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Effects of thiopental in cold ischemia in liver transplantation: An experimental study

Karaciğer transplantasyonunda tiyopental'in soğuk iskemi üzerine etkileri: Deneysel bir çalışma

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Abstract

Aim: The aim of this study is to use the reducing effect of thiopental on metabolic rate to reduce basal metabolic rate and thus energy requirements in organs with doses administered before organ transplantation and in this way to increase organ viability by reducing to a minimum tissue damage occurring during the cold ischemia process.

Methods: The study was started with 20 Wistar albino rats in 2 groups. In Group 1 (Control=Ketamine-Xylazine group) and Group 2 (Thiopental group), rats had a midline incision made after shaving the abdominal region under anesthesia with appropriate agents. The portal vein was entered with a cannula and organ storage solution at +4 °C was injected to the portal vein to perfuse the liver. Then hepatectomy was performed, the livers were placed in Falcon tubes containing +4°C organ storage solution and stored at +4°C. Tissue samples were taken for histopathologic and TUNEL investigation and storage solution samples were taken for biochemical analysis at 12th hour.

Results: In histopathologic evaluation, the mean for hydropic degeneration and sinusoidal dilatation was higher in Group 1 compared to Group 2, but the results weren't statistically significant. Apoptotic Index (AI) values in Group 1 were higher than Group 2; however, there was no statistically significant difference between the median values (3.50 (Min:1.00-Max:16.00) vs. 2.50 (Min:1.00-Max:20.00), respectively p=0.974). The mean values for ALT, AST and ALP were appeared to be higher in Group 1.

Conclusion: In conclusion, thiopental has a protective effect on liver tissue during the cold ischemia process via reducing the mean values of histopathological, apoptotic and biochemical assessment results, but these findings weren't statistically significant.

Keywords: Thiopental, Cold ischemia, Liver transplantation, TUNEL, Histopathology

Öz

Amaç: Bu çalışmanın amacı; tiyopentalin metabolizma hızını azaltıcı etkisinden faydalanarak, organ nakli öncesi uygulanacak olan dozlar ile, nakledilecek organın bazal metabolizma hızını düşürerek enerji ihtiyacını azaltmak ve bu sayede soğuk iskemi sürecinde meydana gelebilecek doku hasarını en aza indirerek organların viabilitesini artırmaktır.

Yöntemler: Çalışmada kullanılan 20 adet Wistar Albino cinsi sıçan iki gruba ayrılmıştır. Grup 1 (Kontrol=Ketamin-Ksilazin grubu) ve Grup 2 (Tiyopental grubu)'deki sıçanlara, kendi grupları için uygun olan ajanlarla anestezi uygulandıktan sonra karın derisi traşlanıp orta hat insizyonu yapıldı. Portal vene bir kanül ile girilerek +4 °C'deki organ saklama sıvısı enjekte edildi. Böylece karaciğer perfüzyonu sağlandı. Ardından hepatektomi yapılarak karaciğerler, içerisinde +4°C'de organ saklama sıvısı bulunan Falkon tüplere konuldu ve yine +4°C'de saklandı. 12 saat saklama süresinin sonunda histopatolojik ve TUNEL değerlendirmeler için doku örnekleri alınırken, saklama sıvısından da biyokimyasal analizler için numune alındı.

Bulgular: Histopatolojik değerlendirme sonucunda, hidropik dejenerasyon ve sinusoidal dilatasyon ortalamaları grup 1'de Grup 2'ye kıyasla yüksek bulundu ancak sonuçlar istatistiksel olarak anlamlı değildi. Apoptotik indeks (AI) değerleri Grup 1'de Grup 2'ye kıyasla yüksekti ancak ortanca değerlerinde istatistiksel olarak anlamlı bir farklılık bulunmadı(sırasıyla 3.50 (Min:1.00-Max:16.00) vs. 2.50 (Min:1.00-Max:20.00) p=0.974). Biyokimyasal ALT, AST ve ALP ortalama değerleri de Grup 1'de yüksek bulundu.

Sonuç: Sonuç olarak, tiyopentalin, soğuk iskemi sürecinde karaciğer dokusu üzerine koruyucu bir etkisi olduğu ve bu etkiyi histopatolojik, apopitotik ve biyokimyasal değerlendirme sonuçlarının ortalama değerlerini azaltmak yoluyla gerçekleştirdiği görülmüştür, ancak bu bulgular istatistiksel olarak anlamlı bulunamamıştır.

Anahtar kelimeler: Tiyopental, Soğuk iskemi, Karaciğer nakli, TUNEL, Histopatoloji

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Introduction

Liver transplantation (LT) is a life-saving treatment method for patients with end-stage liver failure [1]. The first successful LT in humans was performed in 1963 by Dr. Starzl [2]. Developments in surgical methods, in immunosuppressive medications and liver donor protection methods have advanced the topic of LT. Though there can be both deceased donors and live donor liver providers, the number of organs remain insufficient due to the increased number of patients with liver failure. Additionally, it is very important to ensure transplantation of the organ within the appropriate duration for successful transplantations. This requires developing methods of organ preservation and preserving the viability of the organ for long durations [2].

Timely transplantation of organs is one of the most important factors determining success after surgery. The duration from the time the organ is obtained to transplantation into the organ receiver is cold ischemia duration. During the cold ischemia duration, organs are left at temperatures of +1 to +4°C in standard preservation solutions [3]. During the process, hypooxygenation and hypothermia of the tissues is observed. In hypooxygenation situations, cells do not complete oxidative energy production and attempt to produce energy by oxygen-free pathways. In normal conditions the Na^+/K^+ ATPase (adenosine triphosphatase) pump using high amounts of adenosine triphosphate (ATP) is disrupted as the amount of ATP reduces and causes intracellular K^+ loss and Na^+ increases. This situation causes the cells to swell [3,4]. Hypooxygenation affects the intracellular distribution of Ca^{+2} ions. This causes disruption of enzyme activities and of the integrity of the cell membrane [5]. Additionally, the glycolysis process begins and high rates of lactate synthesis are observed accompanied by acidosis [3]. During ischemia, ATP transforms to adenosine diphosphate (ADP) and adenosine monophosphate (AMP) transforms to adenosine and progressively differentiates to reduce to adenine, inosine and hypoxanthine. Hypoxanthine accumulation catalyzes the xanthine transformation and activates xanthine oxidation. This reaction is accompanied by the production of free oxygen radicals causing injury within the cell [6].

Damage occurs to the organ for transplantation linked to these effects explained by hypooxygenation. Hypothermia slows metabolism and reduces the enzyme activities and requirements of cells. In this way, cell death is slowed; however, again energy requirements are higher than the amount of energy produced by glycolysis and oxygen-free pathways [7].

There are a variety of solutions used for organ preservation. The most common of these include University of Wisconsin (UW) solution, histidine-tryptophan-ketoglutarate (HTK), Celsior (CS), Institut Georges Lopez (IGL-1) Collins, Euro-collins, UW-PEG, Polysol, Kyoto, New Kyoto etc. [8,9].

These solutions were developed based on the principle of organ preservation by balancing the basic metabolic requirements in the process after the organ is removed. However, in spite of this, they can only preserve the viability of the organ taken from the donor for limited periods and the search for new methods to slow organ preservation and metabolism and ensure viability for long periods continues [10-19].

Thiopental is a barbituric acid sodium salt and one of the most commonly used intravenous anesthetics. Its effects are due to suppression of the reticular activating system (RAS) controlling consciousness and vital functions. The fat solubility of thiopental is excessive. As a result, the duration of effect is not related to metabolism and elimination but to redistribution. Rates of 80% of thiopental bind to plasma proteins. Due to the solubility in fat and 60% non-ionizing fraction, it reaches high concentration in the brain within 30 seconds. The elimination half-life is 3-12 hours. Thiopental transforms to water soluble inactive metabolites in the liver and metabolites are eliminated by the renal extraction [20,21].

At high anesthetic doses, thiopental reduces oxygen consumption in the brain and significantly lowers the brain's metabolic rate. The reduction in brain metabolic rate causes a reduction in the brain's blood flow requirements and this event ensures vasoconstriction of veins in the brain. The reduction in brain blood flow lowers the blood volume and intracranial pressure in the brain. This effect on brain metabolism protects both the injured and perfused healthy regions of the brain. Due to thiopental reducing brain blood flow, increasing perfusion pressure and lowering metabolic rate, it is a very beneficial agent for anesthesia of patients with increased intracranial pressure [22]. Additionally, it is used with the aim of protecting the brain during incomplete ischemia. Here, the effect is ensured by reducing the metabolic rate and lowering energy requirements to a minimum [22].

There is insufficient information in the literature about whether a similar response to this metabolic effect of thiopental in the brain develops in peripheral organs. If a similar effect to the vasoconstriction accompanying slowed brain metabolism occurs in the periphery, vasoconstriction will increase peripheral vascular resistance and reduce blood flow [23]. Slowing metabolism and reducing energy requirements in peripheral organs may be beneficial to ensure long-term preservation during transplantation.

The aim of this study is to use the reducing effect of thiopental on metabolic rate to reduce basal metabolic rate and thus energy requirements in organs for transplant with doses administered before organ transplantation and in this way to increase organ viability by reducing to a minimum tissue damage occurring during the cold ischemia process.

Materials and methods

Materials and experimental design

The study was approved by the Institutional Animal Use and Care Committee of University and performed in accordance with Turkish Law 6343/2, Veterinary Medicine Deontology Regulation 6.7.26, Guide for the Care and Use of Laboratory Animals, and with the Helsinki Declaration of World Medical Association recommendations on animal studies. Wistar albino rats were obtained from Çanakkale Onsekiz Mart University (COMU) Experimental Research Application and Research Center. Twenty female Wistar Albino rats were used in the study, with a mean age of six months and mean weight of 240–300 g. The rats were housed in stainless steel cages in an animal room maintained at a standard humidity (45%-50%) and temperature 22±2°C with 12 hour light periods (12 hours of

daylight/12 hours of dark). All animals were fed standard food and water and twelve hours before the study procedure feeding was stopped and the rats were only allowed to drink water. The entire experiment was conducted under half-sterile conditions.

Experimental procedure

The study was started with 20 Wistar albino rats in 2 groups as below;

Group 1 (Control group, n=10): Anesthetized with Ketamine (50 mg/kg, intraperitoneally) and Xylazine (15 mg/kg, intraperitoneally)

Group 2 (Thiopental group, n=10): Anesthetized with Thiopental Sodium (85 mg/kg intraperitoneally)

Liver perfusion was performed with a method previously described in the literature [10]. Rats in Group 1 had a midline incision made after shaving the abdominal region under 50 mg/kg ketamine hydrochloride (Ketalar®, Pfizer İlaçları Ltd, Şti, İstanbul, Turkey) and 15 mg/kg Xylazine (Alfazyne 2%, Ege Vet San. Tic, İzmir, Turkey) anesthesia. After observing the liver, the portal vein was entered with a cannula and tied distal of the portal pedicle. Organ storage solution (Bel-Gen Cold Storage Solution, Institut Georges Lopez, Lissieu, France, LOT: SL170190) at +4 °C was injected to the portal vein to perfuse the liver. Perfusion continued until clear fluid came from the hepatic vein. Then hepatectomy was performed. After hepatectomy rats were sacrificed with high dose anesthetic. The livers obtained from the hepatectomy procedure were placed in Falcon tubes containing +4°C organ storage solution and stored at +4°C. Tissue samples were taken for histopathologic investigation of perfused liver at 12th hour. Again, for biochemical analysis of storage solution, samples were taken at 12th hour.

Animals in Group 2 had the procedures explained above performed with intraperitoneal 85 mg/kg thiopental sodium (Pental 1 gr flacon, İ. E. Ulagay İlaç Sanayi Türk A.Ş, İstanbul, Turkey) anesthesia. Similar to Group 1, tissue samples were obtained from livers in cold ischemia at 12th hour for histopathologic investigation. Storage solutions were also sampled for biochemical analysis at 12th hour.

Histopathological examinations

To investigate histopathologic changes, liver tissue samples were consecutively numbered and placed in 10% neutral buffered formaline. Evaluation of the pathology specimens were done by a histology specialist who was blind to the two study groups.

After fixation, dehidratation and clearing processes, liver tissues were embedded in paraffin. The paraffin blocks were cut in 5 mm thickness on Rotatory Microtome (Leica RM2125 RTS) and the sections were stained with hematoxylin and eosin (H&E) method. The histopathologic sections were examined under a light microscope (Zeiss AxioScope A1) for the presence of hydropic degeneration, sinusoidal dilatation, and focal necrosis and rated on a semi-quantitative scale of 0-3 as follows;

0: no damage

1: mild damage

2: moderate damage

3: severe damage

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

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was used to detect apoptosis of the liver tissue. Prepared tissue was fixed with 10% neutral formaline, embedded in paraffin and 4 µm thick sections were cut from each paraffin block. After dewaxing, hydration, and serum blocking, ApopTag Peroxidase in situ Apoptosis Detection Kit (S7100, Merck Millipore, Darmstadt, Germany) was used according to the manufacturer's protocol. In order to determine the apoptotic index (AI), 5 randomly selected regions of the each section was chosen under x400 magnification. Cells, which have nucleuses stained brown, were judged TUNEL-positive apoptotic cells. The AI of hepatocytes was determined as the percentage of TUNEL positive cells with respect to the total number of cells counted using the formula:

Apoptotic index (AI) = (Number of positive cells/Total number of cells counted) x100

Biochemical Evaluation

Samples of 2 ml were taken from the storage solution that the liver tissue of each animal was stored in at 12th hour in biochemistry tubes not containing anticoagulant. The samples had aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels measured with the enzymatic colorimetric method in the Clinical Biochemistry laboratory using a biochemical autoanalyzer.

Statistical analysis

Data were analyzed with the SPSS program version 20.0. For presentation of descriptive data, mean, standard deviation, median, minimum and maximum values were used. The fit of variables to normal distribution according to numbers in the animal groups was investigated with the Shapiro-Wilk test. When sample size and normal distribution tests are investigated, non-parametric tests were chosen for the analysis methods. The chi-square test was used to compare hydropic degeneration, sinusoidal dilatation and focal necrosis degree between the groups. The Mann-Whitney U test was used to compare TUNEL score and biochemical ALT, AST and ALP values between the groups. Situations with $p<0.05$ were accepted as statistically significant.

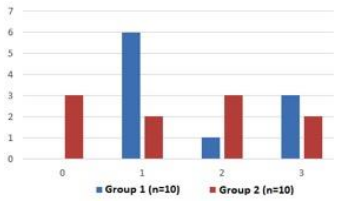
Results

Histopathologically, the hydropic degeneration, sinusoidal dilatation and focal necrosis parameters were assessed in both groups. Accordingly, the mean for hydropic degeneration (Table 1 and Graphic 1) and sinusoidal dilatation (Table 2 and Graphic 2) was higher in Group 1 compared to Group 2, but the results were not statistically significant (p=0.102 and p=0.351, respectively). Histopathologic changes are seen in Figure 1.

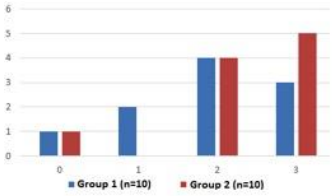
When examined in terms of focal necrosis, 4 subjects in group 1 had different levels of focal necrosis, while only 1 subject in Group 2 had grade 1 focal necrosis (Table 3). Focal necrotic areas can be seen in Figure 2.

AI values in Group 1 were higher than Group 2; however, there was no statistically significant difference between the median values (3.50 (Min:1.00-Max:16.00) vs. 2.50 (Min:1.00-Max:20.00), respectively p=0.974) (Mann-Whitney U Test) (Graphic 3). Apoptotic cells with TUNEL staining can be seen in Figure 3.

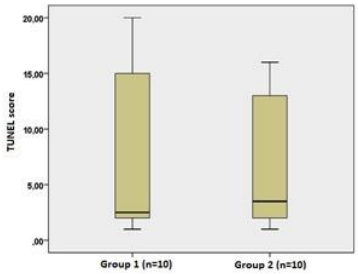
When the ALT, AST and ALP values studied in the organ preservation solutions at 12th hour are compared, the mean values for these parameters appear to be higher in Group 1. However, there was no statistically significant difference between the groups in terms of these parameters (ALT p=0.739, AST p=1.000 and ALP p=0.165) (Table 4).



Graphic 1: Distribution of hidropic degeneration grades between groups



Graphic 2: Distribution of sinusoidal dilatation grades between groups



Graphic 3: Comparison of experimental groups in terms of AI score

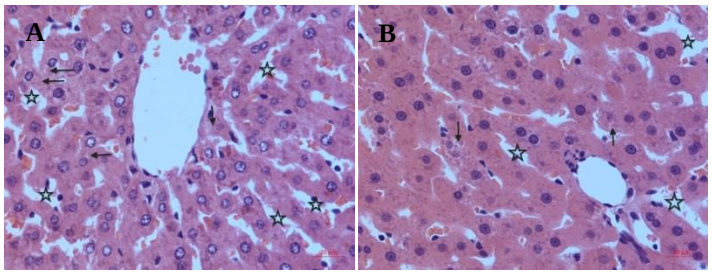


Figure 1: (A) Hematoxylin and Eosin staining of the liver tissues of Group 1 obtained after 12 hours of storage. Hydropic degenerations of hepatocytes (arrows) and sinusoidal dilatation areas (stars) are seen intensely (x400 magnification). (B) Hematoxylin and Eosin staining of the liver tissues of Group 2 obtained after 12 hours of storage. Hydropic degenerations of hepatocytes (arrows) and sinusoidal dilatation areas (stars) are seen less than Group 1 (x400 magnification).

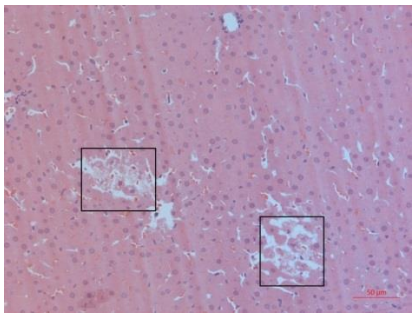


Figure 2: Hematoxylin and Eosin staining of the liver tissues of Group 1. Focal necrosis areas are seen in squares(x200 magnification).

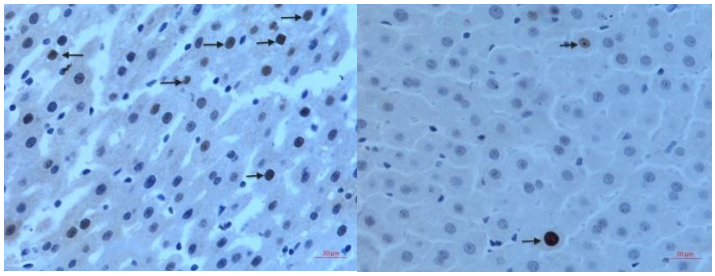


Figure 3: (A) TUNEL staining of the liver tissues of Group 1. Apoptotic hepatocytes' nucleuses are seen in brown-black color (arrow) (x400 magnification). (B) TUNEL staining of the liver tissues of Group 2. There are fewer apoptotic cells than Group 1 (x400 magnification)

Table 1: Comparison of experimental groups in terms of hydropic degeneration

Grade	Group 1 (n=10) n (%)	Group 2 (n=10) n (%)	p value*
0	0 (0.0)	3 (30.0)	
1	6 (60.0)	2 (20.0)	
2	1 (10.0)	3 (30.0)	0.102
3	3 (30.0)	2 (20.0)	

Percentage: Columnar percentage, *:chi-square test

Table 2: Comparison of experimental groups in terms of sinusoidal dilatation

Grade	Group 1 (n=10) n (%)	Group 2 (n=10) n (%)	p value*
0	1 (10.0)	1 (10.0)	
1	2 (20.0)	0 (0.0)	
2	4 (40.0)	4 (40.0)	0.351
3	3 (30.0)	5 (50.0)	

Percentage: Columnar percentage, *:chi-square test

Table 3: Comparison of experimental groups in terms of focal necrosis

Grade	Group 1 (n=10) n (%)	Group 2 (n=10) n (%)	p value*
0	6 (60.0)	9 (90.0)	
1	2 (20.0)	1 (10.0)	
2	1 (10.0)	0 (0.0)	0.402
3	1 (10.0)	0 (0.0)	

Percentage: Columnar percentage, *:chi-square test

Table 4: Comparison of biochemical parameters of 12th hours of experimental and control groups

Variables	Group 1 (n=10) Mean± sd	Median (Min-max)	Group 2 (n=10) Mean±sd	Median (Min-max)	p value
ALT	1064.75± 475.97	1120.60 (525.90-1562.90)	1014.22±374.56	870.95 (631.40-2218.00)	0.739
AST	973.33± 278.71	946.70 (629.20-1829.30)	951.49±355.46	899.15 (584.20-1386.30)	1.000
ALP	3.80± 1.14	3.00 (3.00-6.00)	3.11±0.60	3.00 (2.00-4.00)	0.165

sd: standard deviation, p: Mann-Whitney U Test

Discussion

The most important factor affecting success of organ transplantation is protecting against ischemia-reperfusion (I/R) injury and ensuring continued organ viability [8]. With the aim of reducing organ metabolism, subjecting the organ to cold ischemia is an important method to increase viability and post-transplantation success. However, in spite of this, the researches for additional methods to preserve viability continue [10-12]. The aim of this study is to use the reducing effect of thiopental on metabolic rate to ensure preservations of the liver during the cold ischemia process and to increase the post-transplantation success rates. When examined from this aspect, there was no study found in the literature related to the effect of thiopental on the cold ischemia process in the liver.

In our study, histopathologic assessment, AI and biochemical ALT, AST and ALP analyses were performed during the cold ischemia process on liver tissue after thiopental anesthesia. The results were higher in thiopental group compared to control group. However, these results were not found to be statistically significant.

Thiopental is a barbiturate group anesthetic which is highly soluble in fat [24]. There are studies revealing the antioxidant effect of thiopental by inhibiting lipid peroxidation [24,25]. Thiopental is widely known to have protective effect against cerebral ischemic injury and is routinely used for patients with ischemic attack [26]. Apart from this effect in the brain, the literature contains a variety of studies showing thiopental reduces renal ischemia reperfusion injury [27,28]. These studies biochemically measured malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) values in renal tissue and additionally attempted to determine histopathological injury to renal tissue. The results of both studies revealed that thiopental had a protective effect in the kidney both in terms of biochemical enzyme activity and histopathologically. In our study, a cold ischemia model of liver tissue was studied, without reperfusion. When assessed histopathologically, the group administered thiopental had less histopathological injury compared to the control group. Additionally, lower numbers of apoptotic cells

were found in the thiopental group. However, our study did not examine antioxidant enzyme levels, different to the literature [28]. Apart from this, our study biochemically examined the ALT, AST and ALP levels in organ preservation solution samples taken at 12th hour. ALT levels have been used as a marker of liver injury for a long time [29]. AST is known to be a marker for hepatocyte integrity [30]. ALP is another marker used to assess liver function. Increase in ALP levels, along with increases in ALT and AST show hepatocellular injury, while ALP increase alone may indicate cholestatic injury [31]. In our study, samples taken from preservation solutions at 12th hour had lower ALT, AST and ALP values compared to the control group. However, these values did not reach statistical significance. This result indicates that the protective effect of thiopental which was previously demonstrated for the kidney may also be shown for liver. In our study, we left the organs in cold ischemia for 12 hours after perfusion. In situations with lengthened durations, the fall in liver enzymes may have reached statistical significance. The study duration being 12 hours is a limitation of our research.

In this study, the anesthetic agent in the experimental group was thiopental, while ketamine was used for the control group. Ketamine is a commonly chosen anesthetic agent for surgeries in rats. It is also routinely used in animal studies in our center. Ketamine, a cyclohexylamine, is used as a general anesthetic clinically and enters the dissociative anesthetic class due to some characteristics [32]. Studies have revealed that ketamine has a protective effect against ischemic injury in a variety of tissues [33,34]. Similar to thiopental, the literature shows a protective effect in incomplete cerebral ischemia [34]. In our study, though ketamine has a protective effect against ischemic injury in a variety of tissues, in the thiopental group the histopathological injury levels in the liver were lower and the biochemical ALT, AST and ALP values were lower. This indicates that thiopental may be chosen as anesthetic agent before cold ischemia in transplantation surgeries.

In the literature, two studies compared propofol and thiopental administered to different groups in a testicular I/R injury model and revealed the protective effect of propofol on testis tissue was better compared to thiopental [35,36]. In these studies, the testis was histopathologically assessed and antioxidant enzyme levels were examined, the results showed less injury in the propofol group. In our study, different to these studies, liver tissue was examined and a cold ischemia model was applied. Reperfusion was not performed so antioxidant enzyme levels were not studied. However, the lack of a third group in our study administered propofol anesthesia is a limitation of our study.

In conclusion, our study is the first study to reveal whether thiopental has a protective effect on liver tissue during the cold ischemia process and attempted to show this effect histopathologically and biochemically. The lower AI values in the group administered thiopental, especially, are important in terms of being able to show a cytoprotective effect on the liver during the cold ischemia process. In fact, during the transplantation process, the lower the number of apoptotic cells in liver tissue during cold ischemia, the better the protection of postoperative organ viability. Additionally, biochemically, the mean values of ALT, AST and ALP were low in the thiopental group, which is important for liver preservation and protection of hepatocytes. There is a need for advanced studies in order for our results to reach statistical significance.

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References

1. Li JH, Jia JJ, Shen W, Chen SS, Jiang L, Xie HY, et al. Optimized postconditioning algorithm protects liver graft after liver transplantation in rats. Hepatobiliary Pancreat Dis Int. 2018;17(1):32-8.
2. Jia JJ, Li JH, Jiang L, Lin BY, Wang L, Su R, et al. Liver protection strategies in liver transplantation. Hepatobiliary Pancreat Dis Int. 2015;14(1):34-42.
3. Vajdová K, Graf R, Clavien PA. ATP-supplies in the cold-preserved liver: A long-neglected factor of organ viability. Hepatology. 2002;36(6):1543-52.
4. Lanir A, Clouse ME, Lee RG. Liver preservation for transplant: Evaluation of hepatic energy metabolism by 31P NMR 1. Transplantation. 1987;43(6):786-90.
5. Haddad P, Cabrillac JC, Piche D, Musallam L, Huet PM. Changes in intracellular calcium induced by acute hypothermia in parenchymal, endothelial, and Kupffer cells of the rat liver. Cryobiology. 1999;39(1):69-79.
6. Yokoyama Y, Beckman J, Beckman T, Wheat J, Cash T, Freeman B, et al. Circulating xanthine oxidase: potential mediator of ischemic injury. Am J Physiol Gastrointest Liver Physiol. 1990;258(4):G564-G70.
7. Belzer FO, Southard JH. Principles of solid-organ preservation by cold storage. Transplantation. 1988;45(4):673-6.
8. Szilágyi ÁL, Mátrai P, Hegyi P, Tuboly E, Pécz D, Garami A, et al. Compared efficacy of preservation solutions on the outcome of liver transplantation: Meta-analysis. World J Gastroenterol. 2018;24(16):1812.
9. Demmy TL, Biddle JS, Bennett LE, Walls JT, Schmaltz RA, Curtis JJ. Organ preservation solution in heart transplantation patern of useage and related survival. Transplantation. 1997;63(2):262–9.
10. Akbulut S, Sevmis S, Karakayali H, Bayraktar N, Unlukaplan M, Oksuz E, et al. Amifostine enhances the antioxidant and hepatoprotective effects of UW and HTK preservation solutions. World J Gastroenterol. 2014;20(34):12292.
11. Hosseini F, Naseri MG, Badavi M, Ghaffari MA, Shahbazian H, Rashidi I. Effect of beta carotene on lipid peroxidation and antioxidant status following renal ischemia/reperfusion injury in rat. Scand J Clin Lab Invest. 2010;70(4):259-63.
12. Sanches SC, Ramalho LNZ, Mendes-Braz M, Terra VA, Cecchini R, Augusto MJ, et al. Riboflavin (vitamin B-2) reduces hepatocellular injury following liver ischaemia and reperfusion in mice. Food Chem Toxicol. 2014;67:65-71.
13. Liu A, Dong W, Peng J, Dirsch O, Dahmen U, Fang H, et al. Growth differentiation factor 11 worsens hepatocellular injury and liver regeneration after liver ischemia reperfusion injury. FASEB J. 2018;fj-201800195R.
14. Wang X, Xu M, Jia J, Zhang Z, Gaut JP, Upadhya GA, et al. CD 47 blockade reduces ischemia/reperfusion injury in donation after cardiac death rat kidney transplantation. Am J Transplant. 2018;18(4):843-54.
15. Zhan X, Zhang Z, Huang H, Zhang Y, Zeng Z. Effect of heme oxygenase-1 on the protection of ischemia reperfusion injury of bile duct in rats after liver transplantation. Clin Res Hepatol Gastroenterol. 2018;42:245-54.
16. Rossetti A, Togliatto G, Rolo AP, Teodoro JS, Granata R, Ghigo E, et al. Unacylated ghrelin prevents mitochondrial dysfunction in a model of ischemia/reperfusion liver injury. Cell Death Dis. 2017;3:17077.
17. Zhang W, Li F, Ye Y, Liu Y, Yu S, Cen C, et al. Isoglycyrrhizinate Magnesium Enhances Hepatoprotective Effect of FK506 on Ischemia-Reperfusion Injury Through HMGB1 Inhibition in a Rat Model of Liver Transplantation. Transplantation. 2017;101(12):2862-72.
18. Pizarro MAD, Rodríguez JNV, Mamprin MAE, Fuller BJ, Mann BE, Motterlini R, et al. Protective effects of a carbon monoxide-releasing molecule (CORM-3) during hepatic cold preservation. Cryobiology. 2009;58(3):248-55.
19. Tüfek A, Tokgöz O, Aliosmanoglu İ, Alabalik U, Evliyaoglu O, Çiftçi T, et al. The protective effects of dexmedetomidine on the liver and remote organs against hepatic ischemia reperfusion injury in rats. Int J Surg. 2013;11(1):96-100.
20. Kayhan Z. Lokal / Bolgesel Anestezi Yontemleri, Klinik Anestezi. 2.Baskı. İstanbul: Logos Yayıncılık; 1997. pp.452-98.
21. Morgan GE, Mikhail MS, Muray MS, Larson CP. Regional Anesthesia & Pain Management, Clinical Anesthesiology. 3rd Edition. Los Angeles :The Mc Graw - Hill Companies; 2002. pp. 253-344.
22. Baugman VL, Brain protection during neurosurgery. Anesthesiol Clin North Am. 2002;20:315–27.
23. Morgan GE, Mikhail MS, Murray MJ, Clinical Anesthesiology, third edition, Lange Medical Boks/McGraw-Hill Companies; 2002. pp. 151–177.
24. Yagmurdur H, Ayyildiz A, Karaguzel E, Ogus E, Surer H, Caydere M, et al. The preventive effects of thiopental and propofol on testicular ischemia-reperfusion injury. Acta Anaesthesiol Scand. 2006;50(10):1238-43.

25. Almaas R, Saugstad OD, Pleasure D, Rootwelt T. Effect of barbiturates on hydroxyl radicals, lipid peroxidation, and hypoxic cell death in human NT2-N neurons. Anesthesiology. 2000;92(3):764-74.
26. Hirotani T, Kameda T, Kumamoto T, Shirota S, Yamano M. Protective effect of thiopental against cerebral ischemia during circulatory arrest. Thorac Cardiovasc Surg. 1999;47(04):223-8.
27. Dogan Z, Yuzbasioglu MF, Kurutas EB, Yildiz H, Coskuner I, Senoglu N, et al. Thiopental improves renal ischemia–reperfusion injury. Renal failure. 2010;32(3):391-5.
28. Yuzer H, Yuzbasioglu MF, Ciralik H, Kurutas EB, Ozkan OV, Bulbuloglu E,et al. Effects of intravenous anesthetics on renal ischemia/reperfusion injury. Renal failure. 2009;31(4):290-6.
29. Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology. 2003;37(6):1286-92.
30. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. CMAJ. 2005;172(3):367-79.
31. Lala V, Minter DA. Liver Function Tests. [Updated 2018 Jan 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2018 Jan. PMID: 29494096
32. Anis NA, Berry SC, Burton NR, Lodge D. The dissociative anaesthetics, ketamine and phencyclidine, selectively reduce excitation of central mammalian neurones by N-methyl-aspartate. Br J Pharmacol. 1983;79(2):565-75.
33. Ergün Y, Öksüz H, Atli Y, Kılınç M, Darendeli S. Ischemia-reperfusion injury in skeletal muscle: comparison of the effects of subanesthetic doses of ketamine, propofol, and etomidate. J Surg Res. 2010;159(1):e1-e10.
34. Hoffman WE, Pelligrino D, Werner C, Kochs E, Albrecht RF. Ketamine decreases plasma catecholamines and improves outcome from incomplete cerebral ischemia in rats. Anesthesiology. 1992;76(5):755-62.
35. Yagmurdur H, Ayyildiz A, Karaguzel E, Akgul T, Ustun H, Germiyanoglu C. 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(3):350-7.
36. Yagmurdur H, Ayyildiz A, Karaguzel E, Ogus E, Surer H, Caydere M, et al. The preventive effects of thiopental and propofol on testicular ischemia-reperfusion injury. Acta Anaesthesiol Scand. 2006;50(10):1238-43.