

The protective effect of single dose tadalafil in contrast-induced nephropathy: An experimental study

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ABSTRACT

Objective: Contrast-induced nephropathy (CIN) is one of the most common causes of acute renal failure in hospitalized patients. The direct toxic effect of contrast media; ischemic damage caused by reactive oxygen species; increased perivascular hydrostatic pressure; high viscosity and changes in the activity of vasoactive substances play important roles in the pathogenesis. Tadalafil inhibits the phosphodiesterase enzyme which destroys nitric oxide. Nitric oxide causes renal vasodilatation, increases renal medullar blood flow and mediates the removal of free oxygen radicals. Drugs that increase levels of nitric oxide are expected to reduce the development of contrast nephropathy due to contrast media. We aimed to test the hypothesis that tadalafil reduces the development of contrast nephropathy due to contrast toxicity.

Methods: A total of 24 female Wistar albino rats, three groups of eight, were included in the study. After 48 hours of dehydration, contrast media (meeglumine diatrizoate, 6 mL/kg) was administered to the first group, and contrast media with tadalafil (10 mg/kg) was administered to the second group. The third group served as the control group. Blood and tissue samples were taken 48 hours after this procedure.

Results: Serum cystatin C, serum creatinine and blood urea nitrogen (BUN) values were significantly lower in the contrast with tadalafil group compared to the group given only contrast. Serum and tissue malondialdehyde (MDA) levels were significantly lower in the contrast with tadalafil group than in the contrast only group.

Conclusion: These results demonstrate the protective effect of tadalafil in the prevention of CIN in rats.

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Keywords: contrast-induced nephropathy, nitric oxide, tadalafil, phosphodiesterase-5 inhibitors, cystatin C

Introduction

Contrast-induced nephropathy (CIN) is defined as an acute deterioration in renal function after contrast media exposure. CIN is commonly defined by an elevation in serum creatinine (SCr) levels of 44.2 µmol/L (0.5 mg/dL) or more than 25% of the previous value within 2-5 days after contrast media exposure (1). CIN is an iatrogenic disorder and one of the most common causes of acute renal failure in hospitalized patients (2). In approximately half of patients, CIN occurs after diagnostic and interventional cardiac procedures, and the number of cardiac procedures using contrast media has increasing steadily in recent years (3). There is still no proven method for preventing CIN except for extracellular volume expansion (4).

The direct toxic effect of contrast media on renal tubular cells and renal medullar hypoxia are the main mechanisms

responsible for the pathogenesis of CIN (5). Increased oxidative stress and decreased production of nitric oxide also play important roles in the pathogenesis of CIN (6, 7). Preservation of normal levels of renal cortical and medullar nitric oxide (NO) synthesis may help prevent or lessen contrast-induced renal vasoconstriction (7).

Tadalafil is a long-acting oral drug which inhibits phosphodiesterase enzyme-5 (PDE5). PDE5 hydrolyzes cyclic guanosine monophosphate (cGMP), thus, inhibition of PDE5 with tadalafil increases cGMP levels and nitric oxide mediated vasodilatation (8). Nitric oxide also plays important roles in the pathogenesis of pulmonary arterial hypertension (PAH) and erectile dysfunction (ED). Tadalafil is currently widely used for the treatment of these disorders. Immunohistochemical and reverse transcriptase-polymerase chain reaction analysis have demonstrated the presence of anti-PDE5 antibodies and



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PDE5 transcripts in rat kidney tissue (8). PDE5 inhibitors have been shown to reduce kidney damage in rat renal ischemic-reperfusion models (9, 10). PDE5 inhibitors have also been shown to reduce tubular damage with unilateral ureteral obstruction (11) and to prevent post-cardiopulmonary bypass acute kidney injury in pigs (12). While previous studies have demonstrated that PDE5 inhibitors prevent renal injury in other circumstances, none have shown that PDE5 inhibitors reduce acute renal injury caused by contrast media.

The aim of this study was to investigate the protective effect of long-acting oral PDE5 inhibitor tadalafil in CIN using novel early acute kidney injury marker cystatin C.

Methods

Animals

The experiments were conducted following the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The study included 24 female Wistar albino rats (6 weeks old) with an average weight of 158.88 ± 10.49 g. The rats were born and bred in the Experimental and Clinical Research Center of Gaziosmanpaşa University. The rats were randomly divided into three groups and maintained on a 12-hour light-dark cycle. They were kept in a stainless steel cage at 22-25°C. The study's design and experimental procedures were approved by the Animal Care Committee of Gaziosmanpaşa University (protocol number: 2010/043).

Experimental design

Rats were given unlimited standard rat chow and all rats were deprived of water 48 hours before randomization. At the beginning of the study, experimental animals were marked, and each rat's weight was recorded. After the 48 hour dehydration period, weights were recorded again. Rats were divided into three groups of eight each after dehydration: the first group, the control group (C), underwent no treatment; the second group was injected with contrast media (CM); and the third group was injected contrast media and treated with tadalafil after contrast media (T). Ionic high-osmolar contrast medium (6 mL/kg), meglumine/sodium diatrizoate (Urografin 76%, Schering AG, Germany), was administered intravenously via the tail vein to the CM and T groups after the 48 hour dehydration period. A tadalafil 20 mg tablet (Cialis®, Lilly ICOS LLC, Indianapolis, IN, and Bothell, WA, USA) was converted into a homogeneous solution using a solution preparation machine. The tadalafil solution (10 mg/kg) was administered by oral gavage to group T rats immediately after the administration of contrast. After the procedure, the animals were fed unlimited standard rat chow and water. All animals were sacrificed 48 hours later after procedures. Drug administration, blood sampling and weight measurements were completed between 9:00 and 10:00 a.m. to minimize circadian variation.

Sample collection and biochemical analysis

Blood samples were drawn from the vena cava at the end of the study under general anesthesia using intraperitoneal injec-

tions of xylazine (10 mg/kg; Rompun®, Bayer, İstanbul, Turkey) and ketamine (75 mg/kg; Ketalar®, Pfizer, İstanbul, Turkey). Serum samples were kept at -70°C until analyzed. Serum BUN and SCr levels were measured spectrophotometrically using a Cobas6000 analyzer (Roche Diagnostics GmbH, Mannheim/Germany). Serum cystatin C levels were measured in all groups using the Rat Cystatin C Elisa Kit (Biovendor GmbH, Germany). The Malondialdehyde (MDA) assay was conducted following the method reported by Esterbauer et al. (13). Left kidneys were washed in iced cold NaCl solution (9 gm/L), and samples of 0.4 to 0.5 grams taken from each tissue were homogenized in 20 mM phosphate buffer [pH=7.4 (tissue to buffer ratio, 1:10 w/v)]. To prevent additional sample oxidation, 10 µL of 0.5 M butylated hydroxytoluene per mL of homogenate were added. The homogenate was centrifuged at 4.000 g and 4°C for 10 minutes. To analyze the MDA level in tissue samples, 200 µL of supernatant from each homogenate was used. The MDA measurement was based on the reaction of a chromogenic reagent, and the final MDA content was expressed as µM of MDA per mg protein.

Kidney histology

The right kidneys were preserved in phosphate-buffered 10% formalin, embedded in paraffin wax, cut into 4-5 mm slices, and stained with haematoxylin-eosin. An experienced pathologist who was unaware of the treatment conditions performed the histological evaluation under a Leica DM 200B (Leica DM 2500; Leica Instruments, Nussloch, Germany), light microscope. Histological changes in kidney degeneration, vacuolization of proximal and distal tubules, necrotic changes, and medullary congestion were evaluated by quantitative measurements in randomly selected fields.

Statistical analysis

Analysis were performed with SPSS software version 15.0 for Windows (Chicago, IL, USA). The distributions of the parameters within each group were evaluated using the one sample Kolmogorov-Smirnov test. The parameter distributions were normal for all groups, so parametrical tests were used for analysis. One-way ANOVA was performed, and posthoc multiple comparisons were completed with Bonferroni. Between two independent groups, the Mann-Whitney U test was used for comparison. A p values less than 0.05 were considered statistically significant. Values were expressed as mean±SD.

Results

Rat baseline and follow-up characteristics

The rats tolerated the treatment well, and all survived until the end of the experiment in the C and CM groups. One rat died in the T group on the fourth day of the study prior to sacrifice. Rats in all three groups had similar beginning and post-dehydration weights ($p=0.96$ and $p=0.38$, respectively) (Table 1). All rats experienced significant weight loss after the dehydration period (on average 9%).

Table 1. Initial and post-dehydration rat weights

Weight	Control (n=8)	Contrast (n=8)	Tadalafil (n=8)	P
Initial, g	158.38±15.2	159.75±6.36	158.38±9.42	0.96
After dehydration, g	146.50±7.30	141.25±11.70	147.11±9.19	0.38

Table 2. Renal function parameters in the study groups

	Control (n=8)	Contrast (n=8)	Tadalafil (n=7)*
cysC [†] , mg/dL	1.60±0.29	2.54±0.68	1.61±0.29
BUN [‡] , mg/dL	18±1.82	26±2.42	21±1.03
SCr [§] , mg/dL	0.27±0.04	0.46±0.09	0.32±0.03

BUN - blood urea nitrogen; CysC - cystatin C; SCr - creatinine; P<0.05 was considered statistically significant

*One rat died before sacrifice in the T group. For the comparison between groups more than two, analysis of variance (ANOVA) was applied, and for the post-hoc study, the Bonferroni test was used.

[†]There was a statistically significant difference between the control and contrast groups (P=0.02) and between the contrast and tadalafil groups (P=0.03). No statistically significant difference was found between the control and tadalafil groups (P=1)

[‡]There was a statistically significant difference between the control and contrast groups (P<0.001) and between the contrast and tadalafil groups (P<0.001). A statistically significant difference also occurred between the control and tadalafil groups (P=0.01)

[§]There was a statistically significant difference between the control and contrast groups (P=0.01) and between the contrast and tadalafil groups (P=0.04). No statistically significant difference occurred between the control and tadalafil groups (P=0.92)

Table 3. Oxidative stress marker (MDA) levels in the study groups

	Control (n=8)	Contrast (n=8)	Tadalafil (n=7)*
MDA [†] , nmol/g	4.23±0.75	6.87±1.07	4.98±1.02
MDA [‡] , μmol/L	2.04±0.12	2.61±0.19	2.29±0.24

MDA[†], tissue MDA level; MDA[‡], serum MDA level; P<0.05 was considered statistically significant

*One rat died before sacrifice in the T group

[†]There was a statistically significant difference between the control and contrast groups (P<0.001) and between the contrast and tadalafil groups (P=0.03). No statistically significant difference occurred between the control and tadalafil groups (P=0.432)

[‡]There was a statistically significant difference between the control and contrast groups (P<0.001) and between the contrast and tadalafil groups (P=0.01). No statistically significant difference occurred between the control and tadalafil groups (P=0.056)

Renal function parameters

Serum cystatin C levels were significantly higher in the CM group than in the C and T groups (p=0.002 and p=0.003, respectively). Serum cystatin C levels in C and T groups were not statistically different (p=1.0). BUN levels were significantly higher in the CM group compared to the C and T groups (p<0.001 and p<0.001, respectively). BUN levels were significantly higher in the T group than in the C group (p=0.018). SCr levels were significantly higher in the CM group compared to the C and T groups (p=0.001 and p=0.004, respectively). SCr levels of the C and T groups were not significantly different (p=0.092) (Table 2).

Oxidative stress markers

Serum MDA levels were significantly higher in the CM group compared to the C and T groups (p<0.001 and p=0.013, respectively). Serum MDA levels of the C and T groups were not sig-

nificantly different (p=0.056). Tissue MDA levels were significantly higher in the CM group compared to the C and T groups (p=0.001 and p=0.003, respectively). Tissue MDA levels of the C and T groups were not significantly different (p=0.432) (Table 3).

Histopathological results

No significant histopathological difference between the groups was observed during microscopic examination. Cortical glomeruli showed normal morphology, and similar rates of congestion were observed in the renal medullary layers of the three groups. Sections of the medulla showed collector tubules and calyceal systems of normal morphology.

Discussion

This experimental study is the first study to demonstrate that a long-acting inhibitor of the PDE5 tadalafil may prevent the development of CIN. In this study, unlike other experimental studies, novel acute kidney injury marker cystatin C was used to diagnose CIN. Cystatin C is a reliable marker for early diagnosis and prognosis of contrast-induced acute kidney injury in humans (14, 15). Consequently, the present study showed that cystatin C is a sensitive parameter for detecting acute renal injury in rats due to contrast. At the end of this study, cystatin C, serum BUN and SCr levels were significantly lower in the tadalafil group than in the CM group. Cystatin C and SCr levels were not statistically different between the control and tadalafil groups. Serum BUN levels remained significantly higher in the tadalafil group than in the control group, possibly because BUN levels are more sensitive to dehydration and contrast-induced pre-renal conditions.

Lipid peroxidation due to free oxygen radicals is the most important cause of cell membrane damage and cell destruction. The degree of lipid peroxidation was determined by measuring MDA levels. Previous studies have shown that contrast media increases MDA levels and decreases NO levels in renal tissue (16, 17). Özdeğirmenci et al. (18) showed that tadalafil decreased MDA levels during ischemia reperfusion induced oxidative injury in fetal rat brains. In our study, MDA levels were significantly higher in the CM group compared to the control and tadalafil groups. MDA levels of the control and tadalafil groups were not statistically different. Thus, tadalafil reduced oxidative stress after contrast administration in the kidney tissue.

In our study, we did not observe any of the histopathological changes due to contrast nephrotoxicity reported in earlier experimental studies. In a previous study using a similar contrast agent, rats were dehydrated 4 days before the administration of contrast. In our study, rats were dehydrated 48 hours prior to the administration of contrast (19). In some experimental models, NO and prostaglandin synthesis are inhibited to create the desired contrast nephrotoxicity model (20). In our study, histopathological changes were not observed potentially because tissue samples were taken as early as 48 hours after contrast administration or due to failure of our model to inhibit prostaglandin synthesis to aggravate CIN. In conclusion, no sig-

nificant histopathological difference was identified between groups. Nephrotoxicity due to contrast was shown via biochemical parameters and MDA.

Several experimental models have been used to cause CIN in rats. In some studies, CIN was caused by dehydrating rats prior to the administration of contrast media (21, 22). We used this animal model, and our rats were deprived of water 48 hours before contrast administration, because it is easy to prepare and requires no pharmacological pre-treatment. In a study by Toprak et al. (19), an experimental contrast nephropathy model was created by applying intravenous high osmolarity diatrizoate prior to the dehydration period. In our study, CIN was induced using high dose and high osmolar contrast media meglumine diatrizoate after a 48-hour dehydration period. Tadalafil has been previously tested on models of pulmonary arterial hypertension, ischemic reperfusion in the brain and ovaries, hypoxic brain injury, and penile hypoxic damage. In these studies, the effectiveness of tadalafil was tested using doses of 2-10 mg/kg (18, 23-26). In our study, high doses of tadalafil (10 mg/kg) were used. Long-acting tadalafil is administered as a single dose.

Decreased production of NO and increased oxidative stress play important roles in the pathogenesis of CIN and renal ischemia/reperfusion injury (10, 27). Gasanov et al. (10) demonstrated prevention of renal ischemia/reperfusion injury in rats using tadalafil, but there are no studies on prevention of CIN using PDE5 inhibitors. Erley et al. (28) showed that adenosine antagonists prevent the decline of GFR and renal blood flow in rats with chronic NO deficiency. In several studies, β 1-adrenergic receptor antagonist nebivolol, which has antioxidant and NO mediated vasodilator properties like tadalafil, reduced CIN (29, 30). The beneficial effects of tadalafil in our study may be attributed to the vasodilator and antioxidant effects of NO.

Study limitations

Limitations of this study include that blood and tissue samples were taken a short time after the administration of contrast media. SCr levels are known to reach peak values 48-72 hours after contrast-induced kidney injury and do not reach normal levels for up to 10 days. Changes in renal function parameters and histopathological changes in tissue might have been observed if the study had been longer or immunocytochemistry for inflammatory markers or staining for markers of apoptosis may be helpful for determine histopathological changes. The second limitation of this study was the absence of 24-hour urine analysis. Metabolic cages are generally used for 24-hour urine collection from rats. The use of metabolic cages is expensive and time consuming. In this study, contrast induced acute kidney injury was shown via serum cystatin C levels without the need for 24-hour urine collection.

Conclusion

Efforts are being made all over the world to prevent contrast nephropathy. Nowadays, tadalafil is used to treat erectile dys-

function and pulmonary hypertension. Tadalafil, which has vasodilator and antioxidant properties, may be an alternative treatment to prevent contrast-induced nephropathy in our study. Further studies are needed to evaluate tadalafil for prevention of CIN in humans.

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