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SCIENTIFIC ARTICLE

Tourniquet-induced ischaemia-reperfusion injury: the comparison of antioxidative effects of small-dose propofol and ketamine

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KEYWORDS
Propofol;
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Abstract
Objectives: The aim of the present study was to investigate the preventive effects of propofol and ketamine as small dose sedation during spinal anaesthesia on tourniquet-induced ischaemia-reperfusion injury.
Methods: 30 patients were randomly assigned into two groups of 15 patients. In the propofol group, sedation was performed with propofol 0.2 $mg{\cdot}kg^{-1}$ followed by infusion at a rate of 2 $mg{\cdot}kg^{-1}{\cdot}h^{-1}$. In the ketamine group, a continuous infusion of ketamine 0.5 $mg{\cdot}kg^{-1}{\cdot}h^{-1}$ was used until the end of surgery. Intravenous administration of midazolam was not used in any patients. Ramsay sedation scale was used for assessing the sedation level. Venous blood samples were obtained before propofol and ketamine infusion (T1), at 30 minutes (min) of tourniquet ischaemia (T2), and 5 min after tourniquet deflation (T3) for malondialdehyde (MDA) measurements.
Results: No differences were noted between the groups in haemodynamic ($p > 0.05$) and demographic data ($p > 0.05$). There was no statistically significant difference between the two groups in terms of T1, T2 and T3 periods ($p > 0.05$). There was a statistically increase observed in MDA values respectively both in Group P and Group K between the reperfusion period (1.95 ± 0.59, 2.31 ± 0.48) and pre-ischaemia (1.41 ± 0.38, 1.54 ± 0.45), and ischaemia (1.76 ± 0.70, 1.71 ± 0.38) (μmol^{-1}) periods ($p < 0.05$).
Conclusions: Small-dose propofol and ketamine has similar potential to reduce the oxidative stress caused by tourniquet-induced ischaemia-reperfusion injury in patients undergoing arthroscopic knee surgery under spinal anaesthesia.

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PALAVRAS-CHAVE
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Isquemia-reperfusão

Lesão de isquemia-reperfusão induzida por torniquete: comparação dos efeitos antioxidantes de propofol e cetamina em doses baixas

Resumo
Objetivos: O objetivo do presente estudo foi investigar os efeitos preventivos de propofol e cetamina em sedação com doses baixas durante a raquianestesia sobre lesão de isquemia-reperfusão induzida por torniquete.
Métodos: 30 pacientes foram randomicamente alocados em dois grupos de 15 pacientes cada. No grupo propofol, a sedação foi realizada com 0,2 mg.kg^{-1} de propofol seguida por infusão a uma taxa de 2 mg.kg^{-1}.h^{-1}. No grupo cetamina, uma infusão contínua de 0,5 mg.kg^{-1}.h^{-1} de cetamina foi usada até o final da cirurgia. Midazolam intravenoso não foi administrado em nenhum dos pacientes. A Escala de Sedação de Ramsay (ESR) foi usada para avaliar o nível de sedação. Amostras de sangue venoso foram colhidas antes da administração de propofol e infusão de cetamina (T1), aos 30 minutos (min) de isquemia do torniquete (T2) e 5 min após a desinsuflação do torniquete (T3) para medir os valores de malondialdeído (MDA).
Resultados: Não observamos diferenças entre os grupos em relação à hemodinâmica ($p > 0,05$) e dados demográficos ($p > 0,05$). Não houve diferença estatisticamente significativa entre os dois grupos nos períodos T1, T2 e T3 ($p > 0,05$). Um aumento estatisticamente significativo foi observado nos valores de MDA, respectivamente, no Grupo P e Grupo C entre os períodos de reperfusão ($1,95 \pm 0,59$, $2,31 \pm 0,48$) e pré-isquemia ($1,41 \pm 0,38$, $1,54 \pm 0,45$) e isquemia ($1,76 \pm 0,70$, $1,71 \pm 0,38$) (μmoL^{-1}) ($p < 0,05$).
Conclusões: Propofol e cetamina em doses baixas apresentam potencial semelhante para reduzir o estresse oxidativo causado pela lesão de isquemia-reperfusão induzida por torniquete em pacientes submetidos à artroscopia de joelho sob raquianestesia.

Introduction

Placing the tourniquet proximally is frequently performed in order to ensure an exsanguine area in extremity surgeries. Resupplying the blood flow after loosening the tourniquet results in ischaemia-reperfusion injuries (IRI). Releasing reactive oxygen species (ROS) abruptly and extremely after reperfusion leads to endothelium dysfunction and neutrophile infiltration.[1] While neutrophiles react with the adhesion molecules on their surfaces (CD11/CD18) and the receptors on the endothelium surface (ICAM-I), ROS react with the multiple unsaturated fat acids on the cell membrane and initiate lipid peroxidation.[2] Once the lipid peroxidation starts, it continues as a successive reaction sequence. Malondialdehyde (MDA), which is one of the final products of lipid peroxidation, is quite toxic. MDA not only affects enzyme activities in cells and ion transportation, but also reacts with DNA alkaline and acquire mutagenic characteristics.[3,4]

Antioxidants and free radical scavengers, which are the defence components of body, may limit lipid peroxidation, which mediates tissue injury. In terms of its structure, propofol (2,6-diisopropylphenol) resembles to chemicals that draw away phenol-based free radicals such as butylated hydroxytoluene and endogenous antioxidant α-tocopherol.[5] It not only prevents peroxynitrite accumulation that initiates lipid peroxidation[6] but also accumulates in lipophilic membranes that are sensitive to oxidative damage.[7]

Ketamine is a dissociative anaesthetic[8] and it protects IRI-opponent neurons caused by lipid peroxidation.[9] Studies have revealed that both anaesthetic agents have been compared with the control group and that they reduce lipid peroxidation and ischaemia reperfusion injury.[10,11] However, there have been no studies comparing the effects of propofol and ketamine on MDA in muscular ischaemia reperfusion injury.

In this randomised and prospective study, we compared the small dose infusion effects of propofol and ketamine on ischaemia reperfusion injury among patients who underwent arthroscopic knee surgeries performed with the use of tourniquets under spinal anaesthesia. MDA was measured for this purpose.

Methods

After having received the approval from the ethics committee (ethical approval for this study was provided by the Ethical Committee of Ataturk University Hospitals, Erzurum, Turkey on 04/03/2015-4-2) and informing the patients about the study, 30 adult ASA I patients planned to undergo elective arthroscopic knee surgeries were divided into two randomised groups: Group P (propofol, $n = 15$) and Group K (ketamine, $n = 15$). Patients who had metabolic, renal, and hepatic problems, antioxidant medication histories, chronic pain histories, and were smokers, were excluded from the study. No premedication was administered on

patients before the operation. Heart rate (HR), non-invasive blood pressure (NIBP), and peripheral O_2 (SpO_2) were monitored in the operation room. After placing 20G branule on the dorsum of the hand, 500 mL 0.9% NaCl was administered to all patients before spinal anaesthesia. One venous catheter was inserted on contralateral arm for receiving blood samples. After receiving basal blood sample (pre-ischaemia), spinal anaesthesia was applied with a 25G spinal injector and 10–12.5 mg 0.5% hyperbaric bupivacaine on the L_3–L_4 spinal space. When it was determined that the sensorial block reached the sufficient level by the use of pinprick test, propofol (Propofol 1%, Fresenius) was administered with a dose of 0.2 mg·kg^{-1}, and 2 mg·kg^{-1}·h^{-1} infusion rate was continued in Group P; while ketamine (Ketalar 500 mg/10 ml, Pfizer) was administered with an infusion rate of 0.5 mg·kg^{-1}·h^{-1} in Group K. IV anaesthesia infusion was continued until 5 minutes after reperfusion. Ramsay sedation score (RSS; 1 = awake, anxious and/or crying, 2 = awake, tranquil and cooperative, 3 = asleep but responds to verbal stimuli, 4 = asleep however briskly responds to glabellar tap stimuli, 5 = asleep however sluggishly responds to glabellar tap stimuli, 6 = does not respond to stimuli) was used before and after sedation at the 5th, 10th, 20th, 30th, 45th, 60th and 80th minutes. After reaching a sufficient level of sedation (RSS score 3), infusion dose was reduced to propofol 1 mg·kg^{-1}·h^{-1} in Group P and ketamine was reduced to 0.25 mg·kg^{-1}·h^{-1} in Group K. In addition, potential side effects associated with these anaesthetic agents such as respiration depression, nausea–vomiting, itching and dysphoria (hallucination and dreams) were evaluated and recorded.

The tourniquet was inflated as 2 times of the systolic arterial blood pressure. IV 0.9% NaCl was administered to all patients during the operation. Blood samples were taken before spinal anaesthesia (pre-ischaemia, t_1), 30 minutes after the tourniquet application (ischaemia, t_2), and 5 min after the tourniquet was loosened (reperfusion, t_3). 2 mL of O_2 was given to all patients with nasal cannula.

Blood samples were immediately centrifuged at 8000 × *g* for 5 min. Blood was placed on ice and the supernatant was stored at −20 °C until analysis. For detecting lipid peroxide concentrations of plasma thiobarbituric acid reacting substance, 0.1 mL of trichloroacetic acid (25 g trichloroacetic acid in 10 mL distilled water) was added to 0.5 mL plasma by vigorous shaking. The resulting mixture was reacted with 1 mL of thiobarbituric acid 0.67% and then heated in boiling water for 30 min. The samples were centrifuged at 2000 × *g* for 15 min and the absorption was measured at 532 nm.

Table 2 Ramsay sedation score of groups.

Times	Propofol group ($n = 15$)	Ketamine group ($n = 15$)	*p*-Value[a]
Baseline	2 (1–2)	2 (1–2)	0.355
5 min	2 (2–3)	2 (1–3)	0.859
10 min	2 (2–4)	2 (1–4)	1.000
20 min	2 (2–5)	2 (2–4)	0.862
30 min	2 (2–5)	2 (1–4)	0.327
45 min	3 (2–5)	2 (1–5)	0.054
60 min	3 (2–5)	2 (2–5)	0.083
80 min	3 (2–5)	2 (2–5)	0.213

Values are given as median (min–max).
[a] $p > 0.05$ independent-sample *t* test.

Plasma levels of lipid peroxides were calculated as micromoles per litre.

The primary outcome of the study was the MDA level at reperfusion period. In our preliminary study, we found that the standard deviation was 0.5 in Group ketamine and 0.4 in Group propofol. We aimed to detect a difference between the two groups at least 0.5 μmoL^{-1} on MDA level at reperfusion period. Accordingly, we determined that the number of patients required in every group was 15, based on the power of 83%, alpha error of 0.05 by using Russ Lenth's Piface Java module.

The Kolmogorov–Smirnov test was used to analyse the normal distribution of the variables obtained. Nonparametric tests were performed for data that did not demonstrate a normal distribution. Age was compared between the groups with the Mann–Whitney *U*-test. Student's *t*-test was used for comparing weight, height and duration of tourniquet. Repeated-measures analysis of variance was performed for comparing the time effect and the differences between groups in plasma MDA levels. $p < 0.05$ was considered as statistically significant. All data are presented as mean ± SD or number.

Results

No significant difference was found between groups in terms of age, gender, height, weight and tourniquet time ($p > 0.05$) (Table 1). There was also no significant difference between the groups in terms of haemodynamic changes. There was no difference between the groups in terms of RSS scores (Table 2). No difference was observed

Table 1 Demographic characteristics of patients.

	Propofol group ($n = 15$)	Ketamine group ($n = 15$)	*p*-Value
Age (years)	42.35 ± 9.09	40.30 ± 8.53	0.466[a]
Height (cm)	165.47 ± 8.68	169.73 ± 5.77	0.124[b]
Gender (F/M)	7/8	5/10	0.121[c]
Weight (kg)	76.87 ± 10.12	78.53 ± 8.40	0.625[b]
Tourniquet duration (min)	38 (30–80)	44 (30–80)	0.512[b]

Values are given as number, median (min–max) or mean ± SD.
[a] Mann–Whitney *U*-test.
[b] Independent sample *t* test.
[c] Chi-square test.

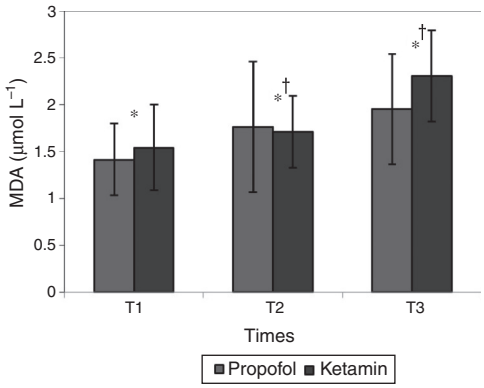

Figure 1 Plasma malondialdehyde (MDA) concentrations of groups (*$p > 0.05$ when comparison was made between groups; †$p < 0.05$ when intragroup comparison was made with the baseline value (T1) of the groups; T1, preischaemia; T2, ischaemia and T3, reperfusion).

between the groups in terms of side effects such as respiration depression, nausea–vomiting and hallucination. There was no statistically significant difference between the two groups in terms of pre-ischaemia, ischaemia and reperfusion periods ($p > 0.05$). However, when the groups studied in themselves, a statistically evident increase was observed in MDA values respectively both in Group P and Group K between the reperfusion period (1.95 ± 0.59, 2.31 ± 0.48) and pre-ischaemia (1.41 ± 0.38, 1.54 ± 0.45), and ischaemia (1.76 ± 0.70, 1.71 ± 0.38) (μmol^{-1}) periods ($p < 0.05$) (Fig. 1).

Discussion

In this study, the small dose infusion effects of propofol and ketamine on ischaemia reperfusion injury among patients who underwent arthroscopic knee surgery performed using tourniquets under spinal anaesthesia were compared. In order to achieve this purpose, MDA levels, which are oxidative stress markers, in blood samples taken in basal; ischaemia and reperfusion periods were measured and compared between the groups. As a result of the study, MDA levels in blood samples taken in basal, ischaemia and reperfusion periods were found to be similar in both groups. Additionally, as a result of the intragroup comparison, MDA levels measured in reperfusion period in both groups were found to be higher than the ischaemia period.

Tourniquet deflation and reperfusion lead to increase in ROS production and this initiates lipid peroxidation. MDA, a lipid peroxidation product, is a marker for tissue damage.[12] It causes more oxidative damage by leading to oxidation of protein molecules.[13]

Propofol reduces the release of stress hormones such as adrenaline and noradrenaline,[14] accumulates in cell membranes and frees hydrogen atoms, which are free radicals, empowers the property of free radical scavenging.[5] Therefore, it protects erythrocytes against oxidative stress and accumulates particularly in lipophilic membranes, and increases the tissue antioxidant defence.[15] Murphy et al., have showed that propofol has a scavenging property of ROS in anaesthetic concentrations.[16]

Antioxidant characteristics of ketamine were revealed through various mechanisms. The main opinion on this subject is associated with the fact that it blocks N-methyl-D-aspartate receptors, prevents Ca^{++} from entering cells and increases blood flow in ischaemic tissue.[17] In addition, reducing adhesion molecules such as p-selectin and ICAM-1 in leucocyte, thrombocyte and endothelial cells is another reason for the antioxidant characteristic of ketamine.[18]

In this study, special attention was paid to patient selection, the anaesthesia method to be applied, and not using midazolam in premedication in order to compare the antioxidant characteristics of small dose propofol and ketamine sedation. Since both induction and inhalation agents administered to apply general anaesthesia and the midazolam administered as an anxiolytic in addition to them have been proved to have antioxidant characteristics in the literature.[19–21] Therefore, we conducted our study under spinal anaesthesia without the use of midazolam.

In the study of Cheng et al.,[10] propofol infusion was applied on patients, to undergo total hip replacement operation under spinal anaesthesia, for sedation purpose and results were compared with the control group. They demonstrated that ROS reduced at both 5th and 20th minutes as being more evident at the 20th minute of the reperfusion, and they concluded that propofol exhibited antioxidant characteristics in membranes due to its accumulation effect. Sarıcaoğlu et al.,[11] administered ketamine for sedation purpose on patients to undergo arthroscopy operations under spinal anaesthesia and compared the results with the control group. Although there was a decrease in the blood MDA concentration in the ketamine group at the 5th minute of the reperfusion in the comparison of groups, this decrease was not statistically significant; while a statistically significant decrease was observed in the tissue MDA concentration in the ketamine group and therefore, ketamine's antioxidant characteristics were exhibited. We used the same doses used in these two studies. Due to the structural quality of propofol and its accumulative effect on membranes, we thought that its antioxidant characteristics would be more distinct than ketamine. In the intragroup comparison, although MDA value was lower in the propofol group than the ketamine group, no statistically significant difference was found between the groups. In addition, due to the fact that the MDA values we obtained in reperfusion period were higher than the ischaemia period, we could not reach the same results with the two referred studies. We think that this is associated with the fact that midazolam helped the antioxidant characteristics of ketamine by giving preoperative midazolam to patients in the study of Cheng et al., and propofol could not accumulate sufficiently in membranes and could not exhibit sufficient antioxidant characteristics by keeping the reperfusion time as 5 minutes in the study of Sarıcaoğlu et al.

In our study, MDA, which is the indicator of cell injury, was found higher in the reperfusion period than the ischaemia period. Ischaemia has detrimental effects on cells. However, the histological changes occurring after three-hour ischaemia and then one-hour reperfusion were worse than the changes observed only in four-hour ischaemia period.[22] In addition, after revascularization, mediators such as ROS in the ischaemic tissue initiate systemic circulation and lead to the release of chemotactic mediators. These chemotactic

mediators lead to the sequestration of inflammatory leukocytes from the primary ischaemic area to other organs.[23] All these changes emphasise that more attention should be paid to reperfusion period than ischaemic period. In their study where they measured MDA concentration in ischaemic, reperfusion and control group rat hearts, Pierro et al.,[24] showed that MDA level increased in the reperfusion period more distinctly than ischaemia period, and exhibited presence of the measurable molecular damage on the tissue. MDA was closely associated with tissue damage caused by IRI, which increased in the reperfusion period.[25] In our study, we also determined that it increased in the reperfusion period by using MDA, which is a good indicator of tissue damage.

Our study has limitations. First of all, due to the nature of surgery, reperfusion period was short. Our second limitation was that we used measurement of MDA as the only indicator in the evaluation of tissue damage.

In conclusion, the results of the present study suggest that small-dose propofol and ketamine has similar potential to reduce oxidative stress caused by tourniquet-induced ischaemia-reperfusion injury in patients undergoing arthroscopic knee surgery under spinal anaesthesia.

Conflicts of interest

The authors declare no conflicts of interest.

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