexmedetomidine)	Group T ($n = 10$) (Thiopental)	p value
Weight	308.3 ± 9.9	312.9 ± 13.8	311.3 ± 11.3	>0.05
Mean arterial pressures				
Initial value	104.9 ± 6.7[c,g]	105.1 ± 4.1[c,g]	105.8 ± 5.5[c,g]	>0.05
Occlusion	36.9 ± 3.9[f]	37.6 ± 4.1[f]	37.3 ± 3.9[f]	>0.05
Reperfusion	96.9 ± 4.4	95.5 ± 5.2	96.1 ± 4.7	>0.05
Heart rate				
Initial value	329.3 ± 20.4[a]	324.5 ± 19.2[a]	332.2 ± 21.1[a]	>0.05
Occlusion	304.6 ± 23.6	304.6 ± 24.9	304.6 ± 28.2	>0.05
Reperfusion	311.2 ± 27.3	311.2 ± 22.8	311.2 ± 26.6	>0.05
Body temperature				
Initial value	37.89 ± 0.17[a]	37.91 ± 0.14[a]	37.82 ± 0.11[a]	>0.05
Occlusion	36.92 ± 0.14[d]	36.09 ± 0.12[d]	36.71 ± 0.15[d]	>0.05
Reperfusion	37.54 ± 0.10	37.62 ± 0.11	37.72 ± 0.09	>0.05
pH				
Initial value	7.37 ± 0.03	7.37 ± 0.04	7.38 ± 0.04	>0.05
Occlusion	7.21 ± 0.07	7.23 ± 0.05	7.20 ± 0.06	>0.05
Reperfusion	7.34 ± 0.04	7.35 ± 0.06	7.36 ± 0.07	>0.05
PO_2 (mmHg)				
Initial value	92.1 ± 3.3[c,g]	94.4 ± 3.9[c,g]	91.9 ± 4.9[c,g]	>0.05
Occlusion	54.2 ± 3.8[e]	52.9 ± 4.6[e]	53.7 ± 3.6[e]	>0.05
Reperfusion	77.2 ± 4.2	76.8 ± 5.1	77.4 ± 4.4	>0.05
PCO_2 (mmHg)				
Initial value	35.7 ± 1.6[b,g]	36.1 ± 0.9[b,g]	35.6 ± 1.3[b,g]	>0.05
Occlusion	40.2 ± 1.2[d]	40.7 ± 1.1[d]	40.6 ± 0.9[d]	>0.05
Reperfusion	37.5 ± 1.7	37.4 ± 1.6	37.1 ± 1.2	>0.05
Glucose (mg/dL)				
Initial value	135.4 ± 2.7[b,g]	137.4 ± 3.1[b,g]	138.4 ± 2.4[b,g]	>0.05
Occlusion	142.9 ± 1.6[d]	143.8 ± 2.8[d]	144.1 ± 2.2[d]	>0.05
Reperfusion	139.7 ± 1.9	139.4 ± 2.4	140.7 ± 1.5	>0.05
HCT				
Initial value	37.4 ± 2.1	37.6 ± 1.9	37.8 ± 2.1	>0.05
Occlusion	37.5 ± 2.4	37.5 ± 2.3	37.5 ± 1.8	>0.05
Reperfusion	38.6 ± 1.9	38.8 ± 2.2	38.5 ± 2.4	>0.05

Significant differences between the values at the beginning and during occlusion. [a]$p < 0.05$, [b]$p < 0.01$ ve [c]$p < 0.001$.
Significant differences between the values during occlusion and reperfusion. [d]$p < 0.05$ ve [e]$p < 0.01$, [f]$p < 0.001$.
Significant differences between the values at the beginning and during reperfusion. [g]$p < 0.05$, [h]$p < 0.01$, [i]$p < 0.001$.

ACM occlusion in rats leads to the formation of focal cerebral ischemia followed by a infarct region. ACM occlusion has been widely used since 1975.[27] This model was used for many times in the focal cerebral ischemia studies and has already proven itself.[24–29]

In order to eliminate the confusion that may occur during interpretation of the results obtained from incomplete cerebral ischemia; the subjects in all three groups receive the same anesthetic agents until the end of *a.carotis* unilateral communis occlusion (the end of ischemia). All subjects in our study underwent isoflurane anesthesia until the end of unilateral arteriacarotiscommunis occlusion due to the presence of studies indicating that isoflurane has no histopathologically protective effect after incomplete cerebral ischemia.

Among the three groups in this study, no statistical difference was found between mean arterial pressure, heart rate, body temperature, hematocrit, blood glucose, hcT, pO_2, pCO_2 and pH at the stages of start before ischemia and occlusion and reperfusion after ischemia. The fact that the mean physiologic values of the laboratory animals were found to be similar at the initial, post-ischemia occlusion and reperfusion stages is an indiction of the homogeneity of groups. In a number of studies, the initial values of the groups were reported to be similar.[14,15,20] For instance, in their studies where they compared early focal cerebral ischemic Injury

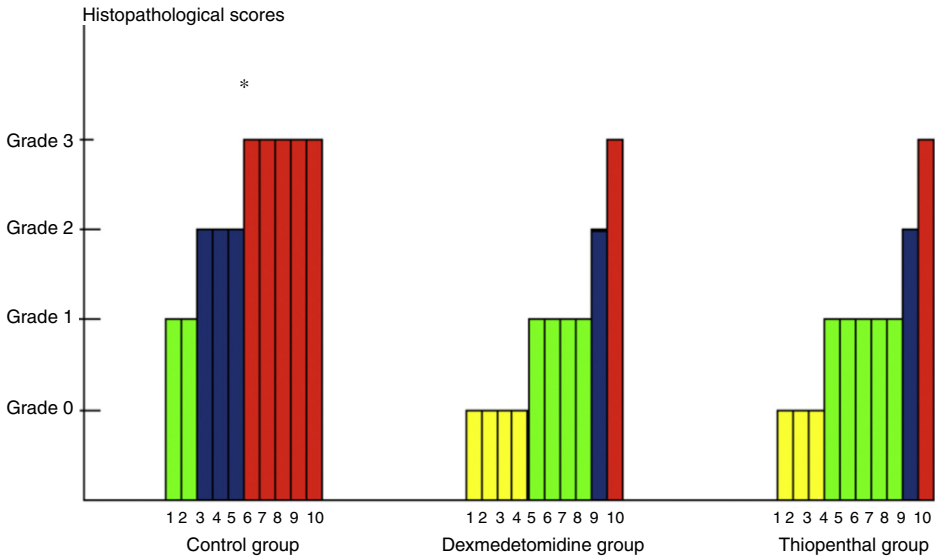

Figure 6 Histopathological score results in the Hippocampus cornuammonis 1 (CA 1) Area: Grade 0 (yellow) = no ischemic neurons, Grade 1 (green) = presence of ischemic neurons less than 10%, Grade 2 (blue) = presence of the ischemic neurons between 10 and 50%, Grade 3 (red) = presence of ischemic neurons more than 50% (*$p < 0.01$ between Group C and Group D and Group C and Group T).

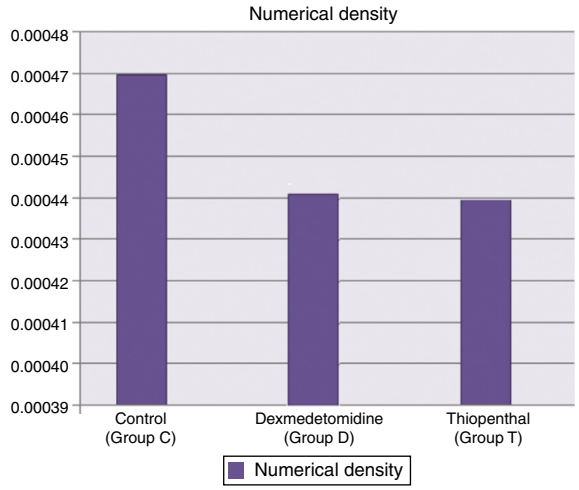

Figure 7 Numerical density of necrotic neuron in the control group and the groups treated with dexmedetomidine and thiopental.

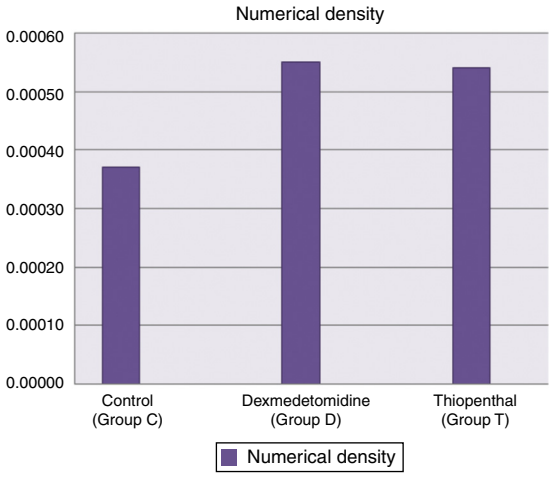

Figure 8 Numerical density of intact neurons in the control group and the groups treated with dexmedetomidine and thiopental.

in rats with thiopental and methoheksital and pentobarbital controls, Cole et al.[14] found that the initial values of pH, PaO_2, $PaCO_2$, mean arterial pressure, hematocrit and glucose levels were similar in all 4 groups they studied.

On the other hand, Drummond et al.[11] could not detect any differences in initial physiological parameters of 4 groups in their studies where they compared the cerebral protective properties of etomidate, isoflurane, and thiopental. The above results seem to verify the results of this study.

In this study, the mean arterial pressure, body temperature, heart rate, pH and PO_2 values were found to significantly decrease in the occlusion and raised again in the reperfusion in three groups when compared to the initial values. However, glucose and pCO_2 values were found to raise in the occlusion and decrease again in the reperfusion in three groups when compared to the initial values.

Kuhmonen et al.[30] studied the effects of dexmedetomidine after temporary and permanent occlusion of middle cerebral artery in rats. They administered 0.9% NaCl intravenously to the control group; these researchers reported that mean arterial pressure, PO_2 and PCO_2 values decreased after ischemia in both temporary and permanent occlusal groups, however, the pH and body temperature remained the same. Nevertheless, they found that the glucose increased.

Engelhard et al.[31] showed that, in the two medicament groups and control groups, the mean arterial pressure and glucose values decreased in ischemia compared to the initial values, however, they raised again in the reperfusion. Still, the level of pCO_2 was found to increase compared to its initial value and then, decreased to its initial value during fallen reperfusion. Engelhard at al.,[32] in another study, studied the α-2 agonist dexmedetomidine's effects on cerebral

neurotransmitter concentrations during cerebral ischemia in rats. In their studies, they demonstrated that the mean arterial pressure value decreased in ischemia compared its initial value and then, raised again in the reperfusion.

Jolkkonen et al.[15] researched in their in vitro studies the effects of dexmedetomidine, NBQX and CGS-19755 after the occlusion and reperfusion of middle cerebral artery according to an infarct volume. They administered 0.9% NaCl intravenously to the control group. They found the initial values of the mean arterial pressure, PO_2, $PaCO_2$, pH and of glucose similar in all the four groups. They also found that PO_2 and $PaCO_2$ decreased during occlusion and reperfusion in all groups while raising in the dexmedetomidine group. The results of this study and the results of the above studies were found to be partially in compliance.

The cause of the cerebral ischemia is the increase in catecholamine concentration in the circulation and the extracellular space. The inventions reducing sympathetic tonus improves neurological outcome.[33,34] Therefore, a treatment administered with agents that reduce the release of norepinephrine in the brain (i.e. α2-agonists) may provide protection against the damaging effects of cerebral ischemia. It has been showed in numerous studies conducted, that dexmedetomidine improves neuronal survival in transient global or focal ischemia in rats.[16,30,35] The mechanism concerning the neuroprotective effect of α2-agonists is not clear yet. Engelhard et al. has demonstrated that dexmedetomidine does not suppress cerebral extracellular catecholamine increase during ischemia and argued that the neuroprotective effect of dexmedetomidine results from modulation of the balance between proapoptotic and anti-apoptotic proteins.[31,32] Many studies has shown that α2-adrenoceptoragonists decrease the excitatory neurotransmitter release (i.e., glutamate).[36,37] As is known, high glutamate levels depolarizes the neuronal membrane and allows calcium to get into the cell. And this triggers a series of events causing cellular damage. Therefore, agents reducing glutamate release are considered to be neuroprotective. It has been observed that administration of dexmedetomidine in focal cerebral ischemia reduces infarct volume by 40% in the cortex, and causes minimal hyperglycemia and hypotension.[15] In incomplete cerebral ischemia, it was observed that, through administration of dexmedetomidine, a decrease occurred in the plasma catecholamine level and histopathological improvement became better than control, depending on the dose.[16] A decrease by 31% in the cortex and 20% in the striatum was reported in the infarct volume after transient occlusion ($15\,\mu g\,kg^{-1}$) in rats given a high dose of dexmedetomidine.[17] It was reported that, during cerebral ischemia, circulating catecholamine decreased while noradrenaline and glutamate concentrations in the brain remained unaffected by dexmedetomidine.[18] In addition, dexmedetomidine was reported to have a neuroprotective effect in the neonatal period and to prevent excitotoxic lesions in the cortex and white matter.[19] Similarly, there are many studies indicating that barbiturates reduce the spread of brain injuria as a result of transient focal cerebral ischemia. While some researchers has stated that thiopental has a protective effect[14,20] some other reported that it has no protective effects.[21,38]

This study was designed to examine whether thiopental and dexmedetomidine which are among the intravenous anesthetics have cerebral protective effects in focal cerebral ischemia in rats and which medication has the most protective effect.

According to the histopathological score, there was ischemia in all subjects in the control group. There was no cerebral ischemia in 4 subjects in the dexmedetomidine group and in 3 subjects in the thiopental group. While Grade 1 cerebral ischemia was present in 2 subjects in the control group and in 4 subjects in the dexmedetomidine group, it was observed in 5 subjects in the thiopental group. Grade 2 cerebral ischemia was found to be in 3 subjects in the control group and only in one subject in the dexmedetomidine and thiopental group. Still, Grade 3 cerebral ischemia was found to be in 3 subjects in the control group and only in one subject in the dexmedetomidine and thiopental group. There was no statistically significant difference between dexmedetomidine and thiopental group in terms of histopathological scores. However, there was a difference between the control group and these two groups (dexmedetomidine group and thiopental group).

Cole et al.[14] used 6–20 week-old 80 male rats weighing 375–425 g in their studies in which they compared thiopental, methoheksital and pentobarbital controls in early focal cerebral ischemic injure. During this study, EEG was continuously recorded through platinum needles placed on the bitemporal configuration. A two-part medication regimen was administered to rats. This first part of the study (EEG burst-suppression) was performed on 40 rats. Each group consisted of 10 rats. 0.9% NaCl was administered intravenously to the controls. As a result of this part, the volume of cerebral injure was found to be similar in the control group ($133 \pm 17\,mm^3$), in the methoheksital group ($126 \pm 19\,mm^3$) and in the pentobarbital group ($130 \pm 17\,mm^3$). However, a lower cerebral injure volume was detected in the thiopental group ($88 \pm 14\,mm^3$). In other words, thiopental was observed to have the most protective effect that reduces the volume of cerebral injury. In contrast, in the second part of the study conducted with barbiturates at a dose of 40%, they significantly reduced only the infarct volume of methoheksital group among the groups created similarly. As a result of the second part of the study, the volume of cerebral injure was found to be similar in the control group ($124 \pm 22\,mm^3$), in the thiopental group ($118 \pm 15\,mm^3$) and in the pentobarbital group ($121 \pm 20\,mm^3$). However, a lower volume of cerebral injure was detected in the methoheksital group ($70 \pm 20\,mm^3$).

Drummond et al.[21] has compared the cerebral protective properties of etomidate, isoflurane and thiopental. After inserting vascular catheter, they divided the 350–400 g 16–20 week-old male spontaneously hypertensive rats into 4 groups. 8 rats were administered thiopental IV (25 mg/mL) while 8 rats were administered etomidate (125 mg/mL) and other 8 rats were administered isoflurane. The control group, on the other hand, consisted of 0.92% halothane. The volume in the cerebral injure was found to be ($99 \pm 13\,mm^3$) in the halothane (control) group, ($56 \pm 10\,mm^3$) in the thiopental group, ($139 \pm 14\,mm^3$) in the isoflurane group and ($145 \pm 11\,mm^3$) in the etomidate group. The brain volume injured was significantly the smallest in the thiopental group compared to all groups. In other words, thiopental was observed to have the most protective effect that reduces the volume of cerebral injury.

Elsaesser et al.[39] administered halothane to 30 Sprague-Dawley rats by creating an occlusion of the middle cerebral artery. They also administered intravenously low and high-dose thiopental to the other two groups. They evaluated the infarct volume after 3 h of reperfusion. When they compared them with the controls, they have found that low-dose thiopental reduced the infarct volume by 28% and the high-dose thiopental reduced the infarct volume by 29%. The authors have indicated that thiopental has cerebral protection and there is no need for extremely high doses in providing this.

Xue et al.[40] created brain ischemia through oxygen-glucose deprivation (OGD) model in cerebral cortical slices in male Sprague-Dawley rats weighting 90–120 g. They compared the effects of ketamine, midazolam, thiopental and propofol on this cerebral ischemia. The evaluated the slices by ELISA method. These researchers have found out that these 4 different IV anesthetics has different effects, low and high doses of ketamine inhibits OGD Injury, high doses of midazolam (10 μmol/L) and thiopental (400 μmol/L) support partially this decrease and again, high dose of propofol (100 μmol/L) also promotes this decrease. As a conclusion, they have reported that ketamine, midazolam in high doses and thiopental have neuroprotective effects on OGD injuria in the cortical slices of the rats.

Lianhua et al.[41] exposed male Sprague-Dawley rats to 24 h reperfusion and 3 h middle cerebral artery occlusion. Then, they compare the infarct volume in rats' brains through giving them midazolam, thiopental and propofol. The discussed the neurological outcomes over a 0–5 grading system. They have observed that ischemic score was relatively low in the rats to which midazolam and propofol were administered. Thus, they have reported that midazolam and propofol have protective effects against reperfusion injury but thiopental shows no protective effect.

In conclusion, we histopathologically discussed whether thiopental and dexmedetomidine which are among the intravenous anesthetics have cerebral protective effects on focal cerebral ischemia in rats. We have detected that thiopental and dexmedetomidine are more neuroprotective compared to the controls. We have shown in the experimental model of focal cerebral ischemia in rats that dexmedetomidine and thiopental have histopathologically cerebral protective effects. However, the clinical accuracy of these effects is not clear yet. Further studies are needed with regard to the use of dexmedetomidine and thiopental in appropriately selected patient populations and specific procedures.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Graham DI. Focal cerebral infarction. J Cereb Blood Flow Metab. 1988;8:769–73.
2. McIlraith DM, Cöte R. Epidemiology and etiology of stroke. Curr Opin Neurol Neurosurg. 1991;4:31–7.
3. Analysis of neuronal damage resulting in the focal cerebral infarct model induced by middle cerebral artery occlusion in rats and Ginkgo Biloba extract's effects on this. Bursa: Uludag University Faculty of Medicine, Department of Neurology. Expertise Thesis; 1994.
4. Ginsberg MD, Busto R. Rodent models of cerebral ischemia. Stroke. 1989;20:1627–42.
5. Garcia JH. Experimental ischemic stroke: a review. Stroke. 1984;15:5–14.
6. Grotta JC. Current medical and surgical therapy for cerebrovascular disease. N Engl J Med. 1987;317:1505–16.
7. Barsan WG, Brott TG, Olinger CP, Marler JR. Early treatment for acute ischemic stroke. Ann Int Med. 1989;111:449–51.
8. Albers GW, Goldgerg MP, Choi DW. NMDA antagonists: ready for clinical trial in brain ischemia? Ann Neurol. 1989;25:398–403.
9. Wong MCW, Haley EC Jr. Calcium antagonists: stroke therapy coming of age. Stroke. 1990;21:494–501.
10. William F. Brain metabolism. In: Cottrell JE, Smith DS, editors. Anesthesia and neurosurgery. third ed. St Louis: Mosby-Year Book; 1994. p. 1–16.
11. Drummond JC, Shipiro HM. Cerebral physiology. In: Miller RD, editor. Anesthesia. fourth ed. Churchill: Livingstone; 1994. p. 689–729.
12. Hoffman WE, Charbel FT, Edelman G, Ausman JI. Thiopental and desflurane treatment for brain protection. Neurosurgery. 1998;43:1050–3.
13. Sano T, Patel PM, Drummond JC, Cole DJ. A comparison of the cerebral protective effects of etomidate, thiopental and isoflurane in a model of forebrain ischemia in the rat. Anesth Analg. 1993;76:990–7.
14. Cole DJ, Cross LM, Drummond JC, et al. Thiopentone and methohexital, but not pentobarbitone, reduce early focal cerebral ischemic injury in rats. Can J Anaesth. 2001;48:807–14.
15. Jolkkonen J, Puurunen K, Koistinaho J, et al. Neuroprotection by the alpha-2 adrenoceptor agonist, dexmedetomidine, in rat focal cerebral ischemia. Eur J Pharmacol. 1999;372:31–6.
16. Hoffman WE, Kochs E, Werner C, Thomas C, Albrecht RF. Dexmedetomidine improves neurologic outcome from incomplete ischemia in the rat. Reversal by the alpha 2 adrenergic antagonist atipamezole. Anesthesiology. 1991;75:328–32.
17. Kuhnomen J, Haapalinna A, Sivenius J. Effects of dexmedetomidine on CO_2 reactivity and cerebral autoregulation in healthy volunteers. In: ASA meeting Abstract Number A-341. 2001.
18. Laudenbach V, Mantz J, Evrard P, Grassens P. Dexmedetomidine protect against neonatal exitotoxic brain injury. In: ASA meeting Abstract Number A732. 2000.
19. Ma D, Hossain M, Franks NP, Maze M. Role of the alpha-2a adrenoceptor subtype in the neuroprotective effect of dexmedetomidine. In: ASA meeting Abstract Number A-832. 2003.
20. Sterio DC. The unbiased estimation of number and sizes of arbitrary particles using the dissector. J Microsc. 1984;134:127–36.
21. Drummond JC, Cole DJ, Patel PM, Reynolds LW. Focal cerabral ischemia during anesthesia with etomidate, isoflurane, orthiopental: a comparison of the extent of cerabral injury. Neurosurgery. 1995;37:472–9.
22. Coyle P. Middle cerebral artery occlusion in the young rat. Stroke. 1982;13:855–8.
23. Zervas NT, Hori H, Negora M, et al. Reduction of brain dopamine following experimental cerebral ischemia. Nature. 1974;247:283–4.
24. Hillered L, Hallström A, Segersuard S, et al. Dynamics of extracellular metabolites in the striatum after middle cerebral artery occlusion in the rat monitored by intracerebral microdialysis. J Cereb Blood Flow Metab. 1989;9:607–16.
25. Makarenko NV. Higher nervous activity in dogs reanimated after long periods of clinical death from drowning and loss of blood. Zurnal Vyssej Nervnoj Dejatelvnosti. 1972;22:82–8.
26. Chen ST, Hsu CY, Hogan EL, et al. A model of focal ischemic stroke in the rat: reproducible extensive cortical infarction. Stroke. 1986;17:738–43.

27. Bederson BJ, Pitts HL. Rat middle cerebral artery occlusion; evaluation of the model and development of a neurologic examination. Stroke. 1986;17:472–6.
28. Kogure K. Molecular and biochemical events within the brain subjected to cerebral ischemia (targets for therapeutical intervention). Clin Neurosci. 1997;4:179–83.
29. Ishiyama T, Dohi S, Iida H, Watanabe Y, Shimonaka H. Mechanisms of dexmedetomidine-induced cerebrovascular effects in canine in vivo experiments. Anesth Analg. 1995;81: 1208–15.
30. Kuhmonen J, Haapalinna A, Sivenius J. Effects of dexmedetomidine after transient and permanent occlusion of the middle cerebral artery in the rat. J Neural Transm. 2001;108: 261–71.
31. Engelhard K, Werner C, Kaspar S, et al. Effect of the α2 agonist dexmedetomidine on cerebral neurotransmitter concentrations during cerebral ischemia in rats. Anesthesiology. 2002;96:450–7.
32. Engelhard K, Wernwer C, Kaspar S, et al. The effect of the α2 agonist dexmedetomidine and the N-methyl-aspartate agonists S+-ketamine on the expression of apoptosis-regulating proteins after incomplete cerebral ischemia and reperfusion in rats. Anesth Analg. 2003;96:524–31.
33. Globus MJ, Busto R, Dietrich WD, Martines E, Valdez I, Ginsberg MD. Direct evidence for acute and massive norepinephrine release in the hippocampus during transient ischemia. J Cereb Blood Flow Metab. 1989;9:892–6.
34. Kuhmonen J, Pokorny J, Miettinen R, et al. Neuroprotective effects of dexmedetomidine in the gerbil hippocampus after transient global ischemia. Anesthesiology. 1997;87:371–7.
35. Maier C, Steinberg GK, Sun GH, Zhi GT, Maze M. Neuroprotection by the α2-adrenoceptor agonist dexmedetomidine in a focal model of cerebral ischemia. Anesthesiology. 1993;79:306–12.
36. Chen Y, Zhao Z, Code WE, Hertz L. A correlation between dexmedetomidine-induced biphasic increases in free cytosolic calcium concentration and energy metabolism astrocytes. Anesth Analg. 2000;91:353–7.
37. Talke P, Chen R, Thomas B, et al. The hemodynamic and adrenergic effects of perioperative dexmedetomidine infusion after vascular surgery. Anesth Analg. 2000;90:834–9.
38. Steen PA, Milde JH, Michenfelder JD. No barbiturate protection in a dog model of complete cerebral ischemia. Ann Neurol. 1979;5:343–9.
39. Elsaesser RS, Schröder M, zausinger S, Hungerhuber E, Baethmann A, Reulen HJ. EEG burst suppression is not necessary for maximum barbiturate protection in transient focal cerabral ischemia in a rat. J Neurological Sci. 1999;162:14–9.
40. Xue Q, Yu B, Wang Z, et al. Effects of ketamine, midazolam, thiopental, and propofol on brain ischemia injury in rat cerebral cortical slices. Acta Pharmacol Sin. 2004;25:115–20.
41. Lianhua C, Qinyan G, Changsi X. Effects of propofol, midazolam and thiopental sodium on outcome and amino acids accumulation in focal cerebral ischemia-reperfusion in rats. Chin Med J. 2003;116:292–6.