Comparative Antineuroapoptotic Effects of Dexmedetomidine and Propofol in Cranial Injury: An Animal Study

Kafa Travmasında Deksmedetomidin ve Propofolün Antinöroapopitotik Etkilerinin Karşılaştırılması: Hayvan Çalışması

Züleyha KAZAK BENGİSUN,[a]
Hakan SABUNCUOĞLU,[b]
Emine Aysu ŞALVIZ,[c]
Önder ÖNGÜRÜ,[d]
Tayfun İDE,[e]
Serdar KAHRAMAN[f]

Departments of
[a]Anesthesiology and Reanimation,
[b]Neurosurgery,
Ufuk University Faculty of Medicine,
Ankara
[c]Department of
Anesthesiology and Reanimation,
İstanbul University
İstanbul Faculty of Medicine, İstanbul
[d]Departmen of Pathology,
Gülhane Military Medical Academy,
[e]Gülhane Military Medical Academy
Research and Development Center,
Ankara
[f]Department of Neurosurgery,
Yeni Yüzyıl University Faculty of Medicine,
İstanbul

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Yazışma Adresi/*Correspondence:*
Züleyha KAZAK BENGİSUN
Ufuk University Faculty of Medicine,
Department of
Anesthesiology and Reanimation,
Ankara,
TÜRKİYE/TURKEY
kazakzuleyha@yahoo.com

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ABSTRACT Objective: Traumatic brain injury (TBI) is a common consequence of accidents, and apoptosis is now recognized as one of its important pathophysiological factors. The primary hypothesis of this study was to show the early antineuroapoptotic effects of propofol and dexmedetomidine by showing the small number of apoptotic cells after mild TBI. **Material and Methods:** Forty five rats, anesthetized with intraperitoneal 50mg/kg ketamine hydrocloride and 5mg/kg xylazine, were randomly assigned into 5 groups. Groups 1 (trauma) and 2 (no trauma) were applied propofol while Groups 3 (trauma) and 4 (no trauma) were applied dexmedetomidine. No additional anesthetics were applied to Group 5 (trauma). The mean arterial pressure (MAP), rectal temperature and blood glucose levels were monitored for 2 hours. Then, the brains of the rats were removed after sacrification and craniectomy, and the apoptotic cell analysis was done in midsagital, parasagittal and hippocampal regions. **Results:** The median values for mean body weight, MAP, and temperature were similar($p>0.05$), but glucose levels were significantly higher in Group 5 in the first 45 min ($p<0.05$). Among the trauma groups, the apoptotic cell number was significantly higher in Group 5 in all regions ($p<0.05$). In contrary, there was no significant difference in the number of apoptotic cells in any of the region in groups without trauma (Groups 2 and 4) ($p>0.05$). **Conclusion:** The number of apoptotic cells in rat brains with mild TBI, in which propofol and dexmedetomidine applied, was smaller. However, these two agents had no superiority to each other in terms of antineuroapoptotic effect. These agents were thought to be protective against the early phase brain damage.

Key Words: Brain injuries; propofol; dexmedetomidine; apoptosis

ÖZET Amaç: Travmatik beyin hasarı (TBH) kazaların sık karşılaşılan bir sonucudur, ve son zamanlarda apoptozun TBH'nin önemli patofizyolojik faktörlerinden biri olduğu anlaşılmıştır. Bu çalışmanın primer hipotezi; hafif TBH sonrası propofol ve deksmedetomidinin erken antinöroapoptik etkinliğini rat beyinlerindeki düşük apoptotik hücre sayısı ile göstermekti. **Gereç ve Yöntemler:** İntraperitoneal 50mg/kg ketamin hidroklorür ve 5 mg/kg ksilazin ile anestetize edilen 45 rat, randomize edilerek 5 gruba ayrıldı. Grup 1 (travmalı) ve Grup 2'ye (travmasız) propofol, Grup 3 (travmalı) ve Grup 4'e (travmasız) deksmedetomidin uygulandı. Grup 5 (travmalı) ratlara ise ek anestetik kullanılmadı. Ratların iki saat süresince ortalama arter basınçları (OAB), rektal ısı ve kan glukoz seviyeleri monitörize edildi. Sakrifikasyon ve kraniektomiden sonra ratların beyinleri çıkartıldı ve midsagittal, parasagittal ve hipokampal bölgelerde apoptotik hücre analizleri yapıldı. **Bulgular:** Tüm ratların ortalama vücut ağırlıkları, OAB ve ısı değerleri benzer ($p>0,05$) iken, ilk 45 dakikada Grup 5'te glukoz seviyeleri anlamlı olarak yüksek bulundu ($p<0,05$). Travma grupları karşılaştırıldığında, Grup 5'te tüm bölgelerde apoptotik hücre sayısı daha yüksekti ($p<0,05$). Tersine, travmasız gruplarda (Grup 2 ve 4) apoptotik hücre sayıları açısından anlamlı bir fark bulunamadı ($p>0,05$). **Sonuç:** Propofol ve deksmedetomidin kullanılan hafif TBH'li ratlarda apoptotik hücre sayısının daha düşük olduğu görüldü. Ancak, bu iki ajanın antinöroapoptotik etkiler açısından birbirlerine üstünlüklerinin olmadığı saptandı. Bu ajanların erken dönemde beyin hasarına karşı koruyucu olabileceği düşünüldü.

Anahtar Kelimeler: Beyin yaralanmaları; propofol; deksmedetomidin; apoptoz

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Traumatic brain injury (TBI) is a common sequence of traffic accidents and incidents at home and work, and it is also the most common cause of death especially among children and young adults.[1] The outcome in patients surviving TBI is mainly determined by secondary events such as cerebral edema, ischemia or neurodegeneration. Acute and chronic neurodegeneration following TBI is characterized by programmed death (apoptosis) of the neuronal cells.[1] Since underlying basic molecular mechanisms of apoptosis resulting in neurological damage have not been well understood yet, several significant attempts to synthesize therapeutic drugs have met with little clinical success. However they have provided some insights for future research.[1-6]

Propofol (2,6 diisopropylphenol), an anesthetic agent, is shown to be an effective neuroprotective agent both in animal models and in clinical practice owing to its positive effects on oxidative stress, inhibition of NADPH oxidase, reduction of infarct volume, and attenuation of apoptosis.[7-11] Some studies have suggested but not directly investigated that dexmedetomidine is (α2-adrenoceptor agonist) also a neuroprotective and antiapoptotic agent.[12-15]

In this study, our primary hypothesis was to show the early antineuroapoptotic effects of propofol and dexmedetomidine by indicating the small number of apoptotic cells in three different regions of rat brains after mild TBI. Our secondary hypothesis was having hemodynamically stable rats in propofol and dexmedetomidine groups in terms of mean arterial pressure (MAP), rectal temperature, and blood glucose levels after mild TBI.

MATERIAL AND METHODS

ANIMAL PREPARATION

This study was approved by the Animal Investigation Committee of Gulhane Military Medical Academy, and we adhered National Institutes of Health guidelines for the use of experimental animals during the study. Forty five six-week-old male Wistar albino rats, weighed between 200.1 -240.9 g (mean 219.7±14.6 g), were housed in individual cages in a cohorted rectal temperature-controlled room (approximately 22°C) with alternating 12 hours light-dark cycles, and the animals were acclimated for 4 days before the study. Food was removed 8 h before the study, but all animals were allowed free access to water.

ANESTHESIA

All rats were given intraperitoneal 50 mg/kg **ketamine hydrochloride** and 5 mg/kg xylazine, as a standard anesthesia. Then, aseptic right internal jugular venous and left carotid arterial lines were inserted to maintain anesthesia, infusion of fluids and to monitor MAP. The rats were randomly assigned into 5 groups, with 9 rats in each. Anesthesia was induced with 10 mg/kg propofol (Abbott Propofol; Abbott Laboratories, Chicago, IL) in Groups 1 (trauma) and 2 (no trauma), and 40 μg/kg dexmedetomidine hydrochloride (Hospira Lake Forest, IL) in Groups 3 (trauma) and 4 (no trauma). Anesthesia was maintained with 20-30 mg/kg/h propofol in Groups 1 and 2, and with 3 μg/kg/min dexmedetomidine in Groups 3 and 4 until the end of the experiment (2 hours). Group 5 (control group-trauma) had only standard anesthesia (Table 1). The MAP, rectal temperature and blood glucose levels were noted at the time of induction, and on 15, 30, 45, 90 and 120th minutes during 2-hour experiment period.

SURGICAL PROCEDURE AND TRAUMATIC BRAIN INJURY

Diffuse head injury was induced in 45 six-week-old male Wistar albino male rats by Marmarou method. A midline incision was done on the scalp and periosteal dissection was performed. A 10 mm metal disc was sticked between coronal and lambdoid sutures. Metal disc (450 g) was dropped from 1 meter high, and a mild cranial injury was consti-

TABLE 1: Experimental setup and the animal groups.

Group	Conditions	n
1	Trauma + propofol	9
2	No trauma + propofol	9
3	Trauma + dexmedetomidine	9
4	No trauma + dexmedetomidine	9
5	Trauma	9

tuted.[16] The rats were sacrificed with cervical dislocation. The rats in Groups 1, 3, 5 were sacrificed 2 hours after the mild cranial injury. The rats in Group 2 and 4 were sacrificed instantly 2 hours after anesthesia induction. After sacrification, a craniectomy was performed and then the brain was removed under the operation microscope. Brain tissue samples from three different regions (midsagital, parasagittal and hippocampal regions) were fixed in 10% formalin solution, and sent to pathology department for assessment of apoptotis.

THE TERMINAL DEOXYNUCLEOTIDYL TRANSFERASE (dUTP) NICK-END LABELING (TUNEL) TECHNIQUE

The terminal deoxynucleotidyl transferase (dUTP) nick-end labeling (TUNEL) technique was applied using "ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit" (Millipore, Billerica, MA, USA), to determine the extent of neuronal cell death in tissue sections. The brain tissue samples were put in 10% formaldehyde for fixation, and after routine tissue processing, embedded in paraffin, cut into 5 μm sections, and deparaffinized by 3 changes of xylene, each wash being 5 min. The sections were next dehydrated in a graded series of ethanol concentrations (100%-70%), and washed in one change of phosphate buffered saline (PBS) for 5 min. For pretreatment, proteinase K (20 μg/mL) was applied at room temperature for 15 min, and washed in two changes of deionized water in a coplin jar, each wash being 2 min. The tissue was quenched in 3.0% hydrogen peroxide in PBS at room temperature for 5 min, and rinsed twice with PBS in a coplin jar, each wash being 5 min. After application of equilibration buffer at room temperature for at least 10 sec, the tissue was incubated with terminal deoxynucleotidyl transferase (TdT) enzyme in a humidified chamber at 37°C for 1 h. The sections were then placed in TdT stop buffer for 10 min, washed again with PBS, incubated at room temperature for 30 min in anti-digoxigenin conjugate solution, and washed with PBS. Peroxidase substrate was applied at room temperature for 5 min, monitoring the color development. After washing with deionized water, tissues were counterstained with methyl green. Slides were washed again with deionized water, and then 3 changes of 100% N-Butanol were done, each wash lasting approximately 1 min. The sections were mounted on slides with mounting media, and coverslipped.

ANALYSIS

Apoptotic cells were counted in 3 different locations with the same methodology. The slides stained with TUNEL method were scanned under a light microscope, and at least 8 pictures were obtained at x400 high power field for saggital, parasaggital and hippocampal regions. Quantitation was achieved with image analysis using ImageJ version 1.38 program.[17] It is a public domain Java image processing program. The equipment used for morphometric analysis included a color video camera (Olympus DP71, Tokyo, Japan) attached to a light microscope (Olympus BX51, Tokyo, Japan), and an IBM compatible computer with Intel core2duo processor, 1 GB RAM and video monitor (BenQ G2400WA, Taipei, Taiwan). Briefly, the analysis of the procedure was as follows: initialize application, open acquired images, process binary, count, and record the measurement. After quantitation of apoptotic cells, a mean value was obtained for saggital, parasaggital and hippocampal regions of each case.

STATISTICAL ANALYSIS

All statistical analysis were performed with SPSS 15.0 statistical package. Data were expressed as mean±standard deviation or median [min-max] as appropriate. MAP, glucose level and rectal temperatures were compared within the groups using Friedman test. Since there were no within group differences according to MAP, glucose levels or temperature, no pairwise comparisons were performed. For glucose, MAP and temperature, we calculated the percentage changes according to the baseline values. These percentage changes were compared between the groups using Kruskal Wallis test. The quantity of apoptosis in groups with trauma (Groups 1,3, and 5) were compared using Kruskal Wallis test, followed by Mann Whitney test with Bonferronni adjustment. Groups without trauma (Groups 2, and 4) were compared with Mann Whitney test. P values less than 0.05 were considered as statistically significant. There are

three comparisons for one region, therefore we adapted the p value of 0.05 by Bonferroni adjustment, dividing 0.05 by 3 (=number of comparisons) which resulted in a p level of 0.017.

RESULTS

Forty five male Wistar albino rats were randomly assigned into 5 groups, with 9 rats in each. The medians of mean body weight of the rats in each group were 224.9, 228.9, 213.3, 216.4 and 210.6 g, respectively (p=0.513). At the time of anesthesia, the MAP (p>0.05) (Figure 1), and rectal temperatures (p>0.05) (Figure 2) were similar. The blood glucose levels were significantly higher in Group 5 compared to the other groups, just in the first 45 minutes of the experiment (p<0.05); however there was no difference within the groups during 2-hour period (p>0.05) (Figure 3).

Apoptotic cells were counted in 3 different regions (midsagital, parasagittal, hippocampal) of rat brains. When trauma groups were compared, apoptotic cells were significantly higher in Group 5 compared to in Groups 1 and 3 (Table 2) (Figure 4). In midsagital region, Groups 1, 3 and 5 had 158.4±17.8 (median=156.6), 151.8±27.8 (median=156.5) and 189.9±18.9 (median=188.5) apoptotic cells, respectively (p=0.001) (Figure 5). In parasagittal region, Group 1 had 61.7±11.5 (median=63.3), Group 3 had 61.8±11.2 (median=60.8), and Group 5 had 78.8±12.1 (median=75.3) apoptotic cells (p=0.023) (Figure 6). Additionally, in hippocampal region, Groups 1,3 and 5 had 8.0±2.2 (median=7.5), 8.1±2.1 (median=8.2) and 14.2±6.1 (median=12.9) apoptotic cells (p=0.019) (Figure 7).

In addition to the comparison of all trauma groups, the groups were also compared with each other one by one (Table 2) (Figure 4). There were statistically significant differences between Groups 1 and 5 in midsagital (p=0.002), parasagittal (p=0.019) and hippocampal regions (p=0.015). Moreover, the differences between Groups 3 and 5 in midsagital (p=0.001), parasagittal (p=0.015) and hippocampal regions (p=0.015) were also statistically significant. However, the number of apoptotic cells were similar in Groups 1 and 3 (p>0.05).

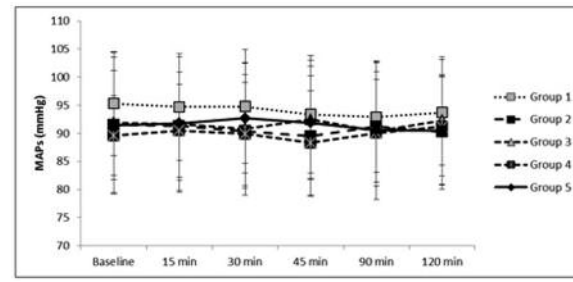

FIGURE 1: The mean arterial pressures (MAPs) during 2-hour experiment period.

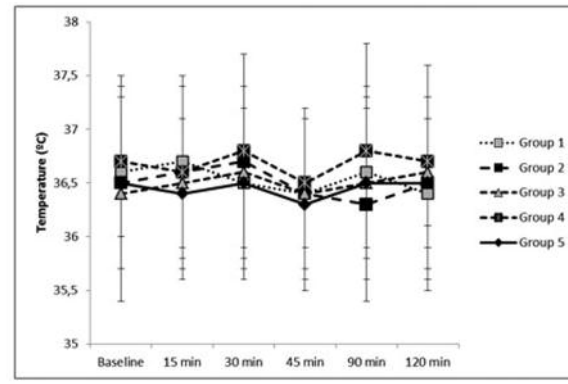

FIGURE 2: The rectal temperatures of the rats during 2-hour experiment period.

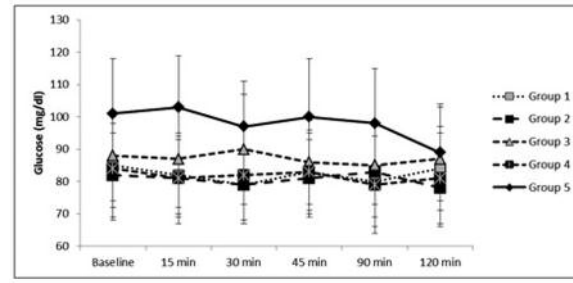

FIGURE 3: The glucose levels of the rats during 2-hour experiment period. The blood glucose levels were significantly higher in Group 5 in the first 45 minutes of the experiment (p<0.05); however there was no differences within the groups during 2-hour experiment period (p>0.05).

The number of apoptotic cells of the groups without trauma (Groups 2 and 4) was very small, and there was no significant differences among the regions (p>0.05) (Table 3).

The variations of MAP, rectal temperature and glucose levels were not statistically significant in the groups (Table 4); however their calculated percentage changes were all differed compared to baseline values in studied groups (Table 5). Accordingly, the percentage changes were found to be comparable in all groups.

TABLE 2: Comparison of the quantity of apoptotic cells in three regions of the brain in groups with trauma.

	Group 1 (n=9)	Group 3 (n=9)	Group 5 (n=9)	P
Midsagital region	156.6 [125.0-192.0]	156.5 [100.0-193.0]	188.5 [167.8-234.6]	0.001* (Group 1-5: p=0.002**) (Group 3-5: p=0.001**) (Group 1-3: p=0.739)
Parasagittal region	63.3 [45.0-75.9]	60.8 [48.2-75.6]	75.3 [66.2-94.6]	0.023* (Group 1-5: p=0.019**) (Group 3-5: p=0.015**) (Group 1-3: p=0.971)
Hippocampal region	7.5 [5.6-12.3]	8.2 [4.9-11.1]	12.9 [7.9-21.6]	0.019* (Group 1-5: p=0.015**) (Group 3-5: p=0.015**) (Group 1-3: p=0.853)

* These statistically significant values show the comparison of all groups (p<0.05).

** The groups were compared with each other one by one with Bonferronni adjustment (p<0.017).

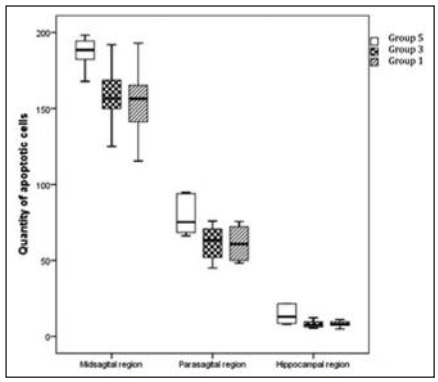

FIGURE 4: The comparison of the quantity of the apoptotic cells in different regions ıf the brain and the groups with trauma. Among the trauma groups (Groups 1, 3, and 5), the apoptotic cells were significantly higher in Group 5 in all regions (p<0.05).

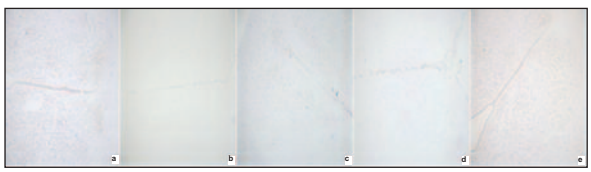

FIGURE 5: Midsagittal region. 1A: Group 1, 1B: Group 2, 1C: Group 3, 1D: Group 4, 1E: Group 5 (TUNEL histochemistry; x400).
(See color figure at http://www.turkiyeklinikleri.com/journal/tip-bilimleri-dergisi/1300-0292/)

DISCUSSION

The outcome in patients surviving TBI is mainly determined by secondary events such as cerebral edema, ischemia or neurodegeneration.[1] Cerebral ischemia and neurodegeneration are associated with an increase in circulating and extracellular brain catecholamine concentrations and apoptosis.[1,18-20] Interventions to reduce sympathetic tone and neuroapoptotic effects may improve neurologic outcome, and therefore propofol and dexmedetomidine

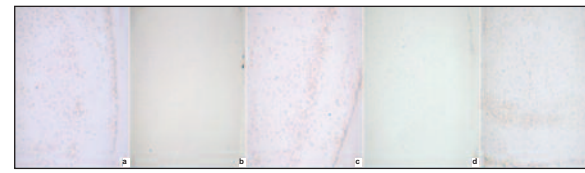

FIGURE 6: Parasagittal region. 2A: Group 1, 2B: Group 2, 2C: Group 3, 2D: Group 4, 2E: Group 5 (TUNEL histochemistry; x400).
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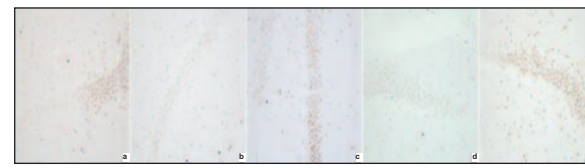

FIGURE 7: Hippocampal region. 3A: Group 1, 3B: Group 2, 3C: Group 3, 3D: Group 4, 3E: Group 5 (TUNEL histochemistry; x400).
(See color figure at http://www.turkiyeklinikleri.com/journal/tip-bilimleri-dergisi/1300-0292/)

TABLE 3: Comparison of the quantity of apoptotic cells in three regions of brain in groups without trauma.

	Group 2 (n=9)	Group 4 (n=9)	p
Midsagital region	3.3 (1.1-6.1)	4.3 (1.3-6.2)	0.315
Parasagittal region	2.7 (1.2-4.6)	2.5 (1.6-4.1)	0.912
Hippocampal region	3.1 (2.3-8.1)	2.7 (1.6-5.9)	0.393

TABLE 4: Glucose, mean arterial pressure and rectal temperature changes in each group.

		Time						
	Group	Baseline	15th min	30th min	45th min	90th min	120th min	Within group p
Glucose								
	Group 1	85 (70-108)	83 (71-111)	86 (72-109)	81 (68-107)	79 (65-102)	80 (71-104)	0.701
	Group 2	82 (70-106)	81 (68-107)	83 (69-109)	78 (69-107)	80 (71-110)	79 (69-102)	0.541
	Group 3	83 (71-107)	82 (70-106)	85 (68-112)	80 (69-106)	81 (69-101)	82 (70-103)	0.815
	Group 4	83 (71-110)	82 (70-111)	84 (70-108)	77 (65-102)	80 (70-108)	81 (70-104)	0.249
	Group 5	100 (81-126)	103 (84-124)	98 (78-119)	100 (80-121)	99 (79-124)	102 (78-125)	0.654
MAP								
	Group 1	93.4 (81.2-108.4)	92.7 (82.5-106.3)	90.4 (79.9-106.8)	92.5 (79.7-110.8)	91.7 (80.7-111.2)	93.6 (82.7-112.3)	0.951
	Group 2	91.5 (79.3-105.2)	90.9 (80.4-103.1)	89.8 (79.7-104.9)	91.8 (79.5-102.8)	90.9 (79.8-104.9)	91.7 (80.8-105.7)	0.825
	Group 3	91.7 (79.1-106.3)	90.7 (80.6-105.4)	89.9 (78.8-105.8)	92.3 (80.5-105.3)	90.8 (80.1-104.7)	91.5 (79.8-103.8)	0.687
	Group 4	89.7 (79.0-105.4)	89.2 (80.1-104.3)	89.9 (80.6-105.8)	90.7 (80.2-103.8)	88.7 (77.6-106.7)	90.6 (78.7-104.2)	0.593
	Group 5	91.2 (79.3-106.4)	91.8 (79.6-104.4)	89.3 (78.4-105.2)	91.6 (79.8-106.6)	90.4 (79.8-104.1)	91.0 (79.0-104.7)	0.785
Rectal temperature								
	Group 1	36.5 (36.1-37.1)	36.4 (36.3-37.2)	36.4 (36.0-37.1)	36.3 (36.0-37.0)	36.5 (36.1-37.0)	36.4 (36.1-37.2)	0.999
	Group 2	36.6 (36.3-37.2)	36.5 (36.2-37.2)	36.4 (36.0-37.1)	36.3 (36.0-37.1)	36.3 (36.0-37.1)	36.4 (36.1-37.3)	0.921
	Group 3	36.5 (36.2-37.0)	36.4 (36.0-37.1)	36.5 (36.1-37.1)	36.5 (36.1-37.1)	36.4 (36.0-37.1)	36.5 (36.2-37.2)	0.984
	Group 4	36.3 (36.0-37.0)	36.4 (36.0-37.1)	36.6 (36.2-37.1)	36.4 (36.0-37.2)	36.5 (36.0-37.2)	36.4 (36.1-37.1)	0.847
	Group 5	36.4 (36.2-37.1)	36.5 (36.3-37.0)	36.5 (36.2-37.2)	36.4 (36.1-37.0)	36.4 (36.0-37.0)	36.3 (36.2-37.2)	0.852

TABLE 5: Glucose, mean arterial pressure (MAP) and rectal temperature changes in percentages in each group.

		Group 1	Group 2	Group 3	Group 4	Group 5	p
Glucose*							
	Baseline-15th min	-0.02 (-0.05-0.08)	-0.01 (-0.05-0.08)	-0.01 (-0.04-0.09)	-0.03 (-0.05-0.10)	0.03 (0.01-0.12)	0.297
	Baseline-30th min	0.02 (-0.01-0.09)	0.02 (0.00-0.08)	0.03 (-0.01-0.10)	0.02 (-0.02-0.12)	0.04 (0.01-0.11)	0.369
	Baseline-45th min	0.01 (-0.03-0.11)	0.00 (-0.03-0.11)	0.02 (-0.04-0.08)	0.04 (-0.02-0.10)	0.02 (-0.01-0.10)	0.854
	Baseline-90th min	0.01 (-0.01-0.08)	0.02 (-0.01-0.08)	0.03 (-0.02-0.11)	0.02 (-0.01-0.11)	0.02 (-0.01-0.13)	0.745
	Baseline-120th min	-0.02 (-0.04-0.08)	0.01 (-0.03-0.09)	-0.01 (-0.03-0.14)	0.02 (-0.05-0.11)	0.04 (0.00-0.14)	0.958
MAP*							
	Baseline-15th min	0.02 (-0.03-0.08)	-0.01 (-0.04-0.10)	-0.01 (0.00-0.09)	-0.03 (-0.05-0.10)	0.04 (-0.01-0.12)	0.745
	Baseline-30th min	0.02 (-0.01-0.09)	0.03 (0.00-0.11)	0.03 (-0.01-0.10)	0.03 (-0.02-0.12)	0.03 (-0.02-0.11)	0.398
	Baseline-45th min	0.01 (-0.02-0.10)	0.00 (-0.03-0.11)	0.02 (-0.03-0.09)	0.04 (-0.03-0.10)	0.02 (-0.03-0.10)	0.785
	Baseline-90th min	0.01 (-0.03-0.12)	0.02 (-0.01-0.08)	0.03 (-0.01-0.11)	0.02 (-0.02-0.11)	0.01 (-0.01-0.11)	0.485
	Baseline-120th min	-0.02 (-0.05-0.08)	0.01 (-0.03-0.09)	-0.01 (-0.05-0.10)	0.02 (-0.04-0.10)	0.05 (0.01-0.16)	0.851
Rectal temperature*							
	Baseline-15th min	0.01 (-0.03-0.08)	-0.01 (-0.04-0.10)	0.01 (-0.01-0.09)	0.02 (-0.05-0.08)	0.03 (-0.01-0.12)	0.658
	Baseline-30th min	0.02 (-0.01-0.09)	0.03 (0.00-0.11)	0.03 (-0.01-0.08)	0.02 (-0.01-0.10)	0.04 (-0.02-0.11)	0.962
	Baseline-45th min	0.01 (-0.02-0.10)	0.00 (-0.03-0.11)	0.02 (-0.03-0.10)	0.03 (-0.03-0.09)	0.05 (-0.03-0.15)	0.997
	Baseline-90th min	0.02 (-0.01-0.10)	0.02 (-0.01-0.08)	0.03 (-0.01-0.11)	0.00 (-0.01-0.10)	0.02 (-0.01-0.10)	0.896
	Baseline-120th min	0.00 (-0.05-0.08)	0.01 (-0.03-0.09)	0.01 (-0.05-0.07)	0.02 (-0.03-0.08)	0.03 (0.01-0.14)	0.924

*Glucose, MAP and rectal temperatures compared to baseline values of changes and percentage changes differed among the groups studied. These statistically significant values show the comparison of all groups ($p<0.05$).

may be used to improve survival. Accordingly, in the current study, we have two main results. First, both propofol and dexmedetomidine significantly decrease the number of apoptotic cells in different regions of the rat brains after mild TBI, and second, these agents have no superiority to each other.

Propofol (2,6-diisopropylphenol) is a short-acting, intravenous hypnotic agent that primarily acts by potentiating the function of the gamma-

aminobutyric acid $(GABA)_A$ and glycine-receptors.[21-23] Propofol's effects on ischemia-reperfusion injury, oxygen-glucose deprivation, apoptosis and neuroprotection have been investigated in both in vivo and in vitro models, and conflicting results were obtained.[9,24-31] Rossaint et al. found that posttraumatic administration of propofol led to a dose-dependent decrease in total injury, and the combination of propofol and hypothermia were additive in regard to neuroprotection.[24] In another study, propofol provided neuroprotection by reversing the increase in glutamate extracellular concentrations and decreasing glutamate uptake after ischemic injury.[25] Adembri et al. reported that propofol had a neuroprotective effect by demonstrating approximately 30% reduced infarct size when it was administered immediately after and up to 30 min after middle cerebral artery occlusion.[9]

On the other hand, dexmedetomidine is an α_2-adrenoceptor agonist that acts by reducing noradrenergic output from the locus coeruleus, decreasing brain norepinephrine levels.[32] Previous studies have reported the ameliorative effect that α_2-adrenoceptor agonists, especially dexmedetomidine, exhibit in acute neuronal injury.[18,33-37] The reason for the neuroprotective effect of dexmedetomidine is thought to be due to its attenuating action for massive release of catecholamines occurring in cerebral hypoxic-ischemia in multiple parts of the brain.[38,39] The administration of dexmedetomidine during the critical phase of synaptogenesis activates the endogenous postsynaptic norepinephrine-mediated trophic system to produce its antiapoptotic effect.[40-42] Several mechanisms have been investigated, however, just one study has mentioned the influence of dexmedetomidine on the number of apoptotic cells. The decreased number of apoptotic neurons with dexmedetomidine, and its neuroprotective effect on hippocampus has been reported.[43] We also obtained similar results suggesting that dexmedetomidine reduced apoptosis after mild cranial injury in midsagital, parasagittal and hippocampal regions, and also compared its antineuroapoptotic effect with propofol. In addition, our current study demonstrated that propofol also prevented apoptosis and tissue injury after mild TBI similar to dexmedetomidine.

In cell culture videomicroscopy studies, the dynamic morphologic changes at the light microscopic level always take place in less than 2 hours.[44,45] Different types of cell death share common mechanisms in the early phases, whereas activation of caspases determines the phenotype of cell death. Detection of apoptotic cells in tissue samples currently relies on the TUNEL assay.[46] Therefore, we decided to investigate the tissue samples of three different regions by TUNEL method at the end of 2-hour period to understand the early apoptotic effects of TBI. Our study is the first one which compared the activities of propofol and dexmedetomidine on apoptosis. This study just intended to find the decreased quantity of apoptotic cells, and no mechanisms were taken into consideration.

Additionally, although our secondary hypothesis was having hemodynamically stable rats in propofol and dexmedetomidine groups in terms of MAP, rectal temperature, and blood glucose levels after mild TBI, there were no differences between the groups during the first 2 hours except for having statistically significant higher blood glucose levels in Group 5 within the first 45 minutes.

CONCLUSION

In conclusion, propofol and dexmedetomidine decrease the formation of apoptotic cells in rats with TBI, and they are beneficial for neuroprotection. The protective effects of these two agents may be a promising for reducing damage in clinical situations, however they have no superiority to each other. Although the results seem to be beneficial, the clinical significance of these results must be elucidated with further studies.

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