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Original Article

The Effects of Thiopental on Cold Ischemic Injury in Renal Transplantation

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Abstract

Introduction: One of the most important factors influencing post-transplant success in kidney transplantation is preserving the viability of the organ from removal to transfer into the recipient.

Aim: This study aimed to reduce the energy requirement with thiopental doses administered before organ transplantation, and to increase the organ viability by minimizing the tissue damage during the cold ischemia process.

Materials and methods: Twenty female Wistar albino rats were divided into two groups: control group (group C), and thiopental group (group T). In group C, a midline incision was performed, and the renal artery was isolated under ketamine and xylazine anesthesia. A standard organ storage solution (cooled to +4°C) was used for kidney perfusion. Nephrectomy was applied, and the removed kidneys were placed into +4°C standard organ storage solution and stored at +4°C for 12 hours. Animals in group T were subjected to the procedures explained above under 85 mg/kg thiopental sodium anesthesia. After 12-hour storage, samples from the kidney tissues were fixed in 10% neutral buffered formalin. Histopathological evaluation and apoptosis detection via TUNEL method were performed.

Results: Tubular necrosis was more extensive in group C compared with that in group T and this difference was statistically significant. Similarly, vacuolization was widely observed in group C, and this increase was also statistically significant. For the 'dilatation of Bowman's space' parameter, a significant decrease was observed in group T compared with group C. When the apoptotic index values of both groups were examined, it was seen that they were lower in group T than those in group C. This result was statistically significant.

Conclusions: These data suggest that thiopental provides protection to the kidney tissue during the cold storage process. Thiopental has been shown to decrease the number of apoptotic cells in the kidney tissue when administered to the donor before organ transplantation, increasing the organ viability.

Keywords

cold ischemia, kidney transplantation, thiopental, animal study

INTRODUCTION

Kidney transplantation is a life-saving treatment option for patients with chronic renal failure.[1] For these patients, kidney transplantation provides better survival and quality of life than dialysis does. The 5-year survival rate is 70% for transplantation patients, whereas this proportion is only 30% for dialysis patients. Comorbidities such as cardiomyopathy can be partially or entirely prevented in patients with end-stage renal failure after successful kidney transplantation.[2,3]

Although a kidney can be supplied from both deceased and living donors, there is still difficulty in finding appropriate organs due to the high demand. The average waiting time for renal transplantation is 1156 days in the UK. Besides, transplantation can be performed to only 20% of the patients in the waiting list.[4]

Preservation of the organ under proper conditions until the time of transplantation is just as important as organ supply. The fact that the donor and receiver are mostly in separate centers causes a prolongation of the time from the removal of the organ to the transplantation into the recipient. Thus, preserving the viability of the organ during this period is one of the most essential factors affecting post-transplant success.[5] The kidney tissue is exposed to ischemia following removal of the organ from the donor, initiating some cellular events.

Hypothermia inhibits intracellular enzymatic activity by reducing the metabolism of cells and, thus, the consumption of adenosine triphosphate (ATP). In this way, the organ is protected from ischemic damage for a longer time.[6] The process that starts at this stage is named as static cold storage (SCS) process. The SCS is an organ preservation method used for all organs. After an organ is removed from the donor, in the first stage, it is necessary to perform perfusion with standard preservation solutions at +4°C for both deceased and living donors. Using this method, the organ is cooled and therefore its metabolism slows down.[6,7]

There are a great variety of preservation solutions used for organ preservation, but the best known one is the University of Wisconsin (UW) solution.[8,9] However, even in optimal conditions, ischemic organ damage increases after 12 hours of kidney preservation.[5] Therefore, the search for novel methods to improve organ viability still continues.

Thiopental, the sodium salt of barbituric acid, is the most widely used intravenous anesthetic. Thiopental shows its effect by suppressing the reticular activating system, which controls the state of consciousness and vital functions.[10] In high doses, thiopental reduces both the oxygen consumption and the metabolic rate of the brain.[8] Furthermore, there is insufficient information in the literature to determine whether a comparable response to thiopental's metabolic effect in the brain has also happened in peripheral organs. A similar effect to vasoconstriction, which occurs with the slowing of the brain metabolism, also takes place in the periphery. Vasoconstriction increases the peripheral vascular resistance followed by a reduction in blood flow.

AIM

This study aimed to reduce the energy requirement by lowering the basal metabolic rate of the donor organ with thiopental doses administered before organ transplantation and, in this way, increase the organ's viability by minimizing tissue damage during the cold ischemia process.

MATERIALS AND METHODS

Materials and experimental design

The study was approved by the Animal Experiments Local Ethics Board of the Çanakkale Onsekiz Mart University (approval No. 2016/04-12) and performed in accordance with Turkish Law 6343/2, Veterinary Medicine Deontology Regulation 6.7.26, and per the Helsinki Declaration of the World Medical Association recommendations on animal studies. The Wistar albino rats were purchased from the Çanakkale Onsekiz Mart University Experimental Research Application and Research Center. Twenty female Wistar albino rats, aged 5 weeks and weighing from 210 to 280 g were used in this study. The rats were housed in standard cages in an animal room maintained at standard humidity (30%-40%) and temperature of 25±2°C with 12-hour light periods (12 hours of light/12 hours of dark). All animals were fed standard food and water. Twelve hours before the study procedure, feeding was stopped and the rats were allowed only to drink water. All study was done in compliance with the NIH guideline for the Care and Use of Laboratory Animals. The data was reported in compliance with the ARRIVE guidelines.

Experimental groups and the surgical procedure

Twenty female Wistar albino rats were randomly divided into two groups: control group (group C) and thiopental group (group T).

Renal perfusion was performed as previously described[11]; in the animals of control group, a midline incision was performed and the renal artery was isolated under 50 mg/kg ketamine (Ketalar®, Pfizer, Turkey) and 10 mg/kg xylazine (Rompun®, Bayer, Canada) anesthesia. A catheter was inserted into the renal artery, and a standard organ storage solution (cooled to +4°C) (Bel-Gen Cold Storage Solution, Institut Georges Lopez, Lissieu, France, LOT: SL170190) was administered, followed by kidney perfusion. The renal vein was cut and kidney perfusion continued until clear fluid came from the renal vein. Then nephrectomy was applied, and the removed kidneys were placed into Falcon tubes containing +4°C standard organ storage solution and stored at +4°C for 12 hours.

Similarly, animals in the thiopental group (group T) were subjected to the procedures explained above under

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the 85 mg/kg thiopental sodium (Pental 1 gr flacon, I. E. Ulagay Ilaç Sanayi Türk A.Ş, Istanbul, Turkey) anesthesia.

After a 12-hour storage, samples from the kidney tissues were fixed in 10% neutral buffered formalin. Additionally, urea and creatinine levels were biochemically assessed in samples taken from the organ storage solutions.

Blinding

In this study, blinding was applied at the stage of histopathological investigations. Histopathological assessments were performed by two histopathologists who had no knowledge about the experimental groups.

Histopathological examinations

After fixation, dehydration was achieved by graded alcohol series. The tissues were subjected to xylene for transparency, and then they were kept in 60°C melted paraffin three times. Tissues were embedded in paraffin blocks. The paraffin blocks were cut in 5-µm-thick slices, and the sections were stained using the Hematoxylin-Eosin (H-E) staining method. Finally, histopathological assessments were performed concerning tubular necrosis, vacuolization, and dilatation of Bowman's space parameters as follows: grade 0: no damage, grade 1: minimal damage, grade 2: moderate damage, and grade 3: severe damage.

Apoptosis assessment with the terminal deoxynucleotidyl transferase dUTP nick end labeling method (TUNEL)

For apoptosis assessment, 4-µm-thick sections were obtained from each paraffin block and stained with the Apoptag Apoptosis Detection Kit (S7100, Merck Millipore, Darmstadt, Germany) following xylene-alcohol changes. The sections were examined under the light microscope (Zeiss AxioScope A1) at 400× magnification, where the brown-black stained nuclei of the apoptotic cells were observed. The apoptotic cell ratio (apoptotic index "AI" values) was calculated in percentages by counting 500 brown-black stained cells:

Apoptotic index (AI) = (Number of positive cells/Total number of cells counted) ×100

Statistical analysis

Statistical analysis was performed using SPSS, version 15 (IBM, Armonk, New York 10504, NY, USA). The Mann-Whitney U test was used to compare the TUNEL scores and the histopathological data of two independent groups. Mean, standard deviation, minimum and maximum values were used for presenting the TUNEL score. The statistical significance level (p) was set at <0.05.

RESULTS

Histopathological assessment

Histopathological, tubular necrosis, vacuolization, and dilatation of Bowman's space parameters were evaluated. Compared to group T, tubular necrosis was more extensive in group C, and this difference was statistically significant (p=0.032). Similarly, vacuolisation was widely observed in group C, and this increase was also statistically significant (p=0.024). Another statistically significant parameter was the dilatation of the Bowman's space, where a significant decrease was observed in group T compared to group C (p=0.014) **(Fig. 1)**. The histopathological results are shown in **Table 1**.

Apoptosis assessment

Apoptotic cell counts were evaluated, and apoptotic index values were counted for each section **(Table 2)**. When the apoptotic indexes of both groups were examined, it was seen that the apoptotic index values were lower in group T compared to group C. This result was statistically significant (p<0.001) **(Fig. 2)**.

The TUNEL stainings of sections belonging to each one of the two groups can be seen in **Fig. 2**. The cells with brown stained nuclei are apoptotic cells. Normal cells' nuclei were stained blue.

DISCUSSION

We demonstrated in the present study that thiopental used in the donor's kidney before organ transplantation increased the organ viability in the cold ischemia process and decreased the number of apoptotic cells. Additionally, it has been reported by the histopathological evaluation that thiopental leads to a decrease in the tubular necrosis, vacuolization, and dilatation of the Bowman's space in the 12-hour SCS process, thereby reducing tissue damage. These data suggest that thiopental provides protection to the kidney during the SCS process and, thus, allows better storage of the organ.

An important consideration in organ transplantation is storing the transplant properly until the time of transplantation. For this reason, it is crucial to reduce the number of apoptotic cells and to maintain organ viability for more extended periods. A wide variety of solutions have been developed for the preservation of the donor organ. The best known among these are the University of Wisconsin (UW) solution, the histidine-tryptophan-ketoglutarate solution (Custodiol HTK), the Collins solution, and EuroCollins solution. Currently, the most commonly used cold storage solution in renal transplantation is the University of Wisconsin (UW) solution. Even with successful perfusion with this solution, the viability of the kidney tissue can be main-

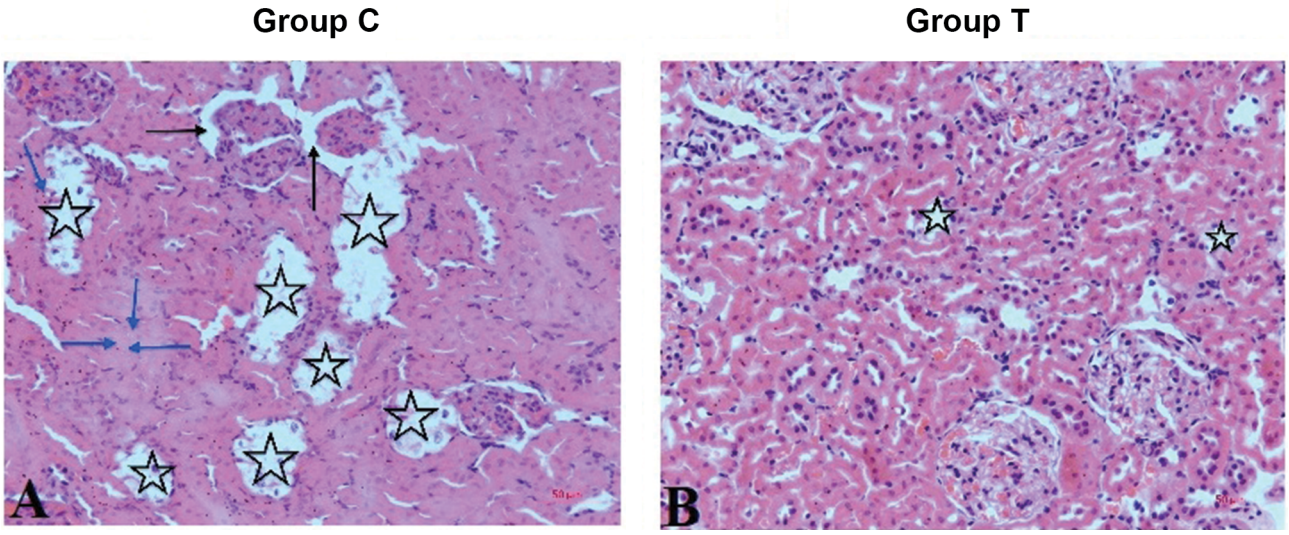

Figure 1. Histopathological evaluation of each study group. **A**. Histopathological section of group C. **B**. Histopathological section of group T. It is shown that the tubular (stars), the dilatation of Bowman's space (black arrows), and vacuolisation (blue arrows) are greater in group C (magnification 200×).

Table 1. Histopathological evaluation and grades of each of the groups

	Grade	Group C n (%)	Group T n (%)	*p*
Tubular necrosis	0	0 (0)	0 (0)	0.032
	1	3 (30)	7 (70)	
	2	3 (30)	3 (30)	
	3	4 (40)	0 (0)	
Vacuolisation	0	0 (0)	0 (0)	0.024
	1	1 (10)	5 (50)	
	2	4 (40)	4 (40)	
	3	5 (50)	1 (10)	
Dilatation of Bowman's space	0	1 (10)	6 (60)	0.014
	1	4 (40)	3 (30)	
	2	4 (40)	1 (10)	
	3	1 (10)	0 (0)	

Percentage: columnar percentage; *p*: Mann-Whitney U test

Table 2. AI scores of two experimental groups

n	Group C										Group T									
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10
AI (%)	65	43	28	36	51	40	46	39	51	43	18	16	9	11	21	19	10	16	21	36
Mean±SD	44±10%										17±7%									
Median (Min-Max)	43 (28-65)										17 (9-36)									
P value	<0.001																			

SD: standard deviation; p: Mann-Whitney U test

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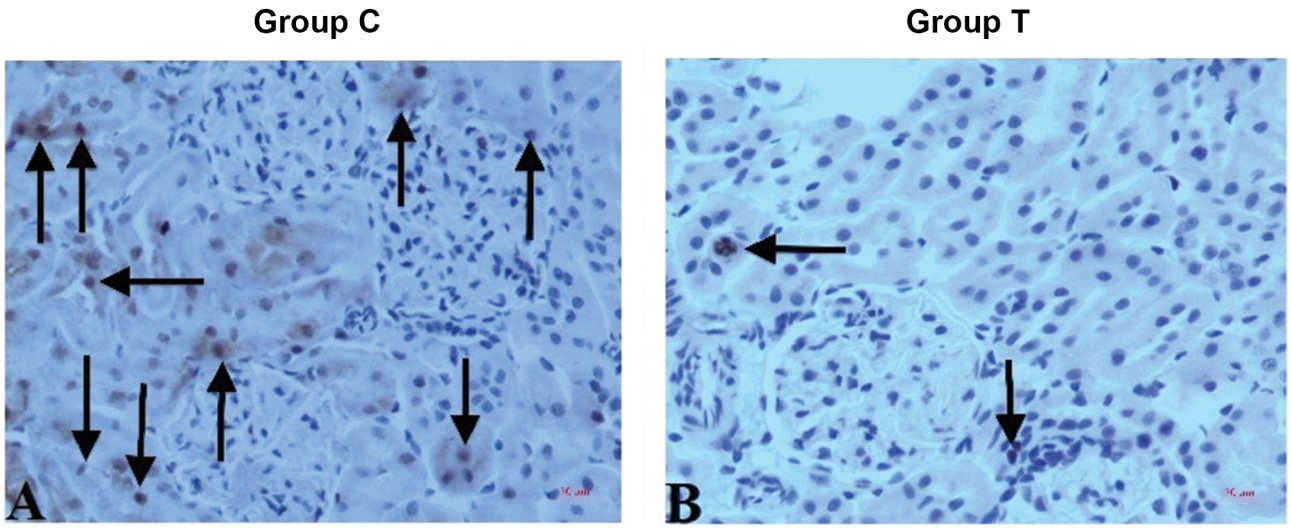

Figure 2. Apoptosis evaluation by TUNEL staining. **A**. Groups C; and **B**. Group T. Arrows mark apoptotic cells (magnification 400×).

tained for only up to 12 hours. At the end of this period, the number of apoptotic cells increases, and the organ is exposed to ischemia.[9] Therefore, it is crucial to develop new options for better protection of the organ during the SCS process. In this study, we showed that the kidney could be preserved from cold ischemia and the number of apoptotic cells could be reduced by a thiopental dose applied to the donor before nephrectomy.

Thiopental is known to have a protective effect on ischemic injury by slowing down the brain metabolism.[10] Studies have also shown that it has a protective effect on the ischemia/reperfusion (I/R) injury in the kidney; however, no information is available to detect its impact on the cold ischemia-induced renal injury.[12-14] In a study, different doses of thiopental were administered, and reperfusion was performed after 60 minutes of ischemia. Then, the levels of interleukin-1-beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and antioxidant enzymes were measured. In conclusion, the cytokine and antioxidant enzyme levels in the thiopental group were found to be lower.[14] In another study, a 60-minute ischemia process was followed by a 60-minute reperfusion process in the kidney, and ketamine, thiopental, etomidate, and propofol were administered to the animals before reperfusion. As a result, thiopental was demonstrated to have a protective effect on the I/R injury.[12] No detailed histopathological examinations were performed in these studies, and apoptosis parameters were not studied. In addition, the I/R injury was induced in rats in all these studies, but SCS was not applied. On the other hand, in our study, cold ischemia was applied after perfusion with standard organ preservation solutions, and the kidneys were kept for 12 hours at +4°C. Additionally, a histopathological evaluation was performed, and it was shown that histopathological injury was less in the thiopental group regarding tubular necrosis, vacuolization, and dilatation of the Bowman's space. One of the most critical parameters affecting the success of organ transplantation is the number of apoptotic cells. In this study, unlike in other studies, the AI was calculated with the TUNEL method, and group T had lower AI values. This, in turn, shows that organ viability may be better after transplantation with thiopental administration.

In this study, thiopental and ketamine were used as anesthetic agents in the experimental and control groups, respectively. Ketamine is the preferred anesthetic agent in experimental studies performed in rats, and it is also used routinely in animal studies. Ketamine, a cyclohexylamine, is also used as a general anesthetic agent in the clinics, and due to some of its properties, it is included in the class of dissociative anesthetics.[15] Studies have shown that ketamine has a protective effect against ischemic damage in various tissues.[16-19] It also has been shown in the current literature that ketamine exerts a protective effect on incomplete cerebral ischemia similar to that induced by thiopental.[20] Although ketamine, which we used for the control group of the present study, is known for its protective effects from ischemic injury in various tissues, the levels of histopathological damage in the thiopental group were lower in the kidney. Additionally, the number of apoptotic cells was significantly lower in group T. This suggests that thiopental may be preferred as an anesthetic agent before cold ischemia in transplant surgery.

Limitations

There are some limitations to our study. First, a 12-hour SCS procedure was performed in the kidney; however, reperfusion was not done at the end of SCS. Therefore, no information could be obtained about how organ functions would be after reperfusion. Second, antioxidant enzyme levels of the kidney tissue had not been measured contrary to the literature. Since we did not perform reperfusion procedure, an antioxidant study was not conducted.

Another limitation of the study is the sample size. We

had 10 animals in both experimental groups. This size should be greater in future studies, thus, the statistical analysis can be more significant.

CONCLUSIONS

Thiopental has been shown to decrease the number of apoptotic cells in the kidney tissue when administered to the donor before organ transplantation, increasing the organ viability. In this way, organ storage times can be increased, and the kidney tissues will be better preserved during the cold ischemia process until transplantation.

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Competing Interests

The authors have declared that no competing interests exist.

REFERENCES

1. Wang X, Xu M, Jia J, et al. CD 47 blockade reduces ischemia/reperfusion injury in donation after cardiac death rat kidney transplantation. Am J Transplant 2018; 18:843–54.
2. Zapolski T, Furmaga J, Wysokiński AP, et al. The atrial uremic cardiomyopathy regression in patients after kidney transplantation - the prospective echocardiographic study. BMC Nephrol 2019; 20(1):152.
3. Yilmaz KC, Akgun AN, Keskin S, et al. The effect of renal transplantation on cardiac functions. Saudi J Kidney Dis Transpl 2020; 31(5):1051–6.
4. Mahendran AO, Barlow AD. Kidney Transplantation. Surgery 2014; 32:364–70.
5. Kotecha R, Toledo-Pereyra LH. Prolonged cold ischemia enhances alloimmune response to liver grafts. J Surg Res 2012; 176:14–6.
6. Ravaioli M, Maroni L, Angeletti A, et al. Hypothermic oxygenated perfusion versus static cold storage for expanded criteria donors in liver and kidney transplantation: protocol for a single-center randomized controlled trial. JMIR Res Protoc 2020; 9(3):e13922.
7. Saidi RF, Kenari SKH. Liver ischemia/reperfusion injury: an overview. J Invest Surg 2014; 27:366–79.
8. Legeai C, Durand L, Savoye E, et al. Effect of preservation solutions for static cold storage on kidney transplantation outcomes: A National Registry Study. Am J Transplant 2020; 20(12):3426–42.
9. Parsons RF, Guarrera JV. Preservation solutions for static cold storage of abdominal allografts: which is best? Curr Opin Organ Transplant 2014; 19:100–7.
10. Sonnay S, Duarte JMN, Just N, et al. Energy metabolism in the rat cortex under thiopental anaesthesia measured in vivo by ^{13}C MRS. J Neurosci Res 2017; 95(11):2297–306.
11. Büyük B, Demirci T, Adalı Y, et al. Can amniotic fluid be an alternative organ preservation solution for cold renal storage? Rev Nefrol Dial Traspl 2020; 40(1):14–24.
12. Yüzer H, Yüzbasioglu MF, Ciralik H, et al. Effects of intravenous anesthetics on renal ischemia/reperfusion injury. Renal Failure 2009; 31:290–6.
13. Dogan Z, Yüzbasioglu MF, Kurutas EB, et al. Thiopental improves renal ischemia-reperfusion injury. Renal Failure 2010; 32:391–5.
14. Coskuner I, Türkay M, Yildiz H. The protective effect of thiopental against renal ischemia/reperfusion damage in rats. Kafkas Univ Vet Fak Derg 2016; 22:659–63.
15. Ergün Y, Öksüz H, Atli Y, et al. Ischemia-reperfusion injury in skeletal muscle: comparison of the effects of subanesthetic doses of ketamine, propofol, and etomidate. J Surg Res 2010; 159:e1–e10.
16. Dourado LFN, Oliveira LG, da Silva CN, et al. Intravitreal ketamine promotes neuroprotection in rat eyes after experimental ischemia. Biomed Pharmacother 2021; 133:110948.
17. Li X, Wang J, Song X, et al. Ketamine ameliorates ischemia-reperfusion injury after liver autotransplantation by suppressing activation of Kupffer cells in rats. Can J Physiol Pharmacol 2018; 96(9):886–92.
18. Xiong Z, Chang L, Qu Y, et al. Neuronal brain injury after cerebral ischemic stroke is ameliorated after subsequent administration of (R)-ketamine, but not (S)-ketamine. Pharmacol Biochem Behav 2020; 191:172904.
19. Chang EI, Zárate MA, Rabaglino MB, et al. Ketamine suppresses hypoxia-induced inflammatory responses in the late-gestation ovine fetal kidney cortex. J Physiol 2016; 594(5):1295–310.
20. Yagmurdur H, Ayyildiz A, Karaguzel E, et al. Propofol reduces nitric oxide induced apoptosis in testicular ischemia-reperfusion injury by downregulating the expression of inducible nitric oxide synthase. Acta Anaesthesiol Scand 2008; 52:350–7.

Влияние тиопентала на холодовую ишемию при трансплантации почки

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Резюме

Введение: Одним из наиболее важных факторов, влияющих на посттрансплантационный успех трансплантации почки, является сохранение жизнеспособности органа от удаления до пересадки реципиенту.

Цель: Это исследование было направлено на снижение потребности в энергии с помощью доз тиопентала, вводимых перед трансплантацией органов, и на повышение жизнеспособности органов за счёт сведения к минимуму повреждения тканей во время процесса холодовой ишемии.

Материалы и методы: Двадцать самок белых крыс линии Вистар были разделены на две группы: контрольную группу (группа К) и группу тиопентала (группа Т). В группе К выполняли срединный разрез и выделяли почечную артерию под анестезией кетамином и ксилазином. Для перфузии почек использовали стандартный раствор для хранения органов (охлаждённый до +4°C). Выполнена нефрэктомия, а удалённые почки помещены в стандартный раствор для хранения органов +4°C и выдержаны при +4°C в течение 12 часов. Животных в группе Т подвергали процедурам, описанным выше, под анестезией 85 mg/kg тиопентала натрия. После 12-часового хранения образцы тканей почек фиксировали в 10% нейтральном забуференном формалине. Была проведена гистопатологическая оценка и обнаружение апоптоза с помощью метода TUNEL.

Результаты: Тубулярный некроз был более обширным в группе К по сравнению с группой Т, и эта разница была статистически значимой. Точно так же вакуолизация широко наблюдалась в группе К, и это увеличение также было статистически значимым. Для параметра «расширение пространства Боумена» в группе Т наблюдалось значительное снижение по сравнению с группой К. При изучении значений индекса апоптоза в обеих группах было видно, что они были ниже в группе Т, чем в группе К. Этот результат был статистически значимым.

Заключение: Эти данные свидетельствуют о том, что тиопентал обеспечивает защиту ткани почек в процессе хранения на холоде. Было показано, что тиопентал снижает количество апоптотических клеток в ткани почки при введении донору перед трансплантацией органа, повышая жизнеспособность органа.

Ключевые слова

холодовая ишемия, трансплантация почки, тиопентал, исследование на животных