Comparison of Cerebral Effects of Thiopental and Propofol Infusion in Traumatic Brain Injured Rats

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Abstract

Objective: To compare the cerebral effects of propofol and thiopental infusions in rats with experimental head trauma.

Methods: This experimental study included a total of 30 rats, and the animals were randomly divided into 3 groups; as control group (Group C), propofol group (Group P) and thiopental group (Group T). Blood samples were taken 4 hours following infusion. A craniotomy was performed, the brain was removed, and it was placed in 10% neutral formalin for histopathological examination. The materials were examined biochemically and histopathologically, and then compared between the groups.

Results: The S100B value between the groups was significantly lower in the thiopental group compared to the control group (p = 0.018). Tau protein levels were significantly lower in the propofol group compared to the control group (p = 0.07). In histological examinations, the numbers of apoptotic cells in the propofol and thiopental groups were significantly lower than in the control group (p = 0.02). There was no significant difference between the propofol and thiopental groups in terms of apoptotic cell numbers (p = 0.3).

Conclusion: Our study demonstrated that thiopental and propofol infusions following a head trauma reduce apoptotic cell death and cause decreases in trauma markers.

Introduction

One of the most important causes of childhood and young adult deaths worldwide is head trauma due to accidents, both inside and outside vehicles. Deaths due to head traumas are responsible for approximately 50% of all deaths due to general. Head trauma is the fourth most common (37%) cause of death in the adults and the leading cause of permanent disability in patients under 40 years of age (Ökten Aİ et al. 1997; Pace MC et al. 2006). Permanent disabilities caused by head trauma not only affect the patients and their families but also cause an increase in medical costs and economic burden. Thus, it is very important to prevent head traumas or provide fast and effective treatments after they occur (İşik HS et al. 2011).

The first stages of brain injury resulting from a head trauma include primary cellular damage in the brain tissues. The secondary brain damage occurs if the the damage has a progression and the early and effective management cannot be provided. The risk of permanent disability due to secondary brain damage is also minimized if some factors that cause brain damage such as bleeding, edema, and increased intracranial pressure can be prevented in the early period. (Dohi et al. 2007, Wilson and Gelb. 2002). Inhibition of free oxygen radicals, which lead to secondary damage, positively affects the poor neurological picture after trauma or ischemia in the central nervous system (Huh P et al. 2000).

Propofol and thiopental are intravenous anesthetic agents that use in general anesthesia. Propofol protects the brain from ischemic damage by preventing lipid peroxidation (Bayona et al. 2004).
Thiopental is an effective anticonvulsant that decreases oxygen consumption of the brain (Büyük and Karakoç 2019). Both agents reduce cerebral blood flow and metabolic rate. The cerebral metabolic rate provides a balance between oxygen delivery and consumption in the brain (Lovell et al.1999). Due to these effects, both agents are used in the cerebral protection.

In this experimental study, we aimed to compare the cerebral effects of propofol and thiopental infusions in a head trauma model created in rats.

**Methods**

After receiving approval from the Bolu Abant Izzet Baysal University local ethics committee (decision number: 2017/04), a total of 30 rats weighing 250–300 g were included in the study. Rats were randomly divided into 3 groups, with 10 rats per group. The head trauma model was a modified trauma model developed by Marmarou et al. (Marmarou et al. 1994). With regard to the trauma device, the main principle was to drop a 200-g piece of metal, using only the force of gravity, from a height of 50 cm onto a metal disk located on the rat's head. Rats were sedated with intramuscular ketamine (90 mg/kg) and xylazine (10 mg/kg). After 5 minutes, the rats were sedated and the trachea and tail areas were shaved. The trachea was explored before the trauma in order to prevent hypoxia and ensure rapid ventilation. For this reason, the neck area was sterilized before the trauma. Soft tissues in the trachea region were dissected after the skin incision. The trachea was explored. A 3/0 suture was passed around the trachea and prepared to bind after cannulation. After head trauma was applied, the trachea was cannulated with a 16 G venous cannula, fixed with 3/0 thread, and rat attached to the mechanical ventilator. This process took a maximum of 30 seconds. A vascular access was then opened with a 24 G intracet from the tail vein. The rat was then placed in the trauma apparatus. A stainlesssteel metal disc was adhered on the vertex of the rat’s head to prevent slippage and to create diffuse cranial damage and provide a wider cranial contact area. When the rat was placed on the platform, the trauma device was pulled upwards and the weight release mechanism was pulled, which allowed a 200-g weight to fall on the metal disk. The rat was then placed in the supine position and the skin in the tracheal region was incised and the trachea was released and intubated at the 4th cartilage ring using a 16 G intracet. Airway safety was assured and then connected to a mechanical ventilator. Intravenous (iv) propofol and thiopental infusion doses were previously prepared for the infusion pumps. The control group \( (n = 10) \), after the head injury, received a 5 ml/kg/h 0.9% NaCl infusion and blood samples were taken at the end of the 4th hour. The propofol group \( (n = 10) \) immediately received a 0.5 mg/kg/min propofol infusion and blood samples were taken at the end of the 4th hour. The thiopental group \( (n = 10) \) immediately received a 140 mcg/kg/h dose of thiopental and blood samples were taken at the end of the 4th hour. After the blood samples, 0.1 mg/kg vecuronium was administered intravenously to provide the necessary muscle relaxation. At the end of the study, ventilation was terminated and the head was dissected between the atlas and axis. The brain was removed with a temporal craniotomy, and the rats were sacrificed. This process took a maximum of 2 minutes.
Biochemical analysis

Phospho tau protein (p\(\tau\)), glial fibrillary acidic protein (GFAP), neuron-specific enolase (NSE), S100 calcium-binding protein B (S100B), Serum Total Antioxidant Level (TAL) and Total Oxidant Level (TOL) analyzed. Blood samples were taken into tubes containing the coagulation activator. Samples were centrifuged at 1500g for 10 min and the sera were separated. Aliquotized sera were stored at -80 °C until biochemical analysis. Samples were gradually thawed prior to analysis. TAS and TOS levels, Rel commercial assay kit (Rel Assay Diagnostics, Gaziantep, Turkey) C8000 using Architect (Abbott, Chicago, IL, USA) were measured in accordance with the manufacturer's instructions otoanalyser. The oxidative stress index (OSI) was calculated using the (TOS / TAS x100) formula. Phospho tau protein, GFAP, NSE and S100B were measured using commercial enzyme-linked immunosorbent measuring kits (Elabscience Biotech, Wuhan, China) according to the manufacturer's instructions.

Histopathological examination:

All histopathological analyzes were performed by a specialist with 30 years of experience (A.K.). The brain tissues were placed as three groups in formol-containing vessels to prevent deterioration. Brain tissue samples were fixed in 10% neutral formalin. Routine histological follow-up was performed and paraffin blocks were prepared. 5 µm thick sections were stained with hematoxylin eosin and evaluated by light microscope. To evaluate apoptotic cells, immunohistochemical staining of the terminal deoxynucleotidyl transferase dUTP nick-end-labeling (TUNEL) (Roche Cat. No: 11684817910, Germany) was performed. TUNEL positive cells were counted in 5 different areas of the cortex and hippocampus regions including frontal, temporal, occipital, parietal and limbic region in the tissue section of each subject with X40 objective magnification.

Statistical analysis

Statistical Package for Social Sciences (SPSS) program version 21 was used for statistical analysis. Data were expressed as mean ± standard deviation. The data were analyzed with Levene Statistic test. One-Way Anova test and Post-Hoc Test-Games-Howell test were used to compare the parameters that did not show normal distribution. Independent sample t test was used to compare the two groups of the parameters that did not show normal distribution. p < 0.05 was considered statistically significant.

Results

Histopathological examination showed that congestion and edema were significantly increased in the control group compared to the propofol and thiopental groups (Figs. 1, 2 and 3).

In the TUNEL immunohistochemical staining performed to evaluate apoptotic cells, apoptotic cells were counted in five different areas of the cortex and hippocampus regions on each subject with X40 objective
enlargement. TUNEL immunohistochemical staining showed that number of apoptotic cells were significantly increased in the control group compared to the propofol and thiopental groups (Fig. 4,5,6).

S100B was significantly lower in the thiopental group in our study (p = 0.018*). TAU protein was significantly lower in the propofol group (p = 0.007*). The oxidative stress index value was significantly lower in the propofol group compared to the thiopental and control groups. (p = 0.04*) (Fig. 7, Table 1).

| Biochemical markers of the groups |
|----------------------------------|
| Propofol | Thiopental | Control | P value |
| NSE (ng/ml) | 18.1 | 27 | 20.2 | 0.200 |
| S100B (pg/ml) | 688 | 501 | 1171 | 0.018 |
| GFAP (ng/ml) | 3.9 | 6.2 | 4.0 | 0.060 |
| TAU Protein (pg/ml) | 145 | 178 | 359 | 0.007 |
| TAS (umol/l) | 1.75 | 1.84 | 1.77 | 0.601 |
| TOS (umol/l) | 3.99 | 5.88 | 5.71 | 0.607 |
| TNAC | 6.82 | 6.95 | 19.78 | 0.020 |

NSE: neuron specific enolase, GFAP: glial fibrillary acidic protein, TAS: total antioxidant status, TOS: total oxidant status NAC: The number of abnormal cells

There was no significant difference in NSE and GFAP values between the groups (Table 1).

Discussion

This experimental study showed that in the acute period after head trauma propofol and thiopental prevented the increase of brain injury, and in the histopathological examination amounts of congestion and edema and number of atypical cell decreased. In addition, it was demonstrated that the expected increases in some trauma markers decreased. However, no any superiorities were identified between propofol and thiopental.

The cranial space is a closed compartment containing brain tissue, cerebrospinal fluid, blood and extracellular fluid. Vascular and hemodynamic mechanisms deteriorate after traumatic brain injury. The intracranial compartment expands with increased tissue edema and increased blood count. Cerebral ischemia leads to cerebral pausing, neuronal damage, and increases in intracranial pressure. This leads to an imbalance between the amount of oxygen delivered to the brain and the amount of oxygen delivered to the brain. The incidence, duration and extent of tissue hypoxia are associated with poor prognosis (Jonston et al.2005; Marik et al. 2002; Stiefel et al. 2006). Therefore, early diagnosis and treatment are important. Antiinflammatory agents, glutamate antagonists, cation homeostasis
modulators, endocannabinoids, free radical scavengers, immunosuppressants, apoptosis and caspase inhibitors have a role in the pharmacological treatment of traumatic brain injuries (Royo et al. 2003).

There are various animal models of head trauma that use experimental studies. While previous animal models mainly took into account the biomechanical characteristics of brain damage, more current models have aimed to improve the understanding of more complex molecular deleterious mechanisms of the trauma. Since the rodents have relatively small size and cost that allows repetitious biochemical and histopathological examinations, many researchers have preferred the rodent models as the most appropriate option in brain trauma investigations in spite of many different ideas concerning the model type to choose (Cernak I 2005). In our study we aimed to establish a head trauma model in rats, without the death of the animal. We used a modified trauma model developed by Marmarou et al. (Marmarou et al 1994) among various animal models of head trauma because we had this model in our laboratory.

Propofol reduces intracranial pressure in patients with normal or increased intracranial pressure by reducing cerebral blood flow (Adembri 2006). In many studies, the neuroprotective effect of propofol has been investigated (Sitar et al. 1999; Zhu et al. 1997). It has been found that it reduces postischemic injury in transient focal ischemia by different mechanisms (Adembri et al. 2006; Gelb et al. 2002). Thiopental causes cerebral protective effect by decreasing cerebral blood flow, intracranial pressure and cerebral metabolic rate (Büyük and Karakoç 2019).

Lipid peroxidation, membrane damage, deoxyribonucleic acid damage, protein denaturation and mitochondrial damage occur due to elevated levels of reactive oxygen species. Oxidative stress develops as a result of the increase of oxidants and decrease of antioxidants. The oxidative stress index (OSI) can be calculated as a result of Total Antioxidant Level (TAL) and Total Oxidant Level (TOL) measurements. A high oxidative stress index (OSI) show that increased oxidative stress (Akkoca et al. 2019). Kaptanoğlu et al. investigated the antioxidant effects of propofol and thiopental in experimental spinal cord trauma. They found that malondialdehyde levels increased as an indicator of lipid peroxidation in rats treated with contusion injury. They showed that thiopental and propofol decreased lipid peroxidation, but propofol did not improve microstructure (kaptanoglu et al. 2002). Öztürk et al. investigated the antioxidant properties of propofol and erythropoietin after closed head trauma in rats. It has been reported that administration of propofol and erythropoietin in acute phase causes significant reductions in oxidative stress metabolites after trauma (Ozturk et al. 2005) Canakci et al. investigated the cerebral protective effects of thiopental and dexmedetomidine by forming a focal cerebral ischemia model in rats. They stated that thiopental and dexmedetomidine have protective effect in cerebral tissue but they could not find a significant difference between them (Canakci et al 2016). In our study, we could not find any difference between the antioxidant properties of thiopental and propofol. But the oxidative stress index value was significantly lower in the propofol group compared to the thiopental and control groups.

Biochemical markers such as S100B, NSE, GFAP and Phospho tau protein are important for the evaluation of post-traumatic damage ( Anand and Stead 2005; Berger 2006; Eng and Ghirnikar 1994; Zemlan et al 1999.) The S100B has a half-life of 60 minutes and is the best predictor of brain damage
There are studies suggesting that NSE peaks after 24 hours rather than the acute period (Anand and Stead 2005). Pelinka et al. looked at GFAP and S100B in a 12-hour period in patients with traumatic brain injury, and compared them with Glasgow Coma Scale (GCS) scores. They did not deviate from the significant differences in these markers and indicated that they could be used in prognosis (Pelinka et al. 2008). Olczak et al. studied TAU protein as a possible biochemical marker of traumatic brain injury in postmortem examination, and revealed that TAU protein levels were significantly higher in patients with head trauma. The authors finally concluded that increase in TAU protein levels could be due to the axonal injury (Olczak et al. 2017). In our study, we could not find a significant difference in GFAP values seen at the end of the 4th hour. S100B was found to be significantly lower in rats given thiopental. TAU protein was significantly lower in propofol group. NSE, we have linked the increase in GFAP to the late increase in GFAP.

TUNEL staining was performed to determine apoptotic cells in histological sections. Wang et al. looked at the beneficial effects of erythropoietin in head trauma-induced rats. TUNEL positive cells were evaluated in brain sections. Rats with erythropoietin had decreased TUNEL-positive stained cells (Wang et al. 2016). Zhang et al. investigated the efficacy of progesterone in head trauma-induced rats. TUNEL positive stained cells were evaluated in brain tissue sections. The number of TUNEL positive cells in progesterone-treated rats was significantly lower (Zhang et al. 2017). We found that the number of apoptotic cells in propofol and thiopental group were significantly lower.

In this study, we limited our infusion time to 4 hours in order to keep the rats alive. We determined the duration of infusion as 4 hours, considering the presence of trauma markers in serum and the survival of the rats (Cotman et al. 2005; Rothermundt et al. 2003). The limitation of our study was short infusion time. In order to understand the antioxidant effects of thiopental and propofol and their effects on brain trauma markers, different studies are needed to increase infusion times.

In conclusion, we think that thiopental and propofol infusion after head trauma reduces apoptotic cell death, decreases congestion and edema, and causes a significant decrease in trauma markers. In order to better understand the effects of thiopental and propofol on brain trauma and markers, further prospective randomized large-scale studies with different infusion doses are required.

**Declarations**

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**Conflict of interest**

The authors declare that they have no conflict of interest.
Authors’ contributions

YK and İY conceived and designed research. AÇ and HY performed the experiments. BTK performed biochemical analysis. AK performed histopathological analysis. İK analyzed the statistics. AD and MB analyzed the data. All authors read and approved the manuscript and all data were generated in-house and that no paper mill was used.

Ethics approval

All experiments were performed according to the ethical committee guidelines of Bolu Abant Izzet Baysal University Local Ethics Committe (Ethical number: 2017/4).

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