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Efficacy of prophylactic calcium dobesilate in renal ischemia-reperfusion injury in rats

Ratlarda böbrek iskemi-reperfüzyon hasarında proflaktik kalsiyum dobesilatın etkinliği

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ABSTRACT

Aim: In this study, the objective was to investigate the protective effect of calcium dobesilate, which has antioxidant and anti-inflammatory properties, on the experimental renal ischemia-reperfusion injury (IRI).
Methods: Twenty-four male Wistar-Albino rats were divided into three groups: Sham group (Group 1), ischemia-reperfusion group (Group 2), and treatment group (Group 3). Before the ischemia-reperfusion procedure, rats in Group 3 received calcium dobesilate through gavage (100mg/kg/day) for 10 days. Groups other than the sham group underwent ischemia for 45 minutes and reperfusion for 24 hours. Plasma urea and creatinine levels, erythrocyte superoxide dismutase and glutathione peroxidase enzyme activity levels were measured. In addition, histopathological changes that may be related to ischemia-reperfusion injury in the renal tissue, were investigated.
Results: The median glutathione peroxidase and superoxide dismutase enzyme levels were higher in Group 2 compared to Groups 1 and 3. However, the differences were not statistically significant. The creatine levels were statistically lower in Group 3 compared to Group 1 and Group 2. The median urea levels were lower in Group 3 than in Group 1 and Group 2, but the differences were not statistically significant. The histopathological examination showed that parameters such as cellular necrosis, flattened tubular epithelial cells, cytoplasmic vacuolization, tubular lumen obstruction, and chronic inflammation, which are indicators of the ischemia-reperfusion injury, were statistically less common in the treatment group compared to the control group.
Conclusion: Our study demonstrated that prophylactic calcium dobesilate had a protective effect on ischemia-reperfusion injury.

Keywords: Renal, ischemia-reperfusion, calcium dobesilate, prophylactic.

ÖZ

Amaç: Çalışmamızda, antioksidan ve antienflamatuvar özellikleri olduğu bilinen, kalsiyum dobesilatın deneysel böbrek iskemi-reperfüzyon hasarı (IRI) üzerindeki koruyucu etkisini araştırmayı amaçladık.
Yöntemler: 24 adet erkek Wistar-Albino rat üç gruba ayrıldı; sham grubu (grup 1), iskemi-reperfüzyon grubu (grup 2) ve tedavi grubu (grup 3). İskemi-reperfüzyon işlemi öncesi Grup 3'e 10 gün boyunca 100 mg/kg/gün kalsiyum dobesilat gavaj yolu ile verildi. Sham grubu haricindeki gruplara 45 dakika iskemi ve 24 saat reperfüzyon uygulandı. Plazma üre ve kreatinin düzeyleri, eritrosit süperoksit dismutaz ve glutatyon peroksidaz enzim aktivite düzeyleri çalışıldı. Ayrıca böbrek dokusundaki iskemi-reperfüzyon hasırına ait olabilecek histopatolojik değişiklikler incelendi.
Bulgular: Grup 2'de ortanca glutatyon peroksidaz ve süperoksit dismutaz enzim düzeyleri Grup 1 ve Grup 3'den daha yüksekti, ancak istatistiksel anlamlı değildi. Grup 3'de kreatinin düzeyleri Grup 1 ve Grup 2'den istatistiksel olarak anlamlı derecede daha düşüktü. Ortanca üre değerleri Grup 3'de Grup 1 ve Grup 2'den daha düşüktü ancak istatistiksel olarak anlamlı değildi. Histopatolojik incelemede; kontrol grubu ile kıyaslandığında tedavi grubunda, hücre nekrozu, tübüler epitelyal hücre düzleşmesi, sitoplazmik vakuolizasyon, tübüler lümen obstrüksiyonu ve kronik inflamasyon gibi iskemi-reperfüzyon hasarının göstergesi olan bu parametrelerin istatistiksel olarak anlamlı derecede daha az olduğu gözlendi.
Sonuç: Çalışmamız, proflaktik kalsiyum dobesilatın böbrek iskemi-reperfüzyon hasarında koruyucu etkilerinin olduğunu göstermiştir.

Anahtar Kelimeler: Renal, iskemi- reperfüzyon, kalsiyum dobesilat, proflaktik

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INTRODUCTION

In the recent years, a pathological condition called ischemia-reperfusion injury (IRI) became a common discussion topic depending on the gradually increasing popularity of surgeries like kidney transplantation, nephron-sparing surgery, renal artery surgery, and aorta surgery, which cause a transient decrease in the renal blood flow.

The blood flow to the kidneys is temporarily obstructed during various surgical interventions comprising the clamping of the renal artery or aorta above the renal artery and kidney transplantation [1]. This obstruction may cause ischemic injury in kidneys. Although organ reperfusion following ischemia may decrease the existing injury, it may adversely affect several cellular processes emerging due to the reperfusion. The severity of the injury may lead to organ dysfunctions [2, 3]. Hypoxia, inflammatory cytokines, and free oxygen radicals are the underlying factors of the renal IRI. The involvement of the increased free oxygen species and inflammation lead to cell death [4,5]. Therefore, inflammatory cytokines, free radicals, and oxidative damage are the target of the therapeutic approaches [6].In this context, several agents had been investigated up to the present.

Calcium dobesilate (CD) (calcium 2,5-dihydroxybenzene sulfonate, CLS 2210) is currently used in three major indications (chronic venous insufficiency, diabetic retinopathy, and hemorrhoidal attacks) depending on its angioprotective and antioxidant properties [7]. It is classified under the venotonic and vasculoprotector agents [8]. In Turkey, calcium dobesilate (Doxium®, 500mg capsule, Abdi İbrahim İlaç Sanayi) is approved for the chronic venous insufficiency and used in the treatment of lower extremity venous insufficiencies and diabetic retinopathy for approximately 30 years. Besides, it is used as an adjuvant agent in the treatment of arteriovenous circulation disorders and hemorrhoidal disorders.

Depending on its protective effect against IRI in the skeletal muscle, small intestine and liver, several studies had been conducted to investigate the effects of CD on the preservation of the ischemic skin flaps, diabetic retinopathy, myocardial infarct, and the heart muscle during the cardiac surgery and erectile disfunction. However, there is no study in the literature related to its protective effects against renal ischemia-reperfusion injury. In our study, our objective was to investigate the effects of CD on antioxidants like superoxide dismutase (SOD) and glutathione peroxidase (GPx); on renal function test parameters like urea and creatine and the histopathological changes in respect of IRI.

MATERIAL AND METHOD

This experimental study was approved by the Local Ethics Committee for the Animal Research of the Medical Faculty at AbantİzzetBaysal University by written permit numbered (2009/35).

Twenty-four adult male Wistar-Albino rats (300-360g) were enrolled in our study. The animals were exposed to 12-hour light and 12-hour darkness and followed in separated cages under room temperature (22°C). All subjects were fed with standard rat food and tap water for 10 days. The cages were cleaned regularly.

The anesthesia induction was performed with intramuscular ketamine HCl (40mg/kg; 50mg/cc; Ketalar®; Parke-Davis) and 2% xylazine HCl (10mg/kg; 23.32mg/cc; Rompun®; Bayer) combination. After the first laparotomy, postoperative paracetamol (1-2mg/ml added to drinking water; Parol®; AtabayKimya) was added to the analgesic effect of xylazine HCl. Renal ischemia was created with an atraumatic microvascular clamp placed on the right renal artery. Blood samples were taken from vena cava inferior for the measurement of hemoglobin, GPx, SOD enzyme, urea, and creatine levels. The samples were stored in a deep-freezer at -80°C.

GROUP 1 (n=8, sham group) and GROUP 2 (n=8, control=ischemia-reperfusion group) were monitored without administration of any treatment for 10 days. GROUP 3 (n=8, treatment group) received calcium dobesilate with a dose of 100mg/kg/day, which was dissolved in 0.5cc drinking water and administrated through intragastric gavage for 10 days. After 10 days, only in GROUP 1, laparotomy was performed with a laparotomic incision and right nephrectomy was carried after 24 hours following blood collection. Ischemia was performed in the renal artery in GROUP

2 and GROUP 3 for 45 minutes. 24 hours later blood samples were obtained from all subjects before the right nephrectomy. Tissue samples were inserted in a 10% formaldehyde solution for the histopathological examination. After the experiment, all animals were sacrificed with decapitation.

Urea and creatinine levels (mg/ml) were measured in an autoanalyzer (Abbott Architect Ci 8200, Chicago, IL, USA) using the kits manufactured by Abbott (Chicago, IL, USA). The erythrocyte enzyme activity was measured with the commercial kitRansod (Randox Laboratories Ltd., SD125 Ransod, UK), the erythrocyte GSH-Px activities were measured in an autoanalyzer (Olympus AU-600, Tokyo, Japan) with the commercial kit Ransel (Randox Laboratories Ltd., 505 Ransel, UK) in U/g Hb.

Regarding the histopathological examination, tissue samples stored in a 10% formaldehyde solution were embedded in paraffin blocks and cut at 5μm thickness. All sections were stained with hematoxylin-eosin and examined under a light microscope by an investigator blinded for the samples. The scoring was done according to a modification of the semiquantitative scale described by Paller et al. for the assessment of the changes in the acute renal failure [9].

According to this scale:

1-Chronic inflammation (none/mild, moderate, severe/diffuse)

2-Flattened tubular epithelial cells (none/mild, moderate, severe/diffuse)

3-Cytoplasmic vacuolization (none/mild, moderate, severe/diffuse)

4-Cellular necrosis and ischemic changes (none/mild, moderate, severe/diffuse)

5-Tubular lumen obstruction (none/mild, moderate, severe/diffuse)

The Statistical Analysis

The normal distribution of the measured levels was analyzed with the Shapiro-Wilk test. We determined that parameters other than SOD and GPx values were not normally distributed. The descriptive statistics were expressed according to the compatibility with the normal distribution and quantitative variables were expressed with mean±standard deviation or median (deviation between quarter values; Interquartile Range-IQR) and qualitative variables were expressed with numbers. The 5-point scoring system, which had been used in the histopathological examinations, was converted to a 3-point scale to enable statistical analysis and facilitate interpretation (0=none or mild change; 1=moderate change; 2=severe/diffuse change). During the intergroup comparisons, normally distributed variables were analyzed with the one-way analysis of variance (ANOVA) and non-normally distributed variables with the Kruskal-Wallis variance test with the non-parametric correspondence. To assess the origin of the detected differences, paired comparisons were performed with the Bonferroni posthoc test or Bonferroni-corrected Mann-Whitney U test. For the intergroup comparison of the categorical variables, Chi-square or likelihood ratio values were used. Statistical analysis was done with SPSS 15.0 (SPSS Inc., Chicago, IL, USA) software package. A score of $P \leq 0.05$ was considered as statistically significant.

RESULTS

We determined that the mean SOD value was higher in the control group compared to the sham group. Even though the mean SOD value was lower in the treatment group than the control group, there was no statistically significant difference. We found that the mean GPx value was higher in the control group than the sham group and lower in the treatment group than the control group. However, there was no statistically significant difference between the groups for the GPx value. Urea levels were highest in Group 1 and lowest in Group 3, which was the treatment group. However, there was no statistically significant difference between the three groups. The creatine levels were higher in Group 2 compared to Group 1 and Group 3. The median creatine level was lower in the treatment group than the sham group (Table 1). It was determined that the median creatine value at least in one of the experimental groups was statistically higher compared to other groups. According to the results of the post hoc paired comparison of the creatine levels in the experimental groups, there

was a statistically significant difference between control and sham and treatment groups (Table 2).

Table 1. The SOD, GPx, urea, and creatinine levels in the study groups.

	Urea level (Median/ IQR)	Creatine level (Median/ IQR)	SOD level (mean±SD)	GPx level (Mean±SD)
Group 1	36/32.5	0.54/0.53	2036.39 ± 103.81	20.58 ± 12.54
Group 2	37.5/34.0	0.57/0.55	2168.97 ± 192.52	24.87 ± 14.16
Group 3	34.5/26.5	0.53/0.50	2076.15 ± 204.88	22.19 ± 8.98
X2*	2.239	11.105		
F**			1.237	0.262
P	0.326	0.004	0.311	0.772

Abbreviations: SOD, superoxide dismutase; GPx, glutathione peroxidase; SD, standard deviation; IQR, Interquartile Range

* The result of the Kruskal-Wallis non-parametric variance analysis

* * The result of the ANOVA one-way variance analysis

Table 2. Post hoc paired comparison of the creatine level per experiment groups

	Group 2		Group 3	
Group 1	Z	P	Z	P
Group 2	2.774	0.005	1.333	0.195
Group 3			2.743	0.005

We found also that there was a statistically significant difference between the groups for the distribution of all histopathological variables (Table 3).

Chronic inflammation: While there was a statistically significant difference between sham and control groups (X2=6.904; p=0.009); and between the control and treatment groups (X2=4.857; p=0.028), there was no statistically significant difference between the sham and treatment groups (X2=0.255; p=0.614). The chronic inflammation scores of the treatment and shame groups were similar. While the control group had the highest chronic inflammation score, the chronic inflammation score of the treatment group was considerably close to the score of the Sham group.

Flattened tubular epithelial cells: While there was a statistically significant difference between the Sham and control groups (X2=22,181; p<0.001); and between the control and treatment groups (X2=15,451; p<0.001), there was no statistically significant difference between the sham and treatment groups (X2=3,059; p=0,080).

Table 3. The distribution of the histopathological changes per experiment groups

		Group 1 (n:8)	Group 2 (n:8)	Group 3 (n:8)	P
Chronic inflammation	None/mild	4	0	3	0.026
	Moderate	4	8	5	
	Diffuse	0	0	0	
Flattened tubular epithelial cells	None/mild	8	0	6	<0.001
	Moderate	0	5	2	
	Diffuse	0	3	0	
Cytoplasmic vacuolization	None/mild	5	0	0	<0.001
	Moderate	3	2	6	
	Diffuse	0	6	2	
Cellular necrosis	None/mild	8	0	1	<0.001
	Moderate	0	5	6	
	Diffuse	0	3	1	
Tubular lumen obstruction	None/mild	8	0	4	<0.001
	Moderate	0	2	4	
	Diffuse	0	6	0	

Cytoplasmic vacuolization: There was a statistically significant difference between the Sham and control groups (X2=15,451; p<0.001); between the control and treatment groups (X2=10,723; p=0,005); and between the sham and treatment groups (X2=4,186; p=0,041).

Cellular necrosis: While there was a statistically significant difference between the sham and control groups (X2=22,181; p<0.001); and between the control and treatment groups (X2=15,902; p<0.001), there was no statistically significant difference between the sham and treatment groups (X2=2,524; p=0,283).

Tubular lumen obstruction: There was a statistically significant difference between the sham and control groups (X2=22,181; p<0.001); between the control and treatment groups (X2=6,904; p=0,009); and between the sham and treatment groups (X2=14,543; p=0,001).

DISCUSSION

IRI is an adverse process that may lead to acute renal failure [10]. IRI may emerge as a result of certain surgical interventions [1]. The re-establishment of blood flow to the tissue following ischemia may only completely prevent the development of injury

but also may increase the tissue injury related to the reperfusion [11]. Regarding the prevention of IRI, several methods such as inhibition of the calcium diffusion into the cells, bonding to calcium, inhibition of the development of the free oxygen radicals, suppression of the neutrophil functions, and inhibition of the lipoxygenase and cyclooxygenase pathways had been suggested [12]. In these context; several agents like antioxidants, carnitine, aminoguanidine, calcium channel blockers, immunosuppressants, PDE3 inhibitors, and vitamin E had been investigated.

Some studies conducted with CD, which is classified under the venotonic and vasculoprotective agents, showed that CD has free oxygen species scavenging and decreasing properties along with the antioxidant capacity [13, 14]. It increases NOS activity in macro- and microvascular endothelial cells and decreases the capillary endothelial cell desquamation depending on its effects on the NO synthesis and release [15]. Furthermore, it inhibits the platelet aggregation, decreases the blood viscosity, erythrocyte aggregation, and rigidity. As a result of these effects, it may prevent blood stasis, vascular obstruction, and ischemia [16, 17].

Several studies had been conducted with CD to demonstrate its effects on the ischemia-reperfusion injury in different doses in organs like heart, eye, lung, muscle, and penis with conflicting results. However, there is no published study in the literature related to its effects on renal ischemia-reperfusion injury. In our study, we preferred a dose range of 100mg/kg/day based on a study on antioxidant and angioprotective effects of CD in diabetic rats [13].

Cihan et al. [18] investigated the effects of CD on the myocardial ischemia-reperfusion injury and observed that the change in the mean coronary artery pressure was significantly higher in the group, in which patients had undergone calcium dobesilate perfusion. They reported that this effect might be explained with the increase of the vasodilator substances originating from the endothelium. In their study, İşkesen et al. [19] investigated the prevention of the myocardial injury during the cardiac surgery and observed that the levels of CK, CK-MB, myoglobin, and troponin-T, which are biochemical markers for the myocardial injury, were lower in the group, in which patients received oral calcium dobesilate for 14 days, compared to the control group. In addition, the lactate level in the blood samples taken after the reperfusion was significantly lower. They concluded that calcium dobesilate had positive effects regarding the reduction of the myocardial injury and preservation of myocardium.

In their study, Rota et al. [20] administered 100mg/kg/day calcium dobesilate to diabetic rats for 10 days and showed that the retinal albumin leak decreased about 70% and retinal VEGF expression about 69.4%. They suggested that calcium dobesilate stabilized the blood-retina barrier in diabetic retinopathy depending on it's in situ antioxidant activity. In a similar study conducted by Szabo et al. [21] on diabetic rats, it was concluded that calcium dobesilate was effective in the prevention of the retinal injury mediated by free oxygen radicals and induced by ischemia-reperfusion.

In a study conducted on Guinea pigs, inflammation was created with the subdermal implantation of microscope glass and then CD was administered to observe its anti-inflammatory effects. They concluded that CD had an anti-inflammatory effect probably depending on the blocking of the activity of the macrophage-activating factors and more probably depending on the stimulation of the faster production of the macrophage-deactivating factors like TGF beta-1 and 2, which decreases the number of the circulating monocytes [22].

In our study, we observed that the plasma levels of urea and creatine, which are indicators of the renal glomerular dysfunction, were lower in the treatment group compared to the control group. These low levels were statistically significant for creatine but not for urea. The SOD and GPx enzyme activities were at the highest level in the control group. This finding depended on that the control group was exposed to the highest level of ischemic injury compared to other groups. Although the SOD and GPx enzyme activities were not statistically significant, their level in the treatment group was lower than the control group and higher than the sham group. As a result of this, it might be suggested that the treatment group

was less exposed to IRI than the control group. Histopathologically; cellular necrosis, flattened tubular epithelial cells, cytoplasmic vacuolization, tubular lumen obstruction, and chronic inflammation, which are markers of the ischemic changes, were less severe in the treatment group compared to the control group. They were similar to the sham group in the treatment group (Figure 1).

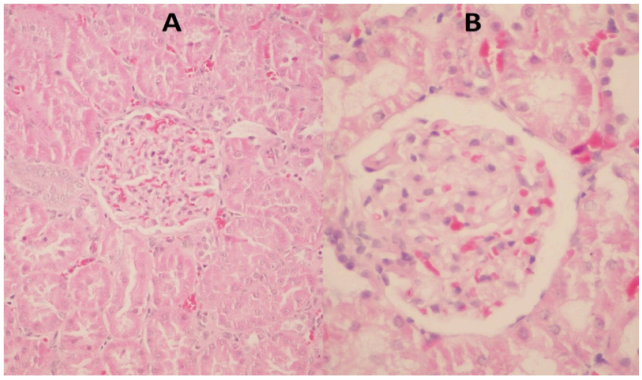

Figure 1:The Sham group showed normal renal cortical tissue structure(HE x 100) (A), Histopathological findings similar to sham group in the treatment group (HE x 200) (B).

The most important limitation of our study is that we did not measure the SOD and GPx levels in the tissue samples. Another limitation of the study is the experimental study.

CONCLUSION

Although there is no study in the literature focused on the prophylactic calcium dobesilate use in the renal ischemia-reperfusion injury yet, the results of our study indicated that prophylactic calcium dobesilate might be used for the reduction of the renal injury caused by ischemia-reperfusion. Further studies with different posology and duration are needed to support our results.

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