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Original Article

Effect of melatonin on kidney cold ischemic preservation injury

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Abstract: Melatonin is a potent free radical scavenger of reactive oxygen species, nitric oxide synthase inhibitor and a well-known antioxidant secreted from pineal gland. This hormone has been reported to protect tissue from oxidative damage. In this study, we aim to investigate the effect of melatonin on kidney cold ischemia time when added to preservation solution. Thirty male Wistar albino rats were divided equally into three groups; Ringer Lactate (RL) solution, University of Wisconsin (UW) solution with and without melatonin. The serum Lactate Dehydrogenase (LDH) activities of the preservation solutions at 2nd, 24th, 36th, and 48th hours were determined. Tissue malondialdehyde (MDA) levels were also measured and a histological examination was performed at 48th hour. Melatonin that added to preservation solution prevented enzyme elevation and decreased lipid peroxidation in preservation solution when compared to the control group ($p<0.05$). The histological examination revealed that UW solution containing melatonin significantly prevented the kidney from pathological injury ($p<0.05$). Melatonin added to preservation solutions such as UW solution seemed to protect the tissue preserved effectively from cold ischemic injury for up to 48 hour.

Keywords: Melatonin, kidney, University of Wisconsin solution, cold preservation

Introduction

Oxidative stress is involved in ischemia-reperfusion injury and allograft rejection after transplantation. To prolong the survival capacity for transplantation, an important preservation strategy is hypothermia and pharmacological inhibition to slow the metabolic processes in the ischemic/anoxic organ [1]. Simple hypothermic organ preservation is an uncomplicated, cost-effective procedure that can be used for almost all solid organs [1]. During hypothermic ischemia, UW solution minimizes cell swelling, prevents acidosis, averts interstitial edema, captures oxygen radicals, and supplies the organ with precursors for energy metabolism [1]. Altering the content of preservation solutions is the most commonly applied strategy in the development of new solutions.

Melatonin (N-acetyl-5-methoxytryptamine) is a highly lipophilic molecule secreted from the pineal gland, retina, and digestive tract. The highest melatonin concentration is found in the hepatobiliary system [2-4].

Melatonin given in combination with a variety of antioxidants acts synergistically to suppress the formation of free radicals [5]. In addition to its direct free radical scavenging action, melatonin may prevent tissue damage by acting cooperatively with other antioxidants and also induce heath shock protein synthesis [6-8]. Gunal et al found that melatonin alleviate cold preservation injury of the liver [9]. But we do not know whether it preserve the kidney from cold preservation. For this reason in this study we have studied a potent antioxidant melatonin as an adjunct to UW solution to investigate the pro-

tective effect for the kidney from cold preservation injury.

Materials and methods

This study was performed at the Physiology Laboratory, Düzce Medical Faculty of Abant İzzet Baysal University, Düzce, Turkey. Experiments were performed according to the protocol of Animal Care and Experimental Research Labarotory of Düzce Medical Faculty of Abant İzzet Baysal University. The principles of laboratory animals care of Helsinky declaration were strictly followed.

Thirty male Wistar albino rats weighing 300-350 grams were divided into 3 groups equally. The group 1 (Group-RL, control group), in which kidneys were perfused with RL; the group 2 (Group-UW), in which kidneys were perfused with UW preservation solution; and the latter group 3 (Group-UW+M), in which kidneys were perfused with UW preservation solution containing melatonin.

All animals were fed with standard rat chow and water ad libitum at 25°C. Food was withheld overnight before surgery.

Anesthesia and surgery

The rats were anesthetized with intraperitoneal ketamine (Ketalar-50 mg/kg-Eczacibaşi, Istanbul, Turkey). The abdominal cavity was opened via a midline abdominal incision. The abdominal aorta, inferior vena cava (IVC), left and right renal vessels were dissected. The aorta was suspended with a 4/0 silk thread and cannulated with 23-gauge polyethylene tube catheter. Two milliliters of physiological saline was infused through the abdominal aorta before blood sampling from the suprahepatic inferior vena cava in order to rehydrate the subject. The IVC was catheterized above the renal veins level to enable the exanguination of blood and perfusion solution.

The kidneys were perfused with the preservation solutions through the aortic cannula continuously at 70 cm H_2O pressure until the both kidneys paled and the output from the IVC cannula was as clear as the perfusate. Finally, bilateral nephrectomy was performed. The rats were sacrified after the surgical procedures by decapitation.

The graft was weightened and put in a container filled with 40 mL of preservation solution. Melatonin was added to the preservation solutions at a concentration of 30 mg/L at Group 3. All the graft tissues were immersed and preserved in preservation solution immediately after harvesting. The grafts were kept in sterile plastic containers at 4°C in a refrigerator. One milliliter of a mixed sample of preservation fluid was taken from the container for enzyme measurements at 2^{nd}, 24^{th}, 36^{th}, and 48^{th} hours.

Biochemical measurements

The LDH activities in the serum and preservation solutions were determined by standard clinical biochemistry methods using an Olympus AU 640 autoanalyzer (Olympus, Tokyo, Japan). Tissue MDA levels were measured at 48^{th} hour using the method described by Uchiyama et al [10].

Histopathological evaluation

Kidney injury was analyzed and reviewed by a pathologist who did not know which preservation solutions used. Samples were fixed and embedded in hematoxylin-eosin (H&E) stained paraffin sections for examination under light microscopy. Samples are also examined for cellular ultrastructure under an electron microscope (JEOL, Tokyo, Japan).

Light microscopic sections were examined for vacuolization, denuded basement membrane, necrosis, tubular dilatation, and cell detachment. Electron-microscopic sections were examined for mitochondria and tubular cell brush border integrity and interstitial and cell edema. Nine basic morphological patterns (apical cytoplasm vacuolization, tubular necrosis, denuded basement membrane, tubular dilatation, cell detachment, intracellular edema, interstitial edema, brush border integrity, mitochondria integrity) were graded on a five-point scale as follows: 1, no abnormality; 2, mild lesions affecting 10% of kidney samples; 3, lesions affecting 25% of kidney samples; 4, lesions affecting 50% of kidney samples; and 5, lesions affecting more than 75% of kidney samples [11].

Statistical analysis

The data were expressed as means±SD. The significance of differences between the three

Table 1. Serum and preservation solutions LDH activities and MDA level of RL, UW and UW+M at different hours

	Group 1 (RL)	Group 2 (UW)	Group 3 (UW+M)
Serum LDH 0th hour (IU/L)	324±122	298±61	312±82
LDH 2nd hour (IU/L)	82±17$^{\alpha,\beta}$	56±6*	48±8*
LDH 24th hour (IU/L)	437±84$^{\alpha,\beta}$	292±59*	264±65*
LDH 36th hour (IU/L)	1323±356$^{\alpha,\beta}$	581±91*	546±78*
LDH 48th hour (IU/L)	2608±587$^{\alpha,\beta}$	1447±372*	1200±322*
MDA (nmol/gr tissue)	24±7$^{\alpha,\beta}$	14±5*	12±4*

Values are given as means±SD. *Significantly different from group 1; $^{\alpha}$Significantly different from group 2; $^{\beta}$Significantly different from group 3 (P<0.05). LDH: Lactate Dehydrogenase, MDA: Malondialdehyde, RL: Ringer Lactate, UW: University of Wisconsin, M: Melatonin.

Table 2. The median score of the injury parameters for kidney tissue at 48th hour

	Group 1 (RL)	Group 2 (UW)	Group 3 (UW+M)
Apical cytoplasm vacuolization	3.5$^{\alpha,\beta}$	1.0*	1.0*
Tubular necrosis	4.5$^{\alpha,\beta}$	1.5*	1.0*
Denuded basement membrane	3.0$^{\alpha,\beta}$	1.0*	1.0*
Tubular dilatation	4.0$^{\alpha,\beta}$	1.5*	1.0*
Cell detachment	4.0$^{\alpha,\beta}$	1.5*	1.0*
Intracellular edema	3.0$^{\alpha,\beta}$	1.5*	1.0*
Interstitial edema	4.5$^{\alpha,\beta}$	1.0*	1.0*
Brush border integrity	4.0$^{\alpha,\beta}$	1.5*	1.0*
Mitochondria integrity	3.0$^{\alpha,\beta}$	1.5*	1.0*

Values are given as medians. *Significantly different from group 1; $^{\alpha}$Significantly different from group 2; $^{\beta}$Significantly different from group 3 (P<0.05).

groups was analyzed through analysis of variance (ANOVA); if the F value was found to be significant, differences between means were then analyzed with the post-ANOVA (Tukey's) test.

Histopathological findings at 48th hour are given as median values and Kruskal-Wallis and Mann-Whitney U-tests were used to evaluate the significance of differences among groups. Values of p<0.05 were considered as statistically significant.

Results

In the present study, the LDH levels in the serum at 0th hour and preservation solutions of all groups at 2nd, 24th, 36th and 48th hours and tissue MDA levels at 48th hour were measured.

In all groups, before perfusion two milliliters of blood were taken from IVC to evaluate the serum LDH levels. Serum LDH levels at 0th hour were similar for all groups. No significant difference was found (p> 0.05).

The LDH levels of preservation solutions at 2nd and 24th hours of group 1 was lower than both group 2 and group 3, and at 36th and 48th hours a further increase was observed in group 1 than group 2 and group 3. It was found as statisticaly significant (p<0.05) (**Table 1**).

The MDA levels of group 1 (24±7 nmol/gr tissue) was higher than group 2 (14±5 nmol/gr tissue) and group 3 (12±4 mol/gr tissue) (**Table 1**). According to tissue MDA levels there is a significant differences between group 1 with group 2 and group 3 (p=0.005, p=0.005, respectively).

In the histopathological evaluation of the samples; apical cytoplasm vacuolization, tubular necrosis, denuded basement membrane, tubular dilatation, cell detachment, intracellular edema, interstitial edema, brush border integrity and mitochondria integrity were all significantly decreased in groups 2 and 3 than group 1. It was also found as statistically significant (p<0.05) (**Table 2**).

Dicussion

Since the beginning of the 1990s, UW solution has been used in heart, lung, liver, pancreas, kidney, and intestinal transplants with great success. Using UW solution for preservation, the ischemia tolerance limit has been extended to 18 hour for liver and 36 hour for kidney [12].

In this present study we use a well-known antioxidant Melatonin that has been reported to protect tissue from oxidative damage [5, 13].

Li and coworkers found that donor preconditioning with melatonin protected kidney donor

grafts from ischemia reperfusion induced renal dysfunction and tubular injury most likely through its anti-oxidative, anti-apoptotic and NF-kB inhibitory capacity [14]. We did not perform any type of preconditioning. However, melatonin seemed to decrease tissue damage significantly beginning at 36th hour of preservation.

The protective effect of melatonin on ischemia reperfusion induced renal injury is might be related to its antioxidant properties rather than induction of proinflammatory cytokines [15]. It was found that Melatonin and 1400 W were efficient in ameliorating experimental ischemia-reperfusion injury of the kidneys [16]. Additionally in rat model melatonin was found to be effective on liver preservation up to 48 hours [9].

Fadillioglu et al speculated that melatonin treatment may prevent liver oxidant stress induced by distant injury of kidney ischemia reperfusion [17]. They suggest that melatonin reduces blood pressure and ischemia reperfusion injury in nitric oxide synthase inhibited rats by L-arginine methyl ester [18].

Exogenous melatonin is able to preserve renal functional status following ischemia reperfusion induced injury by increasing glutathione and reducing lipid peroxidation in the early reperfusion phase, without any apparent effect on neutrophil infiltration in the late reperfusion phase [19].

Kunduzova et al suggests that melatonin may represent a novel therapeutic approach for prevention of ischemia reperfusion injury [20].

As melatonin administration reversed these oxidant responses, improved renal function and microscopic damage, it seems likely that melatonin protects kidney tissue against oxidative damage [21]. In our study; the histopathological evaluation of the microscopic injury scores were all significantly decreased in groups UW and UW+M from group RL. Melatonin was also used as an additive to preservative solutions in graft preservation of liver [22, 23] and pancreas [24].

Chronic melatonin treatment reduces renal injury by reducing lipid oxidation and NO production in diabetic rats exposed to ischemia [25]. Melatonin has made a significantly difference on MDA. We have found decreased level of MDA at melatonin added to UW preservation solution samples.

These results suggest that physiological and pharmacological melatonin concentrations are important for the reduction of ischemia reperfusion induced damage and melatonin even when administrated just before reperfusion, had a protective effect on ischemia reperfusion injury [26].

Raken together, we conclude that adding melatonin into preservation solutions such as UW, may prolong the cold storage time up to 48th hour and contibute to graft preservation effectively.

Disclosure of conflict of interest

None.

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References

[1] Spiegel HU, Palmes D. Organ preservation. In: Hakim NS, Danovitch GM, editors. Transplantation Surgery. London: Springer–Verlag Press. 2001; pp: 265-94.

[2] Sakamoto K, Liu C, Tosini G. Circadian rhythms in the retina of rats with photoreceptor degeneration. J Neurochem 2004; 90: 1019-24.

[3] Bubenik GA, Brown GM. Pinealectomy reduces melatonin levels in the serum but not in the gastrointestinal tract of the rat. Biol Signals 1997; 6: 40-4.

[4] Tan D, Manchester LC, Reiter RJ, Qi W, Hanes MA, Farley NJ. High physiological levels of melatonin in the bile of mammals. Life Sci 1999; 65: 2523-9.

[5] López-Burillo S, Tan DX, Mayo JC, Sainz RM, Manchester LC, Reiter RJ. Melatonin, xanthurenic acid, resveratrol, EGCG, vitamin C and α-lipoic acid differentially reduce oxidative damage induced by Fenton reagents: a study of their individual and synergistic actions. J Pineal Res 2003; 34: 269-77.

[6] Allegra M, Reiter RJ, Tan DX, Gentile C, Tesoriere L, Livrea MA. The chemistry of melatonin's

interaction with reactive species. J Pineal Res 2003; 34: 1-10.
[7] Bonior J, Jaworek J, Konturek SJ, Palik WW. Increase of heat shock protein gene expression by melatonin in ar42j cells. J Physiol Pharmacol 2005; 56: 471-81.
[8] Cabrera J, Quintana J, Reiter RJ, Loro J, Cabrera F, Estévez F. Melatonin prevents apoptosis and enhances HSP27 mRNA expression induced by heat shock in HL-60 cells: possible involvement of the MT2 receptor. J Pineal Res 2003; 35: 231-8.
[9] Gunal O, Cokun A, Aslaner A, Yildirim U. Does melatonin alleviate cold preservation injury of the liver? Turk J Med Sci 2010; 40: 465-70.
[10] Wahlberg JA, Southard JH, Belzer FO. Development of a cold storage solution for pancreas preservation. Cryobiology 1986; 23: 466-82.
[11] Hauet T, Mothes D, Goujon JM, Carretier M, Eugene M. Protective effect of polyethylene glycol against prolonged cold ischemia and reperfusion injury: study in the isolated perfused rat kidney. J Pharmacol Exp Ther 2001; 297: 946-52.
[12] Southard JH, Belzer FO. The University of Wisconsin organ preservation solution: components, comparisons, and modifications. Transplant Rev 1993; 7: 176-90.
[13] López-Burillo S, Tan DX, Mayo JC, Sainz RM, Manchester LC, Reiter RJ. Melatonin, xanthurenic acid, resveratrol, EGCG, vitamin C and alpha-lipoic acid differentially reduce oxidative DNA damage induced by Fenton reagents: a study of their individual and synergistic actions. J Pineal Res 2003; 34: 269-77.
[14] Li Z, Nickkholgh A, Yi X, Bruns H, Gross ML, Hoffmann K, Mohr E, Zorn M, Büchler MW, Schemmer P. Melatonin protects kidney grafts from ischemia/reperfusion injury through inhibition of NF-kB and apoptosis after experimental kidney transplantation. J Pineal Res 2009; 46: 365-72.
[15] Kurcer Z, Oguz E, Ozbilge H, Baba F, Aksoy N, Celik H, Cakir H, Gezen MR. Melatonin protects from ischemia/reperfusion-induced renal injury in rats: this effect is not mediated by proinflammatory cytokines. J Pineal Res 2007; 43: 172-8.
[16] Ersoz N, Guven A, Cayci T, Uysal B, Turk E, Oztas E, Akgul EO, Korkmaz A, Cetiner S. Comparison of the efficacy of melatonin and 1400W on renal ischemia/reperfusion injury: a role for inhibiting iNOS. Ren Fail 2009; 31: 704-10.
[17] Fadillioglu E, Kurcer Z, Parlakpinar H, Iraz M, Gursul C. Melatonin treatment against remote organ injury induced by renal ischemia reperfusion injury in diabetes mellitus. Arch Pharm Res 2008; 31: 705-12.
[18] Deniz E, Colakoglu N, Sari A, Sonmez MF, Tugrul I, Oktar S, Sahna E. Melatonin attenuates renal ischemia-reperfusion injury in nitric oxide synthase inhibited rats. Acta Histochem 2006; 108: 303-9.
[19] Rodríguez-Reynoso S, Leal C, Portilla-de Buen E, Castillo JC, Ramos-Solano F. Melatonin ameliorates renal ischemia/reperfusion injury. J Surg Res 2004; 116: 242-7.
[20] Kunduzova OR, Escourrou G, Seguelas MH, Delagrange P, De La Farge F, Cambon C, Parini A. Prevention of apoptotic and necrotic cell death, caspase-3 activation, and renal dysfunction bymelatonin after ischemia/reperfusion. FASEB J 2003; 17: 872-4.
[21] Sener G, Sehirli AO, Keyer-Uysal M, Arbak S, Ersoy Y, Yeğen BC. The protective effect of melatonin on renal ischemia-reperfusion injury in the rat. J Pineal Res 2002; 32: 120-6.
[22] Zaoualí MA, Reiter RJ, Padrissa-Altés S, Boncompagni E, García JJ, Ben Abnennebi H, Freitas I, García-Gil FA, Rosello-Catafau J. Melatonin protects steatotic and nonsteatotic liver grafts against cold ischemia and reperfusion injury. J Pineal Res 2011; 50: 213-21.
[23] Vairetti M, Ferrigno A, Bertone R, Rizzo V, Richelmi P, Bertè F, Reiter RJ, Freitas I. Exogenous melatonin enhances bile flow and ATP levels after cold storage and reperfusion in rat liver: implications for liver transplantation. J Pineal Res 2005; 38: 223-30.
[24] García-Gil FA, Albendea CD, Escartín J, Lampreave F, Fuentes-Broto L, Roselló-Catafau J, López-Pingarrón L, Reiter RJ, Alvarez-Alegret R, García JJ. Melatonin prolongs graft survival of pancreas allotransplants in pigs. J Pineal Res 2011; 51: 445-53.
[25] Kurcer Z, Parlakpinar H, Vardi N, Tasdemir S, Iraz M, Fadillioglu E, Baba F, Gül M. Protective effects of chronic melatonin treatment against renal ischemia/reperfusion injury in streptozotocin-induced diabetic rats. Exp Clin Endocrinol Diabetes 2007; 115: 365-71.
[26] Sahna E, Parlakpinar H, Ozturk F, Cigremis Y, Acet A. The protective effects of physiological and pharmacological concentrations of melatonin on renal ischemia-reperfusion injury in rats. Urol Res 2003; 31: 188-93.