The Protective Effect of Thiopental Againts Renal Ischemia/ Reperfusion Damage in Rats

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Abstract

The aim of this study was to determine optimal protective thiopental dose in renal ischemia/reperfusion (I/R) damage. This study was carried out on 42 rats. After abdominal midline incision, left renal artery was occluded. After 60 min of ischemia and reperfusion, a left nephrectomy was performed. Surgical intervention was made for the six rat groups (excluding sham group). In control group, 60 minutes of ischemia and reperfusion were applied to left kidneys. Thiopental (3, 10, 30, 60, 80 mg/kg) was applied intra-peritoneally to the rats except sham and control groups 15 minutes before reperfusion. After reperfusion, IL-1α, IL-6, TNF-α levels; SOD activity; MDA and NO levels were measured. There was no decrease in IL-6 or TNF-α levels in groups at all doses. IL-1α levels only decreased in the group receiving 3 mg/kg thiopental. Only the group receiving 80 mg/kg observed decrease in MDA levels. The rats receiving 30 mg/kg showed significant increase in SOD levels. The rats receiving 3 mg/kg detected significant increase in glutathione peroxidase (GPx) levels. The groups receiving 10, 30, and 60 mg/kg thiopental showed decrease in nitric oxide (NO) levels. According to study results, different doses of thiopental affected each biochemical indicator in renal I/R damage.

Keywords: Renal Ischemia/Reperfusion, Thiopental, Rat

Ratlarda Böbrek İşemi/Reperfüzyon Hasarına Karşı Tiyopentalin Koruyucu Etkisi

Özet

Çalışmada ratlarda farklı dozlarda uygulanan tiyopentalin renal I/R hasarına karşı koruyucu optimal dozunun saptanması amaçlandı. Bu çalışma 42 ratta yapıldı. Abdominal orta hat insizyonu sonrası sol renal arter okluzyonu yapıldı. Altmış dakika işemi ve reperfüzyondan sonra sol nefrektomi yapıldı. Altışar rattan oluşan gruplarda (sham grup dışında) cerrahi prosedür uygulandı. Kontrol grubunda sol böbreklere 60 dk işemi ve reperfüzyon uygulandı. Sham ve kontrol grubu dışındaki ratlara reperfüzyondan 15 dk önce tiyopental (3, 10, 30, 60, 80 mg/kg) intra-peritoneal olarak uygulandı. Reperfüzyon sonunda IL-1α, IL-6, TNF-α seviyeleri; SOD aktivitesi; MDA ve NO düzeyleri ölçüldü. Gruplarda IL-6 ve TNF-α seviyelerinde azalma olmadı. Yalnızca 3 mg/kg tiyopental uygulanan grupta IL-1α seviyesi azaldı. MDA düzeylerinde düşme yalnızca 80 mg/kg tiyopental uygulanan grupta görüldü. 30 mg/kg tiyopental uygulanan ratlarda SOD düzeylerinde anlamlı yükseklik saptandı. 3 mg/kg tiyopental uygulanan ratlarda glutatyon peroksidaz (GPx) düzeylerinde anlamlı yükseklik saptandı. Nitrik oksit seviyesindeki düşme 10, 30 ve 60 mg/kg tiyopental uygulanan gruplarda görüldü. Çalışma sonuçlarına göre, renal işemi reperfüzyon hasarında her bir biyokimyasal belirteci tiyopentalin farklı dozları etkiledi.

Anahtar sözcükler: Renal İşemi/Reperfüzyon, Tiyopental, Rat

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INTRODUCTION

Ischemia is defined as the circulatory system's inability to provide the oxygen and metabolites needed by tissues to remove waste products [1]. It is caused by arterial and/or venous blockage. Shock, sepsis, renal transplantation, some vascular surgeries, myocardial infarction, cerebrovascular events, liver rejection, and trauma are among the important causes of ischemia [2,3].

Reperfusion is when circulation is provided again to ischemic tissue. During reperfusion, while the oxygen and metabolic needs of the tissue are met [4], introducing oxygen into the cell creates free oxygen radicals, and more severe damage occurs compared to the damage caused by the ischemia [5]. Free oxygen radicals deform the structure of the cell membrane and disrupt the function of the transport system in the cell membrane due to lipid peroxidation. DNA is damaged by the oxidative modification of proteins, and the energy production of the cell is hindered [6]. In addition to the characteristic emerging of free oxygen radicals [7] in ischemia/reperfusion (I/R) damage, polymorphonuclear leucocytes activation [8], increase in eicosanoid release [9], activation of the complement system [10], and increase in cytokine release [11] are observed.

With the developments in renal transplantation surgery, renal I/R damage has become an important clinical problem [12]. Tubular epithelial cell damage and ischemic acute renal failure can occur due to renal I/R damage, and this is accompanied by high rates of mortality [13]. The protective effects of various intravenous anesthetics, such as ketamine, propofol, etomidate, and thiopental [14-16], against renal I/R damage have been studied [12,13]. Thiopental is known to reduce the release of free oxygen radicals from neutrophils in renal I/R damage, inhibit lipid peroxidation, and demonstrate antioxidant effects by reducing hemolysis of red blood cells caused by free oxygen radicals [17]. However, there are no studies in the literature that posit at what doses thiopental is effective against renal I/R damage. In our study, we aimed to detect the optimal protective dose of thiopental against renal I/R damage.

MATERIAL and METHODS

Animals

This study was approved by the Animal Experimentation Ethics Committee of Kahramanmaraş Sütçü İmam University (05.04.2011, No: 7), and all of the surgical procedures were performed in accordance with the rules of the National Institutes of Health, Guide for the Care and Use of Laboratory Animals. All observations were conducted by a researcher blinded to the study groups.

The weights of the animals were ranging from 250 to 280 g; totally 42 male rats were used in this study. The rats were brought to the research center one week before the beginning of the study to become accustomed to the environment, which was heat-controlled cages with 12-h night and day cycles. They were fed standard water and food.

Experimentation Design

After the rats were anesthetized with intraperitoneal 1 g/kg urethane (99% Ethyl carbamate: Sigma-Aldrich; 94300-50 g), the abdominal area was shaved and sterilized with povidone iodine. A midline incision was made, and the abdominal viscera were moved to the right. The left renal hilus was dissected and renal artery occluded with a microvascular clamp, and the intestines were replaced in the abdominal cavity. After 60 min of ischemia, the clamp was removed and 60 minutes of reperfusion were provided; then, a left nephrectomy was performed. The rats were randomized into seven random groups, with six animals in each group:

- Group SG (sham group, n=6) rats received the described surgical procedure, except for renal I/R,

- Group CG (control group, n=6) rats received 60-min ischemia followed by 60-min reperfusion in the left kidney,

- Group TG 3 (n=6) rats received 3 mg/kg intraperitoneal thiopental (Pental, 0.5 g, İbrahim Etem Ulugay İlaç Sanayi Türk A.Ş.) 15 min before the reperfusion phase,

- Group TG 10 (n=6) rats received 10 mg/kg intraperitoneal thiopental 15 min before the reperfusion phase,

- Group TG 30 (n=6) animals received 30 mg/kg intraperitoneal thiopental 15 min before the reperfusion phase,

- Group TG 60 (n=6) rats received 60 mg/kg intraperitoneal thiopental 15 min before the reperfusion phase,

- Group TG 80 (n=6) rats received 80 mg/kg intraperitoneal thiopental 15 min before the reperfusion phase.

At the end of the reperfusion period, the tissue was incised for biochemical evaluation. IL-1α, IL-6, TNF-α levels; SOD activity; MDA and NO levels; were measured.

Antioxidant Study

In order to determine antioxidant levels, 1x1 cm² diameter tissue samples were taken. The samples were maintained in a deep freezer until the examination. The tissues were homogenized with 1.15% KCl at ice coldness in three volumes. Antioxidant enzyme activities and lipid peroxidation levels were measured in the supernatant obtained using a 14.000 rpm (18.400Xg) centrifuge. Superoxide dismutase (SOD) activity was measured using the method described by Fridovich [18]. Lipid peroxidation levels in the tissue samples were emphasized with malondialdehyde (MDA) and measured according to the procedure of Okhawa et al.[19]. Protein concentration was determined according to Lowry's [20] method.

Statistical Evaluation

In evaluating the data, the Kruskal-Wallis test was used for comparisons between groups and Dunn's multiple comparison test was used for sub-group comparisons, in addition to supplementary statistical methods (mean, standard deviation, median, interquartile range). The results were evaluated at a significance level of $P<0.05$ and confidence interval of 95%.

RESULTS

IL-1α, IL-6, and TNF-α means of the groups are shown in *Table 1*.

IL-1α levels in Group SG were found to be significantly lower than those of Group CG ($P=0.002$). There was no statistically significant difference in IL-1α levels between Group CG and Group TG 3 ($P=0.655$); however, the levels were found to be significantly higher in Group CG than in Group TG 10, Group TG 30, Group TG 60, and Group TG 80 ($P=0.002$, $P=0.003$).

While there was no statistically significant difference in IL-6 levels between Group CG and Group SG ($p=0.055$), the IL-6 level was statistically significantly higher in Group CG than in Group TG 3, Group TG 10, Group TG 30, Group TG 60, and Group TG 80 ($P=0.003$, $P=0.002$).

TNF-α levels in Group SG were lower compared to Group CG ($P=0.002$). TNF-α levels were significantly higher in Group CG than in Group TG 3, Group TG 10, Group TG 30, Group TG 60 and Group TG 80 ($P=0.002$) *Table 2*.

MDA, SOD, glutathione peroxidase (GPx), and nitric oxide (NO) means of the groups are shown in *Table 3*.

While there were no statistically significant differences in MDA nmol/mg prot values between Group CG and Group SG, Group TG 3, Group TG 10, Group TG 30, and Group TG 60 ($P=0.949$, $P=0.406$, $P=0.565$, $P=0.848$, $P=0.180$), there was a statistically significant difference between Group CG and Group TG 80 ($P=0.018$).

While there was no significant difference in SOD levels between Group CG and Group TG 3, Group TG 10, Group TG 60, and Group TG 80 ($P=0.406$, $P=0.565$, $P=0.655$, $P=0.749$), SOD level was significantly higher in Group CG than in Group SG and Group TG 30 ($P=0.002$).

While GPx levels were significantly higher in Group CG than in and Group SG and Group TG 3 ($P=0.013$, $P=0.035$), there were no statistically significant differences between Group CG and Group TG 10, Group TG 30, Group TG 60, and Group TG 80 ($P=0.848$, $P=0.225$, $P=0.565$, $P=0.180$).

While there were no statistically significant differences in NO levels between Group CG and Group SG, Group TG 3, and Group TG 80 ($P=0.565$, $P=0.277$, $P=0.406$), there was

Table 1. IL-1α, IL-6 and TNF-α means of groups			
Tablo 1. Grupların IL-1α, IL-6 ve TNF-α ortalamaları			
Group	IL-1α (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
SG	90.37±19.23	92.63±9.29	48.31±5.57
CG	160.5±27.52	110.33±18.82	123.41±26.05
TG 3	182.34±71.82	218.57±77.39	292.9±54.87
TG 10	250.21±37.38	381.7±46.84	271.77±41.8
TG 30	321.93±60.85	203.81±37.85	337.73±39.35
TG 60	640.67±80.68	527.29±163.88	593.07±68.4
TG 80	387.57±49.99	452.94±40.77	495.53±108.24
P	0.0001*	0.0001*	0.0001*
* P<0.05, (means±SD)			

Table 2. Comparison of IL-1α, IL-6 and TNF-α means of groups with the control group			
Tablo 2. Grupların IL-1α, IL-6 ve TNF-α ortalamalarının kontrol grubu ile karşılaştırılması			
Dunn's Multiple Comparison Test	IL-1α (pg/mL)	IL-6 (pg/mL)	TNF-α (pg/mL)
Group SG / Group CG	0.002*	0.055	0.002*
Group CG / Group TG 3	0.655	0.003*	0.002*
Group CG / Group TG 10	0.003*	0.002*	0.002*
Group CG / Group TG 30	0.002*	0.002*	0.002*
Group CG / Group TG 60	0.002*	0.002*	0.002*
Group CG / Group TG 80	0.002*	0.002*	0.002*
* P<0.05			

a statistically significant difference between Group CG and Group TG 10, Group TG 30, and Group TG 60 ($P=0.003$, $P=0.009$, $P=0.002$) *Table 4*.

DISCUSSION

In ischemia/reperfusion injury; oxygen deficiency in ischemic period and also cytotoxic events which occurs during reperfusion cause cell damage and apopitosis [21]. Prophylactic antiapoptotic treatment can be an effective therapeutic strategy for the prevention of I/R injury [22]. Thiopental is a highly lipid-soluble anesthetic, which has indicated antioxidant effect by inhibiting lipid peroxidation in ischemia/reperfusion injury [17]. Protective effects of thiopental for renal ischemia/reperfusion injury was searched in several and compared with the other intravenous agents [13,17,23]. Yüzer et al.[13] reported that propofol and thiopental anesthesia protects against biochemical, and morphological damage better than control in renal I/R injury. They also reported in the same study; thiopental decreases the kidney I/R damage via free oxygen radicals in rats, and this effect is significant compared to etomidate [13]. Basu et al.[23] studied oxidative stres after renal transplantation and inflamatory response and they compared propofol and thiopental. As a result they reported that propofol depressed the inflamatory response [23].

Table 3. MDA, SOD, GPx and NO means of the groups			
Tablo 3. Grupların MDA, SOD, GPx ve NO ortalamaları			
Group	**MDA (nmol/mgprot)**	**SOD (U/mgprot)**	**GPx (U/mgprot)**	**NO (U/mgprot)**
SG	2.05±0.83	48.55±20	0.33±0.07	0.06±0.04
CG	1.91±0.88	9.45±2.28	0.21±0.07	0.06±0.03
TG 3	1.24±0.63	10.99±3.62	0.29±0.06	0.06±0.07
TG 10	1.41±0.8	8.75±3.46	0.2±0.06	0.15±0.05
TG 30	1.85±0.65	103.78±33.07	0.26±0.1	0.33±0.26
TG 60	2.44±0.9	8.47±2.42	0.18±0.03	0.57±0.33
TG 80	0.85±0.58	9.2±2.52	0.24±0.03	0.12±0.09
P	0.029*	0.0001*	0.002*	0.0001*
* P<0.05, (means±SD)			

Table 4. Comparison of MDA, SOD, GPx and NO means of the groups to the control group			
Tablo 4. Grupların MDA, SOD, GPx ve NO ortalamalarının kontrol grubu ile karşılaştırılması			
Dunn's Multiple Comparison Test	**MDA (nmol/mgprot)**	**SOD (U/mgprot)**	**GPx (U/mgprot)**	**NO (U/mgprot)**
Group SG / Group CG	0.949	0.002*	0.013*	0.565
Group CG / Group TG 3	0.406	0.406	0.035*	0.277
Group CG / Group TG 10	0.565	0.565	0.848	0.003*
Group CG / Group TG 30	0.848	0.002*	0.225	0.009*
Group CG / Group TG 60	0.180	0.655	0.565	0.002*
Group CG / Group TG 80	0.018*	0.749	0.180	0.406
* P<0.05			

Contrary to this Doğan et al.[17] reported that thiopental has beter antioxidant effects than propofol according to their comparative study.

Li et al.[24] detected a significant increase in MDA levels in renal I/R damage. Plasma and tissue MDA levels are an indicator of increased free oxygen radical production and cell membrane damage as a result of the reperfusion process [19]. In our study, only the Group that received the highest dose of thiopental, 80 mg/kg, showed a decrease in MDA levels. Doğan et al.[17] reported that antioxidant effects of thiopental against renal ischemia/reperfusion injury is more prominent in high dose. Our study has the same result. It's reported that for other intravenous agents like ketamine and propofol, high doses increase the antioxidant effect [17].

It is known that I/R damage leads to an increase in levels of cytokines such as IL-1α, IL-6, and TNF-α [11]. In our study, IL-1α, IL-6, and TNF-α levels were lower in Group SG than in the control group. However, the difference in IL-6 levels was not statistically significant. We associated this finding with the fact that I/R was not applied in Group SG. However, in the thiopental doses we applied, there was no decrease in IL-6 or TNF-α levels in any of the groups. Only in the group receiving 3 mg/kg thiopental did IL-1 α level increase compared to the control group. This result leads us to think that 3 mg/kg thiopental is enough to prevent IL-1α release.

Superoxide dismutase, present in all cells that metabolize oxygen, is a defense mechanism that works with the catalase and glutathione system against the damage caused by oxygen radicals [26,27]. This enzyme protects aerobic organisms against the harmful effects of superoxide [27]. There was only a significant increase in SOD levels in the rats that received the 30 mg/kg dose of thiopental.

The GPx enzyme that carries selenium as the prosthetic group in erythrocytes and other tissues catalyzes the decomposition of hydrogen peroxide and lipid peroxides by reduced glutathione [28]. There was a significant increase in GPx levels in the group that received the 3 mg/kg dose of thiopental.

Nitric oxide is a free radical. Although it is a weak reducing agent, it causes peroxynitrite formation through interaction with the peroxide radical. The peroxynitrite radical is a highly reactive agent and leads to lipid peroxidation and protein damage [29,30]. In our study, a decrease in nitric oxide levels was observed in the groups that received 10, 30, and 60 mg/kg doses of thiopental. Although the NO level was suppressed in those dosage groups, we are of the opinion that the lowest thiopental dose, 10 mg/kg, is enough to prevent lipid peroxidation.

In conclusion, according to the results of our study, different doses of thiopental were found to be effective for each biochemical indicator in renal I/R damage. We

are of the opinion that further studies are required on this subject.

CONFLICTS OF INTEREST

None of the authors have any conflicts of interest to declare.

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