Kaohsiung Journal of Medical Sciences (2016) 32, 292–301

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ORIGINAL ARTICLE

Effectiveness of sugammadex for cerebral ischemia/reperfusion injury

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Received 17 February 2016; accepted 2 May 2016
Available online 23 June 2016

KEYWORDS
Hypoxia;
Ischemia;
Neuroprotections
sugammadex

Abstract Cerebral ischemia may cause permanent brain damage and behavioral dysfunction. The efficacy and mechanisms of pharmacological treatments administered immediately after cerebral damage are not fully known. Sugammadex is a licensed medication. As other cyclodextrins have not passed the necessary phase tests, trade preparations are not available, whereas sugammadex is frequently used in clinical anesthetic practice. Previous studies have not clearly described the effects of the cyclodextrin family on cerebral ischemia/reperfusion (I/R) damage. The aim of this study was to determine whether sugammadex had a neuroprotective effect against transient global cerebral ischemia. Animals were assigned to control, sham-operated, S 16 and S 100 groups. Transient global cerebral ischemia was induced by 10-minute occlusion of the bilateral common carotid artery, followed by 24-hour reperfusion. At the end of the experiment, neurological behavior scoring was performed on the rats, followed by evaluation of histomorphological and biochemical measurements. Sugammadex 16 mg/kg and 100 mg/kg improved neurological outcome, which was associated with reductions in both histological and neurological scores. The hippocampus TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) and caspase results in the S 16 and S 100 treatment groups were significantly lower than those of the I/R group. Neurological scores in the treated groups were significantly higher than those of the I/R group. The study showed that treatment with 16 mg/kg and 100 mg/kg sugammadex had a neuroprotective effect in a transient global cerebral I/R rat model. However, 100 mg/kg sugammadex was more neuroprotective in rats.
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Conflicts of interest: All authors declare no conflicts of interest.

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http://dx.doi.org/10.1016/j.kjms.2016.05.002
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Introduction

Reperfusion after cerebral ischemia may cause secondary neurological dysfunction and neuronal death by multiple pathophysiological mechanisms during a variety of surgical procedures such as cardiopulmonary bypass and carotid and cerebral aneurysm [1]. Specifically during general anesthesia, the most frequently used inhalation agents and intravenous anesthetic medications are neuroprotective against cerebral ischemia/reperfusion (I/R) injury, and their roles in neuroprotection have been shown [2]. For many years protecting the patient's brain during the operation has been the most important factor for anesthetists [3,4]. To achieve this goal, selection of the most appropriate agents during the perioperative period and research to achieve this aim have been performed [2].

Sugammadex is a modified γ-cyclodextrin molecule that has recently begun to be used in clinical neuromuscular pharmacology. Sugammadex, which already plays an important role in anesthetic administration despite its novelty, is an agent with controversial effects on cerebral I/R injury. The neuroprotective effects of cyclodextrins have been shown previously [5,6]. In addition, no study assessing the neuroprotective effect of sugammadex in an *in vivo* cerebral I/R injury model has been conducted. Although a variety of experimental studies have shown the neuroprotective characteristics of other cyclodextrins, the adverse effect of sugammadex on neuronal cell cultures has led to debate on this topic [7]. Their molecular pathogenic mechanisms are not clear. However, studies on this topic have led to a variety of results indicating that the mechanisms are at both the receptor and neuronal membrane level.

The hypothesis of this study is that, similar to other cyclodextrins, a γ-cyclodextrin will reduce cerebral I/R injury in a global cerebral hypoxia model in rats and may have neuroprotective effects. In the current study, we aimed to test this hypothesis using a temporary global cerebral I/R model induced by bilateral common carotid occlusion.

Methods

After obtaining permission from the Dokuz Eylul University School of Medicine (DEUSM) Local Animal Experiments Ethical Committee, Izmir, Turkey (05.09.2015, Number: 15/2015, Yılmaz O), the study was carried out at the DEUSM Animal Experiment Laboratories, Izmir, Turkey. The study included 28 Wistar Albino rats that weighed between 250 g and 300 g. The rats were housed in temperature-controlled (22—24°C) rooms that were lit on a daily schedule (12:12 hours light/dark) before the study. All of the rats were fed standard rat pellets. During the experimental period, the care of the laboratory animals was conducted in accordance with international guidelines.

Experimental design

Male rats were fasted for 12 hours before operation. The weights and ages of all of the rats were recorded. The rats were randomly divided into four groups of seven rats: Sham group, I/R group, I/R + S 16 group, and I/R + S 100 group. Using sterile techniques, all of the rats had a 24 G (Vasofix Safety FEP, Braun, Germany) cannula placed in the lateral tail vein for the administration of 5% dextrose (1 mL total volume) and medications during the study.

Bilateral common carotid artery occlusion

The experimental model was based on a previously reported model, with several modifications [8,9]. The animals were anesthetized with 80 mg/kg ketamine hydrochloride and 12 mg/kg xylazine administered intraperitoneally. After the rats were anesthetized, the bilateral common carotid arteries were exposed and carefully separated from the carotid sheath and the cervical sympathetic and vagal nerves through a ventral cervical incision. The bilateral common carotid arteries were occluded with nontraumatic aneurysm clips in ischemic rats but were not clamped in sham-operated rats. Complete interruption of the blood flow was confirmed by observing the central artery in the retina with an ophthalmoscope. The body temperature of the rats was maintained at 37.0 $\pm$ 0.5°C during this procedure. In this model, 10 minutes of ischemia and 24 hours of reperfusion were induced. After the ischemic period, the rats were treated with sugammadex 16 mg/kg (S 16 group) and sugammadex 100 mg/kg (S 100 group) (intravenous and total volume 1 mL) in the 5th minute of reperfusion. Sugammadex was diluted with normal saline (0.9% NaCl). The same surgical procedure was performed in the I/R group, but the rats were not treated with sugammadex. The neck incision was closed with sutures (3.0 silk), and the animals were allowed to awaken. After 24 hours, the neurological condition of the rats was assessed with a scoring system [9,10]. The sugammadex doses were determined according to doses used in previous experimental studies [11—14].

Assessment of neurological behavior

After the 24-hour reperfusion period, behavior was assessed before sacrifice. The neurological examination assessed rats with a six-stage test scoring system as described by Garcia et al. [9] and with a beam-walking test [10].

Neurological scoring system of Garcia et al.

1, spontaneous activity; 2, symmetrical movement in four extremities; 3, forepaw outstretching; 4, limb placement test, 5, climbing; 6, body proprioception; and 7, response to contact with whiskers. At the end of the assessment, the points given to the rat for each of the six tests were added together. The lowest neurological score was 3 and the highest was 18.

Beam-walking test

0, rat falls; 1, rat does not traverse the beam, but continues to sit on the beam; 2, rat begins to walk and falls (rat falls when walking); 3, rat traverses the beam but this is not accompanied by movement of the affected hindlimb; 4, rat traverses the beam but expends $>$ 50% greater effort (rat traverses with $>$ 50% footslips); 5, rat traverses the beam and shows less excess effort (rat has few footslips); and 6, rat traverses the beam without footslips. In all groups of rats with completed neurological assessment, anesthesia

was administered, and the brains were rapidly removed for histological examination of brain tissue slices. For biochemical testing, blood was taken by cardiac puncture and, at the same time, this method was used to end the study by sacrificing the rats by exsanguination.

Histomorphological evaluation

The brain tissues were fixed in 10% formalin solution, processed by routine histological methods, and embedded in paraffin blocks. Blocks were sectioned coronally into sequential 5-μm sections. Each sample was subjected to estimation of neuron number by taking three consecutive coronal sections through the hippocampus [CA1, CA2, CA3, and gyrus dentatus (GD) regions] and parietal cortex that corresponded approximately to Plates 22 and 23, respectively, in the rat atlas of Paxinos and Watson [15]. All sections were stained with cresyl violet for stereological and histomorphological evaluations [16].

Estimation of neuron density in hippocampus and parietal cortex

The images were analyzed using a computer-assisted image analyzer system consisting of a microscope (Olympus BX-51; Olympus, Tokyo, Japan) equipped with a high-resolution video camera (Olympus DP-71). The number of neurons in CA1, CA2, CA3, and GD of the hippocampus and parietal cortex regions were counted with the help of a counting frame of 15,800 μm^2 viewed through a 20× lens (Olympus U) on the monitor. The counting frame was randomly placed three times on the image analyzer system monitor. The number of neurons was counted (UTHSCA Image Tool for Windows, version 3.0, Comprehensive Dentistry, Twain), and the average was taken. Hippocampal, prefrontal, and parietal cortex neuron densities were calculated [16].

In situ cell death detection

To detect DNA fragmentation in cell nuclei, the TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling) reaction was applied to paraffin sections. The DeadEnd Colorimetric TUNEL system kit (*In Situ* Cell Death Detection Kit; Roche Diagnostics, Mannheim, Germany) was used for detection of apoptotic cells. Serial 5-μm thick paraffin-embedded sections were deparaffinized, rehydrated in graded alcohol, and pretreated in proteinase K (20 μg/mL) for 15 minutes at 37°C. After washing in phosphate-buffered saline (PBS), specimens were incubated with fluorescein-labeled dUTP and TdT at 37°C for 1 hour. Then, converter peroxidase (POD) solution was applied to the slides at 37°C for 30 minutes. Sections were stained with diaminobenzidine (Roche Diagnostics), counterstained with Mayer's hematoxylin, and analyzed using a light microscope [16].

Immunohistochemistry was also performed for active caspase-3 (AB3623; Millipore, Temecula, CA, USA, and polyclonal antibody). After deparaffinization and rehydration, the sections were treated with 10mM citrate buffer (Cat No. AP-9003-125 Labvision, Fremont, CA, USA; pH 6) in a microwave oven for 5 minutes. The sections were washed with PBS and incubated in a solution of 3% H_2O_2 for 5 minutes at room temperature to inhibit endogenous peroxidase activity. After washing with PBS, the sections were incubated with normal serum blocking solution at 37°C for 30 minutes. The sections were again incubated in a humid chamber for 18 hours at 4°C with antibody active caspase-3 (1/100); thereafter, the sections were incubated with biotinylated immunoglobulin G and then with streptavidin conjugated to horseradish peroxidase at 37°C for 30 minutes (Invitrogen-Plus Broad Spectrum 85-9043; Invitrogen, Carlsbad, CA, USA). The sections were finally stained with diaminobenzidine (Roche Diagnostics), counterstained with Mayer's hematoxylin, and analyzed using a light microscope [16]. For the quantitative measurement of the number of cells that underwent apoptosis, 1000 cells were randomly counted including cells showing apoptotic morphology in the hippocampus and parietal cortex, and apoptotic cell percentages were calculated [16].

Measurement of antioxidant enzyme activity and malondialdehyde content

For all groups, intracardiac blood was analyzed by the DEUSM Biochemistry Department Central Laboratory using the following tests.

Total antioxidant status measurement

Total antioxidant status (TAS) was studied using the Rel Assay trade kit (Cat No. RL0017, Gaziantep, Turkey). The principle of the method is based on transformation of dark green—blue-colored 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radicals to the colorless form of ABTS in the presence of antioxidants. The ABTS product becomes colorless in proportion to the amount of antioxidant present. The Trolox used for calibration is a vitamin E analog. The results are given as Trolox equivalents. The method was carried out in a Beckman Olympus AU 5800 device (California, USA) with autoanalysis using spectrophotometric methods. The measurement interval was 0—2.75 Trolox equivalent/L. The managed measurement interval was 0—2.75 Trolox equivalent/L [17].

Superoxide dismutase activity measurement

Superoxide dismutase (SOD) activity was measured using a trade reactive kit (RANSOD, catalogue No. SD125, Randox Laboratories, Crumlin, County Antrim, BT29 4QY, UK) based on spectrophotometric measurement at 505 nm of red formazan stain formed by a chemical reaction between oxygen radicals, formed by xanthine and xanthine oxidase, with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT). The procedure was carried out by setting a Beckman Olympus AU 5800 device for spectrophotometric autoanalysis. The measurement interval of the method was 0.06—4.52 U/mL [17].

Glutathione peroxidase activity measurement

Glutathione peroxidase (GPx) was studied using a BioVision trade kit (Catalogue No. K762-100; BioVision Corporation, Milpitas, CA, USA). The procedure was carried out by setting a Beckman Olympus AU 5800 device for spectrophotometric autoanalysis. The principle of the method is based on the reduction of cumene hydroperoxide by GPx. In this process, glutathione (GSH) was oxidized to glutathione

disulfide (GSSG). To reduce GSSG to GSH again, NADPH is used. As a result, there was a direct correlation with the decrease in NADPH, depending on how much GPx was present in the sample. This decrease was spectrophotometrically monitored at 340 nm. The minimum detection limit was 0.5 mU/mL [17].

Malondialdehyde content measurement

Malondialdehyde (MDA) was measured using a trade reactive kit (Lipid Peroxidation Colorimetric Assay Kit, Catalogue No. K739-100; BioVision Corporation) based on spectrophotometric measurement at 532 nm of pink color formed by reaction with thiobarbituric acid. The measurement interval was 0.1—20.0 nmol/L [17].

Statistical analysis

For statistical analysis, we used SPSS version 15.0 (SPSS Inc., Chicago, IL, USA). To evaluate the significance of the comparisons between groups, Mann—Whitney *U* test and χ^2 tests were used. A p value < 0.05 was considered statistically significant.

Results

The Sham group included six rats because one of the rats died during the experimental surgery procedures.

Neurological scores

The evaluation of neurological scores among the groups revealed that, in the Sham group, body proprioception, response to vibrissae touch, and total neurological score were significantly higher than in the I/R group ($p < 0.001$). In the S 16 and S 100 groups, body proprioception, response to vibrissae touch, and total neurological scores were significantly higher than in the I/R group ($p < 0.05$; Table 1).

The total neurological score obtained by adding the total neurological score to the beam-walking score was found to be significantly higher in the Sham group than in the I/R group ($p < 0.001$). These values were also found to be significantly higher in the S 16 and S 100 treatment groups than in the I/R group ($p < 0.05$; Table 2).

Histological assessment

Histological evaluation assessed the hippocampus (CA1, CA2, CA3, and GD regions) and parietal cortex regions in all rats included in the study in terms of neuron counts and immunohistochemistry (TUNEL and caspase; Figures 1 and 2). In the Sham group, the total neuron counts in the hippocampus CA1, CA2, CA3, and GD regions were significantly higher than those in the I/R group ($p < 0.05$; Figures 1 and 2). This result indicates that the I/R model was successfully induced in the rats. The total neuron counts in the hippocampus CA1, CA2, CA3, and GD regions in the S 16 and S 100 treatment groups were significantly higher than those in the I/R group ($p < 0.05$; Table 3).

Evaluation of the immunohistochemistry scores from both the TUNEL and caspase assays in the hippocampus of the Sham group revealed that they were significantly lower than those in the I/R group ($p < 0.05$). The results obtained from the parietal cortex were similar. The hippocampus TUNEL and caspase results in the S 16 and S 100 treatment groups were significantly lower than those in the I/R group ($p < 0.05$; Table 4).

Biochemical measurements

Six blood samples were taken; therefore, there was a lack of serum from the groups. All measurements of antioxidant enzyme activity (TAS, GPx, and SOD) and MDA content in the Sham group were significantly different from those in the I/R group ($p < 0.05$). The TAS, GPx, SOD, and MDA values in the Sham group were not significantly different from those in the S 16 and S 100 groups ($p > 0.05$). The TAS, GPx, SOD, and MDA values in the I/R group were not significantly different from those in the S 16 and S 100 groups ($p > 0.05$; Table 5).

Discussion

This study evaluated the neuronal effects of sugammadex in a transient global cerebral ischemia model in rats. Examination of the histological findings and neurological behavior scores showed that doses of 16 mg/kg and 100 mg/kg sugammadex reduced cerebral I/R damage.

The evaluation of the functional outcome, in addition to histological changes, is increasingly acknowledged in the experimental cerebral I/R model because improvement of the functional outcome is a primary goal of cerebroprotective therapies. Previous studies aiming to reduce I/R injury have been conducted using a variety of medications and treatment models.

Zhou et al. [18] showed that mild and moderate hypercapnia had neuroprotective effects in rats with cerebral I/R injury. Kakkar et al. [19] reported the protective role of curcumin against cerebral ischemic insult, provided that it is suitably packaged for improved brain delivery. Other researchers [20] have shown the neuroprotective effect of dauricine after cerebral I/R injury. They suggested that the mechanism of the neuroprotective effect of dauricine after cerebral I/R injury may partly be related to inhibition of neuronal cell apoptosis in the penumbra. There is a study evaluating the effects of cerebral I/R at the receptor level. Discoidin domain receptor 1 has been shown to mediate matrix metalloproteinase-9 secretion and to degrade all extracellular matrix compounds in mammalian tumor cells. Discoidin domain receptor 1 may represent a molecular target for the prevention of blood—brain barrier (BBB) disruption after cerebral I/R injury [21]. Reactive oxygen species play a significant role in neuron loss after cerebral ischemic injury. Carnosine and punicalagin have both antioxidant and neuroprotective effects against reactive oxygen species [22,23]. Currently, there is a trend in which researchers investigate the neuroprotective effects of the most commonly used agents in clinical and surgical practice. Lithium and lamotrigine, which are used to treat mood disorders, are among these. Ozkul et al. [24] found that

Table 1 Evaluation of neurological scoring system in groups.

	Spontaneous Activity	Symmetry in the movement of 4 limbs	Forepaw outstretching	Limp placement test	Climbing	Body proprioception	Response to vibrissae touch	Total neurological score
Sham group ($n = 6$)	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	2.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0[b]	3.0 ± 0.00[b]	20.0 ± 0.00[b,c]
I/R group ($n = 7$)	2.50 ± 0.83	2.66 ± 0.51	2.33 ± 0.51	1.33 ± 0.51	2.16 ± 0.98	1.0 ± 0.0[a,c,d]	1.0 ± 0.0[a,c,d]	13.0 ± 2.0[a,b,d]
S 16 group ($n = 7$)	3.0 ± 0.0	2.71 ± 0.48	2.85 ± 0.37	1.42 ± 0.53	2.85 ± 0.37	2.85 ± 0.37[b]	2.85 ± 0.37[b]	18.57 ± 1.71[a,b]
S 100 group ($n = 7$)	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	2.0 ± 0.0	3.0 ± 0.0	2.85 ± 0.37[b]	2.85 ± 0.37[b]	19.71 ± 0.75[b]

Data are presented as mean ± standard deviation.
I/R = ischemia–reperfusion.
[a] $p < 0.05$, compared with Sham group, Mann–Whitney *U* test.
[b] $p < 0.05$, compared with I/R group, Mann–Whitney *U* test.
[c] $p < 0.05$, compared with S 16 group, Mann–Whitney *U* test.
[d] $p < 0.05$, compared with S 100 group, Mann–Whitney *U* test.

Table 2 Total neurological score and beam-walking score.

	Total neurological score	Beam walking	Total neurological score
Sham group ($n = 6$)	20.0 ± 0.0[b,c]	6.0 ± 0.0[b]	26.0 ± 0.0[b,c]
I/R group ($n = 7$)	13.0 ± 2.0[a,c,d]	3.33 ± 0.51[a,c,d]	16.33 ± 2.50[a,c,d]
S16 group ($n = 7$)	18.57 ± 1.71[a,b]	5.28 ± 0.75[b]	23.85 ± 2.26[a,b]
S 100 group ($n = 7$)	19.71 ± 0.75[b]	5.71 ± 0.75[b]	25.42 ± 0.97[b]

Data are presented as mean ± standard deviation.
I/R = ischemia–reperfusion.
[a] $p < 0.05$, compared with Sham group, Mann–Whitney *U* test.
[b] $p < 0.05$, compared with I/R group, Mann–Whitney *U* test.
[c] $p < 0.05$, compared with S 16 group, Mann–Whitney *U* test.
[d] $p < 0.05$, compared with S 100 group, Mann–Whitney *U* test.

lithium and lamotrigine treatments decreased spatial learning memory deficits in global cerebral ischemia without any significant effect of dosage in a global cerebral ischemia rat model. Their findings suggest that lithium and lamotrigine decrease spatial learning memory deficits, accompanied by lower oxidative nitrosative stress in global cerebral ischemia [24].

In previous studies, although there have been many experimental agents evaluated for neuroprotective effects in the global cerebral I/R models, many are not accessible, not easy to use, or not licensed for use in routine clinical practice. The neuroprotective and neurotoxic effects of both volatile inhalation and intravenous agents used for anesthesia during surgical operations have been studied for many years. To better understand the causes and mechanisms of these effects, it is important to continue this research [2].

A variety of studies have shown the degree of neuroprotective effect and how brain metabolism and neurological results are affected by volatile inhalation agents. These data indicate that the neuroprotective effect mechanisms of volatile agents may be related to factors including activation of ATP-dependent potassium channels, upregulation of nitric oxide synthase, reduction of excitotoxic stressors and cerebral metabolic rate, augmentation of peri-ischemic cerebral blood flow, and upregulation of antiapoptotic factors and mitogen-activated protein kinases [25]. There are many studies researching the neuroprotective/neurotoxic features of intravenous agents. Propofol has a lower suppressive effect on neuronal injury during ischemic depolarization compared to thiopental [26]. Propofol causes oxygen glucose destruction in the rat hippocampus that is linked to concentration. Propofol provides neuroprotective effects when present in the incubation medium during oxygen-glucose deprivation and during the following 24-hour recovery period [2]. However, the role of propofol in traumatic brain damage is less well known, and there are limited experimental studies on this topic [27]. In fact, some animal studies have noted dose-linked neurodegenerative characteristics of propofol in the developing rat brain [28].

Many *in vivo* and *in vitro* experimental ischemia models have emphasized the protective effects and importance of barbiturates against ischemic damage. While the first studies of barbiturates proposed that the neuroprotective effects were related to reducing metabolic requirements, currently, it is known that all of the factors increasing

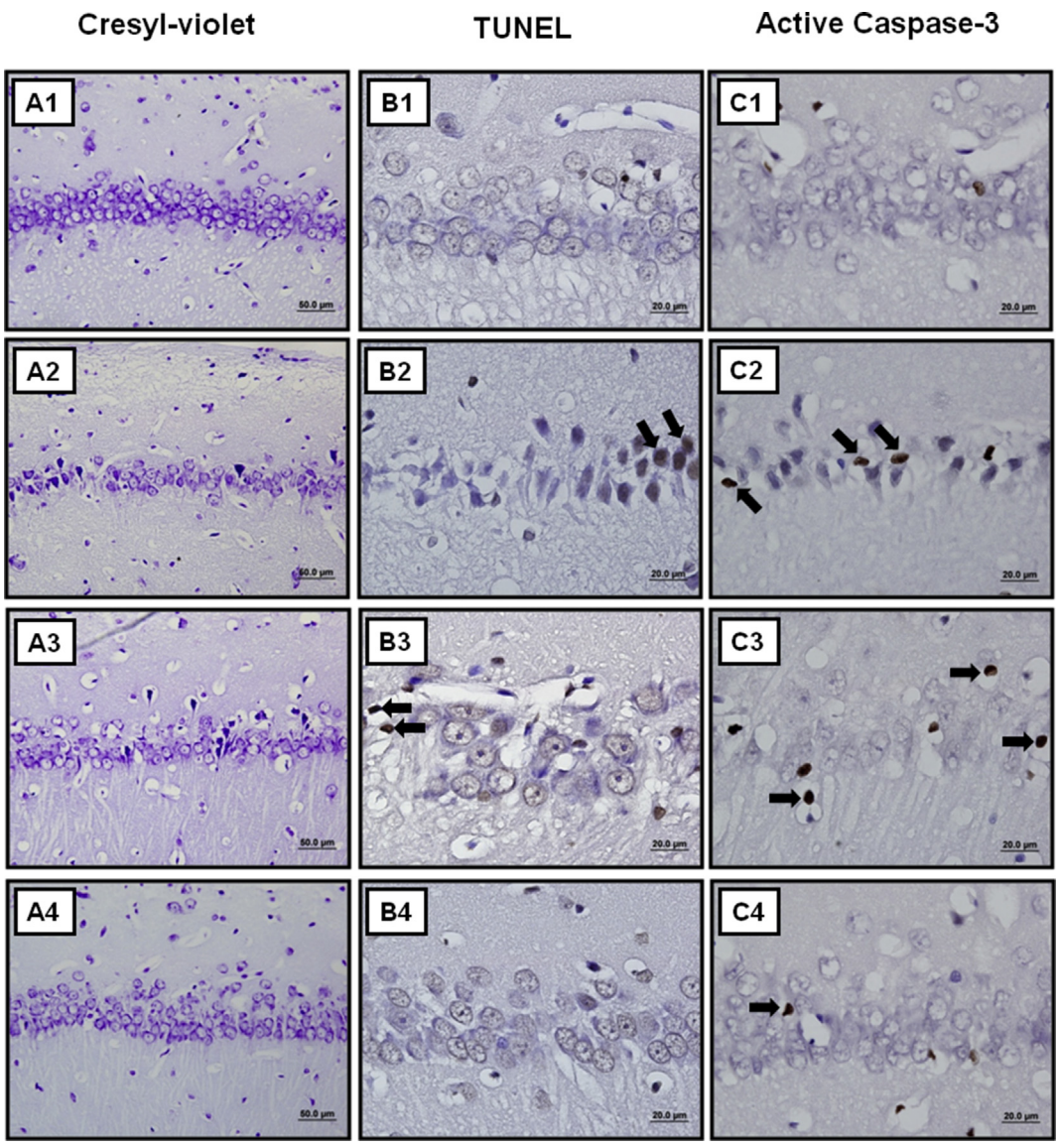


Figure 1. Effects of sugammadex treatment on neuronal density, crezyl-violet staining (A1—4), TUNEL (B1—4), and active caspase-3 immunoreactivity (C1—4) in the CA1 region of the hippocampus. Neuronal density was significantly less in the IR group (A2). TUNEL and active caspase-3-positive cells enhanced in the IR group (B2—C2). Sugammadex (100 mg/kg) treatment significantly reduced the number of apoptotic neurons (B4—C4). Arrows indicate TUNEL-positive and active caspase-3 immune-positive cells. IR = ischemia—reperfusion; TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling.

protein synthesis, GABA-nergic activity and antioxidant activity play a role together [29]. Additionally, the adenosine-induced excitator synaptic transfer inhibition contributes to these mechanisms [30]. Although ketamine is a well-known old agent and noncompetitive N-methyl-D-aspartic acid (NMDA) receptor agonist with neuroprotective effects, it did not provide neuroprotection in an experimental spinal cord ischemia model [31]. In fact, the latest experimental data indicate that neurodegenerative effects are linked to dose and time exposure in developing animals [32]. Frequently chosen for anesthetic administration during surgery in recent years in addition to use in patients under intensive care conditions, dexmedetomidine has been shown to be neuroprotective in experimental studies [33,34]. Dexmedetomidine preconditioning is known to protect against cerebral I/R injury by hypoxia-inducible factor-1α upregulation and vascular endothelial growth factor expression [34]. Jeon et al. [1] studied the neuronal effects of dexmedetomidine and sevoflurane used together in a transient global cerebral ischemia model in rats and found that preischemic dexmedetomidine and postconditioning sevoflurane administration did not provide additional neuroprotective benefits.

While evidence-based knowledge of the neuronal effects of anesthetic agents used from the past to the present is available, the scientific data regarding new generation agents is limited. Sugammadex is one of these. Sugammadex has recently begun to be used in clinical neuromuscular pharmacology and is a modified γ-cyclodextrin molecule. The unique molecular structure of sugammadex

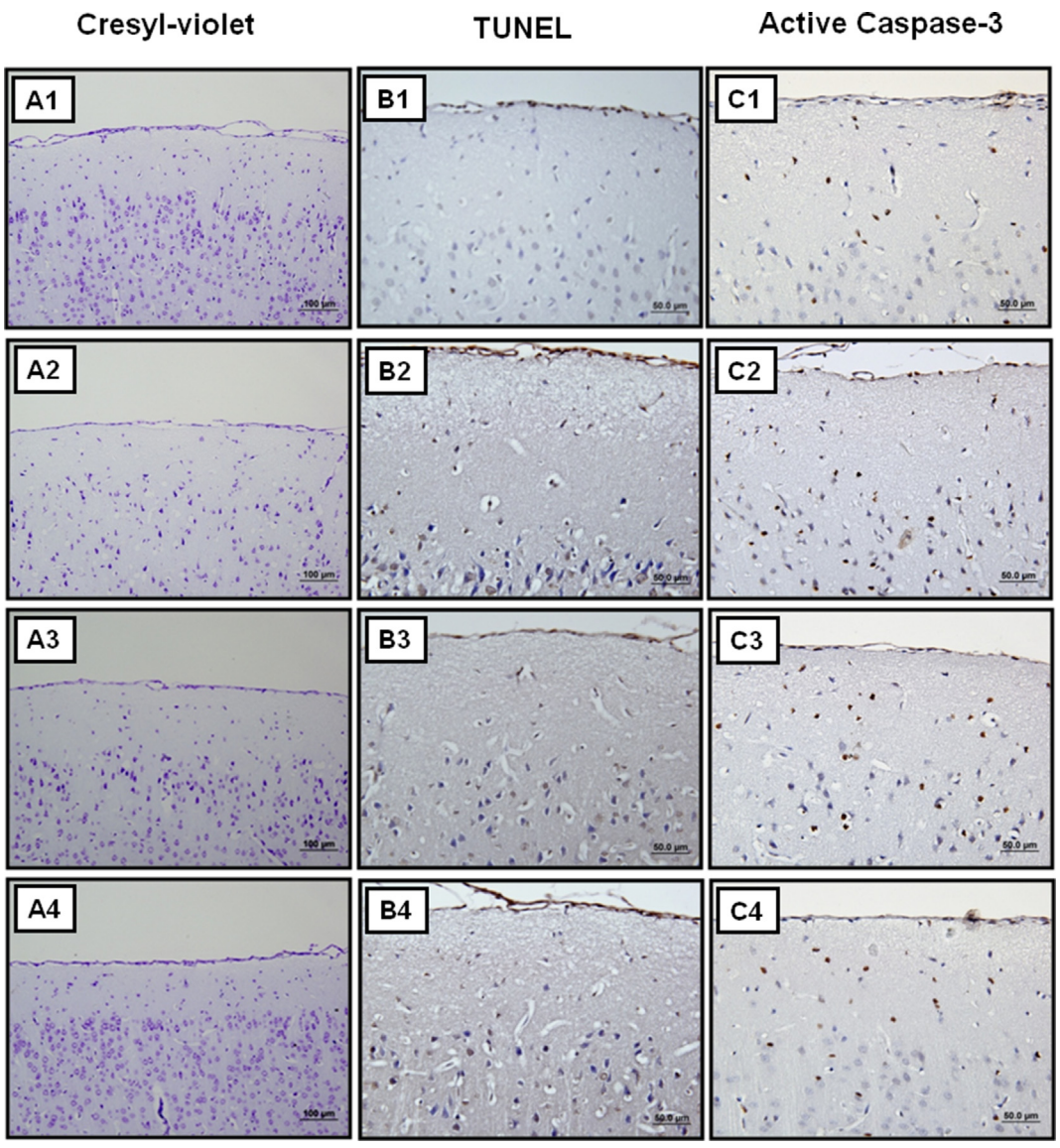


Figure 2. Effects of sugammadex treatment on neuronal density, crezyl-violet staining (A1—4), TUNEL (B1—4) and active caspase-3 immunoreactivity (C1—4) in the parietal cortex. The neuronal density is significantly less in the IR group (A2). TUNEL and active caspase-3-positive cells enhanced in the IR group (B2—C2). Sugammadex (100 mg/kg) treatment significantly reduced the number of apoptotic neurons (B4—C4). IR = ischemia—reperfusion; TUNEL = terminal deoxynucleotidyl transferase dUTP nick end labeling.

encapsulates the neuromuscular blocker rocuronium, removing it from the muscle—nerve junction, and it is used for the selective and rapid reversal of neuromuscular blockage [11—13,35]. Cyclodextrins are frequently used in the medicinal and food industries. Specifically, cyclodextrin molecules are used to convert lipophilic agents to hydrophilic forms. Some cyclodextrins can also alter the receptor stability in membranes and the structure of the lipid stack, affecting receptor functions and removing cholesterol from the cell membrane. The three-dimensional structure of sugar rings contained in cyclodextrins (D-glucopyranose units) form a donut-like ring with an internal hollow truncated cone or hydrophobic cavity and a hydrophilic outer structure. They are named according to the number of rings; α contains six, β contains seven and γ contains eight sugar rings. There are narrow and wide openings caused by primary and secondary faces. The negative hydroxyl groups on the primary and secondary faces make the molecule water soluble. The α1-4 bonds inside and their carbon atoms provide the lipophilic cavity. Thus, the water-soluble molecule surrounds a lipophilic core. This structure encapsulates lipophilic medications of appropriate size and increases water solubility. Noncovalent thermodynamic interactions form inclusion complexes (molecular encapsulation). The size of the cavity is larger in γ-cyclodextrins than in α and β ones and is 0.8 nm. Thermodynamic, Van der Waals, hydrophobic, hydrogen and load transfer interactions contribute to the formation of inclusion complexes (host—guest complexes). The inclusion complex encapsulates a lipophilic molecule [11—13,35].

Table 3 Effect of sugammadex treatment on neuronal density of the hippocampus (CA1, CA2, CA3 and GD regions) and parietal cortex of rats with IR brain injury.

Hippocampus					
	CA1	CA2	CA3	GD	Parietal cortex
Group Sham ($n = 6$)	52.06 ± 2.05[b,c]	36.06 ± 2.51[d]	29.30 ± 3.16[b,c,d]	72.0 ± 2.40[b,c]	34.45 ± 2.10[b,c,d]
Group I/R ($n = 7$)	33.01 ± 3.49[a,c,d]	29.40 ± 2.27[a,c]	19.46 ± 1.56[a,d]	55.06 ± 3.98[a,b,d]	25.06 ± 1.04[a,d]
Group S 16 ($n = 7$)	40.10 ± 2.43[a,b,d]	34.15 ± 2.47[b]	21.11 ± 1.81[a,d]	62.05 ± 1.95[a,b,d]	27.18 ± 1.78[a,d]
Group S 100 ($n = 7$)	50.75 ± 1.72[b,c]	32.08 ± 2.57[a]	24.20 ± 1.98[a,b,d]	70.10 ± 1.84[b,c]	31.04 ± 1.34[a,b,d]

Data are presented as mean ± standard deviation.
I/R = ischemia–reperfusion.
[a] $p < 0.05$, compared with Sham group, Mann–Whitney *U* test.
[b] $p < 0.05$, compared with I/R group, Mann–Whitney *U* test.
[c] $p < 0.05$, compared with S 16 group, Mann–Whitney *U* test.
[d] $p < 0.05$, compared with S 100 group, Mann–Whitney *U* test.

Table 4 The Effect of sugammadex treatment on TUNEL- and caspase-3-positive cells of the hippocampus and parietal cortex of rats with IR brain injury.

	Hippocampus		Parietal cortex	
	TUNEL-positive cells	Caspase-3-positive cells	TUNEL-positive cells	Caspase-3-positive cells
Sham group ($n = 6$)	1.91 ± 0.11[b,c,d]	0.90 ± 0.07[b,c,d]	4.60 ± 0.32[b,c,d]	3.81 ± 0.33[b,c]
I/R group ($n = 7$)	10.50 ± 0.69[a,b,d]	7.10 ± 0.43[a,d]	11.13 ± 0.47[a,b,d]	7.90 ± 0.62[a,b,d]
S 16 group ($n = 7$)	8.32 ± 0.94[a,b,d]	6.85 ± 0.39[a,d]	9.80 ± 0.44[a,b,d]	6.24 ± 0.39[a,b,d]
S 100 group ($n = 7$)	4.70 ± 0.61[a,b,d]	3.48 ± 0.38[a,b,d]	5.91 ± 0.53[a,b,d]	4.08 ± 0.26[b,d]

Data are presented as mean ± standard deviation.
I/R = ischemia–reperfusion.
[a] $p < 0.05$, compared with Sham group, Mann–Whitney *U* test.
[b] $p < 0.05$, compared with I/R group, Mann–Whitney *U* test.
[c] $p < 0.05$, compared with S 16 group, Mann–Whitney *U* test.
[d] $p < 0.05$, compared with S 100 group, Mann–Whitney *U* test.

Table 5 Biochemical measurements.

	TAS	GPx	SOD	MDA
Sham group	1.93 ± 0.34[b]	242.12 ± 47.54[b]	0.58 ± 0.29[b]	1.15 ± 0.45[b]
I/R group IR	1.19 ± 0.61[a]	165.71 ± 57.87[a]	0.21 ± 0.12[a]	2.51 ± 1.53[a]
S 16 group	1.49 ± 0.24	177.28 ± 30.51	0.31 ± 0.03	1.83 ± 0.75
S 100 group	1.53 ± 0.29	204.98 ± 15.79	0.40 ± 0.28	1.70 ± 0.34

Data are presented as mean ± standard deviation.
GPx = glutathione peroxidase; MDA = malondialdehyde; SOD = superoxide dismutase; TAS = total antioxidant status.
[a] $p < 0.05$, compared with Sham group, Mann–Whitney *U* test.
[b] $p < 0.05$, compared with I/R group, Mann–Whitney *U* test.

Many cyclodextrin groups protect cortical neurons, cultured without normal calcium signal effect, against glutamate excitotoxicity and NMDA and oxygen-glucose deprivation [36]. Methyl-β-cyclodextrin has been found to protect the hippocampal areas from the effects of anoxia [37]. Abulrob et al. [5] studied the cyclodextrin 2-hydroxypropyl-b-cyclodextrin (HP-CD) and showed that it had neuroprotective features. Yao et al. [38] conducted a study in rats in which they administered HPCD and determined that cyclodextrins had clear neuroprotective effects in their studies. In experimental studies, HP-CD is used as a carrier for lipophilic medications and as an odor remover [35]. Other lipophilic medication carriers such as ethanol and dimethylsulfide have neuroprotective characteristics [36,37]. Frank et al. [6] reported that, as with other β-cyclodextrins, methyl-β-cyclodextrin reduced neuronal excitability in the hippocampal area as methyl-β-cyclodextrin treatment can separate cholesterol in the postsynaptic area, causing disruption of NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-linked glutamate transmission. There is a decrease in the level of membrane cholesterol attenuated transporter-mediated glutamate release from nerve terminals. Therefore, lowering cholesterol may be used for neuroprotection in stroke, ischemia, and traumatic brain injury, which are associated with an increase in glutamate uptake reversal.

Rivers et al. [39] found that HP-CD administration 30 minutes after variable hypoxia—ischemia significantly reduced brain injury in rats. The researchers found that the protective effect of HP-CD continued effectively for at least 15 days and was clearest in the striatum. They shared the opinion that this may be key to motor functions in the brain. They emphasized that this feature of cyclodextrins provided hope for cerebral ischemia injury.

Due to the unique molecular structure and high molecular weight (2178.01) of sugammadex, its transmission across the BBB and the placenta is low [35]. This characteristic appears to prevent any significant toxicity of sugammadex derivatives in the central nervous system. However, BBB permeability may change in different situations such as in the presence of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, traumatic brain/spinal cord injury, ischemia, and infections or immature nervous systems. Under these conditions, sugammadex may pass the BBB in specific areas [7]. Palanca et al. [7] showed that sugammadex causes apoptosis/necrosis and neuron death in primary cell cultures linked to concentration. In cell cultures, sugammadex increases the expression of the monoclonal cytochrome C protein (CytC), Apoptosis-inducing factor (AIF), anti-Smac/Diablo (1:500), Sigma-Aldrich Biotech (Barcelona, Spain), and CASP-3 proteins. They proposed that this role of sugammadex may be related to changing cholesterol hemostasis with oxidative stress and causing apoptotic activation. This is considered to be due to differences in resistance/sensitivity to oxidative stress between neuronal cell types [7].

The hypothesis of this study is that, similar to other cyclodextrins, a γ-cyclodextrin will reduce cerebral I/R injury in a global cerebral hypoxia model in rats, and may have neuroprotective characteristics. In our study, to test this hypothesis, the role of sugammadex in transient global cerebral I/R injury induced by bilateral common carotid occlusion in rats was examined. In our study, compared with the Sham group, histological scores and behavioral neurological test results of cerebral and biochemical parameters in the I/R group showed that the experimental cerebral I/R model was successfully induced. In the treatment groups, in both the hippocampus and the parietal cortex, immunohistochemical assessment showed that the results of the TUNEL and caspase assays were lower than those in the cerebral I/R group. These results appeared better in the S 100 group. The high dose of sugammadex was found to be more neuroprotective. This result is in accordance with the total score obtained from behavioral neurological evaluation tests in rats.

We did not evaluate antioxidant enzyme activity (TAS, GPx, and SOD) and MDA content in tissues.

There is a need for pharmacological applications that are cheap and simple, if possible, to reduce and/or prevent the negative and unwanted results of hypoxia. These applications should reduce neurological damage and improve patient prognosis. Sugammadex is frequently used in routine anesthesia administration. Our hypothesis is that sugammadex may have neuroprotective effects. The results of this study found that sugammadex had a protective effect against I/R injury. Therefore, studies focusing on the identification of potential therapeutic drugs for treatment and on understanding the mechanisms of cerebral ischemia/reperfusion injury are of great importance. Repeating our study with different models and classes of animals such as mice, rabbits, and swine will increase the literature on this topic and will provide a greater understanding of the effect of sugammadex on neuronal injury.

References

[1] Jeon YT, Hwang JW, Lim YJ, Park SK, Park HP. Postischemic sevoflurane offers no additional neuroprotective benefit to preischemic dexmedetomidine. J Neurosurg Anesthesiol 2013; 25:184—90.

[2] Schifilliti D, Grasso G, Conti A, Fodale V. Anaesthetic-related neuroprotection: intravenous or inhalational agents? CNS Drugs 2010;24:893—907.

[3] Mortier L, Struys M, Herregods L. Therapeutic coma or neuroprotection by anaesthetics. Acta Neurol Belg 2000;100: 225—8.

[4] Toner CC, Stanford LA. General anaesthetics as neuroprotective agents. Baillieres Clin Anesthesiol 1996;10:515—33.

[5] Abulrob A, Tauskela JS, Mealing G, Brunette E, Faid K, Stanimirovic D. Protection by cholesterol-extracting cyclodextrins: a role for N-methyl-D-aspartate receptor redistribution. J Neurochem 2005;92:1477—86.

[6] Frank C, Rufini S, Tancredi V, Forcina R, Grossi D, D'Arcangelo G. Cholesterol depletion inhibits synaptic transmission and synaptic plasticity in rat hippocampus. Exp Neurol 2008;212:407—14.

[7] Palanca JM, Aguirre-Rueda D, Granell MV, Aldasoro M, Garcia A, Iradi A, et al. Sugammadex, a neuromuscular blockade reversal agent, causes neuronal apoptosis in primary cultures. Int J Med Sci 2013;10:1278—85.

[8] Dave KR, Saul I, Busto R, Ginsberg MD, Sick TJ, Pérez-Pinzón MA. Ischemic preconditioning preserves mitochondrial function after global cerebral ischemia in rat hippocampus. J Cereb Blood Flow Metab 2001;21:1401—10.

[9] Garcia JH, Wagner S, Liu KF, Hu XJ. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation. Stroke 1995;26:635.

[10] Zausinger S, Hungerhuber E, Baethmann A, Reulen HJ, Schmid-Elsaesser R. Neurological impairment in rats after transient middle cerebral artery occlusion: a comparative study under various treatment paradigms. Brain Res 2000;863:94—105.

[11] Ozbilgin S, Ozbilgin M, Kucukoztas B, Kamaci G, Unek T, Yurtlu BS, et al. Evaluation of the effectiveness of sugammadex for verapamil intoxication. Basic Clin Pharmacol Toxicol 2013;113:280—5.

[12] Tomak Y, Yılmaz A, Bostan H, Tümkaya L, Altuner D, Kalkan Y, et al. Effects of sugammadex and rocuronium mast cell number and degranulation in rat liver. Anaesthesia 2012;67:1101—4.

[13] Hanci V, Ozbilgin S, Boztaş N, Yurtlu DA, Onat L, Kamaci G, et al. Evaluation of the Effectiveness of sugammadex for teophylline/aminophylline intoxication: an experimental study. Lat Am J Pharm 2014;33:1290—6.

[14] Bostan H, Kalkan Y, Tomak Y, Tumkaya L, Altuner D, Yılmaz A, et al. Reversal of rocuronium-induced neuromuscular block with sugammadex and resulting histopathological effects in rat kidneys. Ren Fail 2011;33:1019—24.

[15] Paxinos G, Warson C. The rat brain in stereotaxic coordinates. 7th ed. 2002. http://booksite.elsevier.com/9780123919496/.

[16] Özbal S, Cankurt Ü, Tuğyan K, Pekçetin Ç, Şişman A, Gündüz K, et al. The effects of alpha-lipoic acid on traumatic brain injury in immature rats. Biotechnic Histochem 2014;90:206—15.

[17] Kiliç K, Hanci V, Selek S, Sözmen M, Kiliç N, Citil M, et al. The effects of dexmedetomidine on mesenteric arterial occlusion-associated gut ischemia and reperfusion-induced

gut and kidney injury in rabbits. J Surg Res 2012 Nov;178: 223—32.
[18] Zhou Q, Cao B, Niu L, Cui X, Yu H, Liu J, et al. Effects of permissive hypercapnia on transient global cerebral ischemia-reperfusion injury in rats. Anesthesiology 2010;112:288—97.
[19] Kakkar V, Muppu SK, Chopra K, Kaur IP. Curcumin loaded solid lipid nanoparticles: an efficient formulation approach for cerebral ischemic reperfusion injury in rats. Eur J Pharm Biopharm 2013;85(3 Pt A):339—45.
[20] Yang X, Zhang L, Jiang S, Gong P, Zeng F. Effect of dauricine on apoptosis and expression of apoptogenic protein after transient focal cerebral ischemia—reperfusion injury in rats. Zhongguo Zhong Yao Za Zhi 2009;34:78—83.
[21] Zhu M, Xing D, Lu Z, Fan Y, Hou W, Dong H, Xiong L, Dong H. DDR1 may play a key role in destruction of the blood-brain barrier after cerebral ischemia-reperfusion. Neurosci Res 2015;96:14—9.
[22] Pekcetin C, Kiray M, Ergur BU, Tugyan K, Bagriyanik HA, Erbil G, et al. Carnosine attenuates oxidative stress and apoptosis in transient cerebral ischemia in rats. Acta Biol Hung 2009;60(2):137—48.
[23] Yaidikar L, Byna B, Thakur SR. Neuroprotective effect of punicalagin against cerebral ischemia reperfusion-induced oxidative brain injury in rats. J Stroke Cerebrovasc Dis 2014; 23(10):2869—78.
[24] Ozkul A, Sair A, Akyol A, Yenisey C, Dost T, Tataroglu C. Effects of lithium and lamotrigine on oxidative-nitrosative stress and spatial learning deficit after global cerebral ischemia. Neurochem Res 2014;39:853—61.
[25] Matchett GA, Allard MW, Martin RD, Zhang JH. Neuroprotective effect of volatile anesthetic agents: molecular mechanisms. Neurol Res 2009;31:128—34.
[26] Kobayashi M, Takeda Y, Taninishi H, Takata K, Aoe H, Morita K. Quantitative evaluation of the neuroprotective effects of thiopental sodium, propofol, and halothane on brain ischemia in the gerbil: effects of the anesthetics on ischemic depolarization and extracellular glutamate concentration. J Neurosurg Anesthesiol 2007;19:171—8.
[27] Adombri C, Venturi L, Pellegrini-Giampietro DE. Neuroprotective effects of propofol in acute cerebral injury. CNS Drug Rev 2007;13:333—51.
[28] Loepke AW, Soriano SG. An assessment of the effects of general anesthetics on developing brain structure and neurocognitive function. Anesth Analg 2008;106:1681—707.
[29] Adachi N. Brain protection by anesthetics. Masui 2006;55: 542—51 [in Japanese].
[30] Narimatsu E, Niiya T, Kawamata M, Namiki A. The mechanisms of depression by benzodiazepines, barbiturates and propofol of excitatory synaptic transmissions mediated by adenosine neuromodulation. Masui 2006;55:684—91.
[31] Lips J, de Haan P, Bodewits P, Vanicky I, Dzoljic M, Jacobs MJ, et al. Neuroprotective effects of riluzole and ketamine during transient spinal cord ischemia in the rabbit. Anesthesiology 2000;93:1303—11.
[32] Istaphanous GK, Loepke AW. General anesthetics and the developing brain. Curr Opin Anaesthesiol 2009;22:368—73.
[33] Hanci V, Erdoğan G, Okyay RD, Yurtlu BS, Ayoğlu H, Baydilek Y, Turan IO. Effects of fentanyl-lidocaine-propofol and dexmedetomidine-lidocaine-propofol on tracheal intubation without use of muscle relaxants. Kaohsiung J Med Sci 2010 May;26(5): 244—50. http://dx.doi.org/10.1016/S1607-551X(10)70035-8.
[34] Ding XD, Zheng NN, Cao YY, Zhao GY, Zhao P. Dexmedetomidine preconditioning attenuates global cerebral ischemic injury following asphyxial cardiac arrest. Int J Neurosci 2006; 126:249—56.
[35] Challa R, Ahuja A, Ali J, Khar RK. Cyclodextrins in drug delivery: an updated review. AAPS PharmSciTech 2005 Oct 14; 6(2):E329—57.
[36] Rufini S, Grossi D, Luly P, Tancredi V, Frank C, D'Arcangelo G. Cholesterol depletion inhibits electrophysiological changes induced by anoxia in CA1 region of rat hippocampal slices. Brain Res 2009;1298:178—85.
[37] Rivers JR, Maggo SDS, Ashton JC. Neuroprotective effect of hydroxypropyl-b-cyclodextrin in hypoxia-ischemia. Neuro Report 2012;23:134—8.
[38] Yao J, Ho D, Calingasan NY, Pipalia NH, Lin MT, Beal MF. Neuroprotection by cyclodextrin in cell and mouse models of Alzheimer disease. J Exp Med 2012;209(13):2501—13. http://dx.doi.org/10.1084/jem.20121239. Epub 2012 Dec 3.
[39] Rivers JR, Sutherland BA, Ashton JC. Characterization of a rat hypoxia ischemia model where duration of hypoxia is determined by seizure activity. J Neurosci Methods 2011;197:92—6.