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The effect of long-acting PDE-5 inhibitor on renal ischemia/reperfusion injury in Wistar rats; a study on serum cystatin C and renal histopathological findings

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ABSTRACT

Background: Ischemia/reperfusion (I/R) is often a complication of bleeding shock, renal dysfunction and renal vessel operation. Nitric oxide (NO) as an important vasodilator is produced by endothelial cells. NO stimulates the generation of cyclic guanosine monophosphate (cGMP). Phosphodiesterase (PDE) is an intracellular enzyme which hydrolyzes cGMP into an inactive metabolite. It effectively decreases cGMP level. PDE is an intracellular enzyme which hydrolyzes cGMP into an inactive metabolite. It effectively decreases cGMP level. Therefore, an inhibition of PDE can increase cGMP level. PDE5 inhibitor is a compound which inhibits or acts antagonistically against biosynthesis or act of PDE. PDE5 inhibitor is now commonly used for the treatment of pulmonary artery hypertension and erectile dysfunction. According to some latest researches, long-acting PDE5 inhibitor (Tadalafil) reduces renal I/R injury in experiments with Wistar rats.

Objectives: The purpose of this study was to determine the effect of long-acting PDE5 inhibitor on renal I/R injury in Wistar rats.

Materials and Methods: Rats were divided into three groups; sham group, a right nephrectomy was performed. Control group, a right nephrectomy was performed followed by an occlusion on left renal pedicle for 60 minutes and a perfusion was performed for 60 minutes. Tadalafil group; the same treatment was performed as to group control, plus administering tadalafil as a PDE5 inhibitor (10 mg/kg), given by a nasogastric tube 60 minutes before the operation. A left nephrectomy was performed on the mice to determine the value of cystatin C level and histopathology.

Results: The mean necrosis of tubular renal cells indicates that highest mean necrosis of tubular renal cells was at group control (mean score, 8.6 ± 0.84), and the lowest mean necrosis of tubular renal cells was at sham group (mean score, 4.4 ± 0.52) which indicates a significant difference between the sham and control groups ($P < 0.05$). For the tadalafil group mean score of renal tubular necrosis cell was 6.9 ± 1.45 , which also indicates a significant difference between this group with sham group and control ($P < 0.05$). Highest mean cystatin C levels related to group control, mean score was 1.51 ± 0.13 mg/dL, which indicates a significant difference with the sham group ($P < 0.05$), but there is no significant difference with the tadalafil group.

Conclusion: The results of this study showed that the administration of PDE5 inhibitor (tadalafil) improves reperfusion ischemic injury. Although it did not decrease the level of cystatin C, it significantly reduced tubular necrosis.

Original Article

Implication for health policy/practice/research/medical education:

Renal I/R injury may lead to acute kidney injury, a disease that contributes to high mortality rate in humans. PDE5 inhibitor improved significantly the condition of renal I/R injury in Wistar rats.

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1. Background

Acute renal failure (ARF) due to ischemia/reperfusion (I/R) is a serious clinical injury. I/R is often complication of as renal transplantation (1), bleeding shock, cardiac arrest, renal artery reconstructions or suprarenal aneurysms (2,3).

Endothelial dysfunction plays an important role in post-ischemic injury. Although its exact mechanism is not clear, different factors have been suggested in this regard. Nitric oxide (NO) is probably one of the main factors involved in this way. The role of NO as a vascular dilator in vascular tone regulation is well-defined. It also plays an important role in the prevention of I/R injury (4,5). NO stimulates the generation of cyclic guanosine monophosphate (cGMP), not only in vessel but also in renal tubule, including proximal tubule, loop of Henle, and collecting tubules (3). Also cGMP is cGMP-dependent protein kinase (PKG) activator. PKG phosphorylates numerous cellular proteins that causes changes in both activities and functions of kidney, like vasodilatation, anti-inflammation, anti-fibrosis, and anti-apoptosis (6). Phosphodiesterase (PDE) is an intracellular enzyme which hydrolyzes cGMP into an inactive metabolite (6,7). It effectively decreases cGMP level. Therefore, an inhibition of PDE can increase cGMP level. PDE5 inhibitor is a compound which inhibits or acts antagonistically against biosynthesis or act of PDE. PDE5 inhibitor is now commonly used for the treatment of pulmonary artery hypertension and erectile dysfunction (8,9). According to some latest researches, long-acting PDE5 inhibitor (Tadalafil) reduces renal I/R injury in experiments with Wistar rats (10).

Some studies have revealed that cystatin C is more sensitive than serum creatinine in identifying decreases in renal function. In addition, cystatin C is not affected by inflammatory process, sex, diet, and nutrient status. However, given that the substance is relatively new as a biomarker of the decrease of renal function, few studies have been conducted on it (10,11). Hence we will use cystatin C as a biomarker for evaluating renal damage. It is a protein derived from a chain 120 amino acid and produced by a majority of cell nucleus. The gene for cystatin C synthesis is in chromosome 20. Cystatin C is contained in a systemic circulation at a high concentration and considered as an important extracellular inhibitor of protease (11,12).

Based on the above description, the central theme of the present research was that renal ischemia is one of the causes of acute renal failure. Additionally, to assess the damage of tissues, it can be inferred from some parameters like cystatin C levels and histopathological

picture of renal tissue. Long-acting PDE5 inhibitor (Tadalafil) can reduce renal I/R injury in experiments with Wistar rats.

2. Objectives

The aim of this study is to determine the effect of long-acting PDE5 inhibitor (Tadalafil) on renal I/R injury by assessing the parameters of renal tissue damage, namely cystatin C and histopathological picture of kidney.

3. Materials and Methods

The present research conducted at Bandung Eijkman veterinary laboratory, Anatomic Pathology and Clinic Pathology laboratory, Division of Urologic Surgery, FKUP/RSHS Bandung, from July to December 2015. The research was a laboratory-based preliminary research, substantially falling into a basic research of male Wistar rats, 250-300 g weight (13). These rats were housed at a temperature of 23–25°C. They had free access to water and rat chow. They were also acclimatized to their diet for at least one week prior to the experiment. Treatment was performed by an anesthesia and an injection of ketamine 75 mg/kg intraperitoneal. The rats were divided into three groups, each 10 rats. For the sham group, a right nephrectomy was performed. For the control group, a right nephrectomy was performed followed by an occlusion on left renal pedicle for 60 minutes and a perfusion was performed for 60 minutes (13-15). The final group the same treatment was performed as to group control, plus administration of tadalafil (10 mg/kg) solved in a saline solution (Tadalafil group), given by a nasogastric tube 60 minutes before the operation (12,16,17). Toward the end of the research, blood samples were taken from the animal's heart to determine the level of cystatin C also a left nephrectomy was performed for the rats to determine histopathology examination (15,16). The left kidney was fixated by formalin and placed in paraffin and stained by hematoxylin and eosin (H&E). All samples were assessed by a single pathologist. The parameters assessed were glomerular, tubular, and interstitial morphologies and leucocyte infiltration (13).

3.1. Ethical issues

The research was approved by ethical committee of Padjajaran University. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Padjajaran University. The research followed the tenets of the Declaration of Helsinki.

3.2. Statistical analysis

Statistical analysis by one-way analysis of variance (ANOVA) test and followed by Tukey HSD test using SPSS software to compare histopathological findings and cystatin C serum levels between groups and P values <0.05 were statistically significant.

4. Results

The mean necrosis of tubular renal cells indicates that highest mean necrosis of tubular renal cells was at group control (mean score: 8.6 ± 0.84). On the other hand, the lowest mean necrosis of tubular renal cells was at the sham group; 4.4 ± 0.52 . Mean necrosis of tubular renal cells indicated a significant difference between the sham and the control groups ($P < 0.05$). For the tadalafil group, mean score of renal tubular necrosis cell was 6.9 ± 1.45 , which also indicates a significant difference ($P < 0.05$) between this group with the sham and the control groups (Figure 1).

The mean cystatin C levels showed the highest mean cystatin C levels related to control group, 1.51 ± 0.13 mg/dL, which indicated a significant difference with the sham group ($P < 0.05$), but there was no significant difference with the tadalafil group. The lowest mean cystatin C level was at sham group 1.27 ± 0.27 mg/dL, For tadalafil group, mean value was 1.47 ± 0.18 mg/dL (Table 1).

5. Discussion

The most important results of this study, i.e., use of PDE5 inhibitor (tadalafil) improves mean necrosis of tubular renal cells.

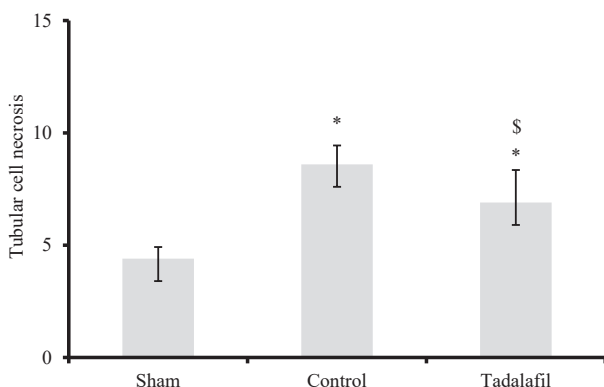


Figure 1. Renal tubular cell necrosis score. Sham group, a right nephrectomy was performed. Control group, a right nephrectomy was performed followed by an occlusion on left renal pedicle for 60 minutes and a perfusion was performed for 60 minutes. Tadalafil group, the same treatment was performed as to group control, plus administration of tadalafil (10 mg/kg). * indicates a significant difference with the sham group and \$ indicates a significant difference with the control group.

Table 1. The mean value of cystatin C for each group

Group	Mean	SD	Minimum	Maximum
Sham	1.27	0.27	0.74	1.58
Control	1.51*	0.13	1.23	1.69
Tadalafil	1.47	0.18	1.17	1.76

Sham group, a right nephrectomy was performed. Control group, a right nephrectomy was performed followed by an occlusion on left renal pedicle for 60 minutes and a perfusion was performed for 60 minutes. Tadalafil group, the same treatment was performed as to group control, plus administration of tadalafil (10 mg/kg). *indicates a significant difference with the sham group.

In acute I/R injury, renal ischemia caused cell damage and cell death by the depletion of cellular energy, where there occurred an accumulation of sodium, calcium, and reactive oxygen in intra-cells, and activation of multiple enzyme systems (18). Though reperfusion is vital in preventing tissue death, it may also cause secondary local damage due to acute inflammatory responses involving a tissue infiltration activated by platelet and polymorphonuclear leukocytes (19). The tissue damage is mediated by cytokine, a local imbalance in nitrite oxide levels, endothelium cell molecule adhesion, platelet activation factor, and free radicals (20). The formation of reactive oxygen at this stadium was one of the causes of tissue damage.

PDE is an intracellular enzyme which hydrolyzes cGMP and guanosine 3,5-cyclic phosphate into an inactive metabolite (adenosine monophosphate and guanosine monophosphate). PDE effectively decreases cGMP level. In addition, the inhibition of PDE5 could increase intra-cell cGMP levels (21). PDE inhibitors available in the market include sildenafil, vardenafil, and tadalafil (one of the latest generations), now commonly used for the treatment of erectile dysfunction (22).

Experimental studies performed by PDE5 inhibitor showed that these substances can promote endothelium function and decrease ischemia areas after the occurrence of a myocardial infarction (23). The protective effect of tadalafil on myocardial infarction in mouse reported by Sesti et al, which detected the cardio-protective effect of PDE5 inhibitor, usually used overcome erectile dysfunction (22). Verit et al showed that tadalafil has an advantageous effect on cardiovascular system by decreasing oxidative stress level in serum (18). It was also shown that administering tadalafil orally (10 mg/kg BB) 2 hours before performing arterial occlusion for 30 minutes produced a smaller size of infarction area than that of other groups (22).

PDE inhibitors are expressed lots in the vessel, platelet, kidney, and other tissues of the body. In a study by Dousa et al (19), PDE5 was localized in glomeruli, mesangial

cell, cortical tubules, and modular collecting duct cells in mouse kidney. It initiated the occurrence of cGMP and an inhibition of PDE5 increased cGMP level (23). The study gave a rise to a question on whether an anti-infarct effect of PDE5 on renal I/R injury is existed (23). To recognize, we investigated the effect of tadalafil, one of the latest generations of PDE5 inhibitor on renal I/R injury, inferred from both cystatin C serum biomarker parameter and renal histopathologic pictures.

Cystatin C is a protease inhibitor expressed by all nucleated cells and excreted by the kidney. This biomarker is independent of muscular catabolism. The concentration of cystatin C plasma is more accurate than creatinine in measuring glomerulus filtration rate in patients with diabetes and cachexia. In addition, unlike creatinine, cystatin C has advantageous characteristics. First, its productive process is quite stable and not affected by non-renal factors, such as protein intake, dehydration, gastrointestinal bleeding, infection, or steroid consumption. In addition, the biochemical characteristics of cystatin C allow free filtration in renal glomerulus, as well as total reabsorption and catabolism by proximal tubules without entering blood circulation. It shows the advantage of cystatin in detecting acute kidney injury (AKI) and it is more sensitive than serum creatinine (22). Cystatin C has also a great clinic potential as a biomarker for acute renal damage. A study involving 85 patients with a severe disease and a risk of an occurrence of acute renal damage found that an increase in cystatin C levels in a serum can be predictive of an occurrence of AKI, 1-2 days before the occurrence of increases in creatinine (22). Therefore, cystatin C indicates a clinical potential as a biomarker to detect AKI. To see a picture of acute ischemia-reperfusion injury, a histopathological examination was also performed in order to evaluate glomerulus, tubular, and interstitial morphologies. The tubular morphology was subdivided into 4 score groups; normal, light abnormal brush border (leveling, irregular shape and size), moderate abnormal brush border (more than 50% tubules are involved), and tubular necrosis (loss of all microvilli and tubules).

Tadalafil differed from both sildenafil and vardenafil in term of pharmacokinetic profile, by halftime of 17.5 hours, maximal plasma concentration was reached 2 hours after being administered orally, and efficacy was going on for 36 hours. These differentiate tadalafil from other PDE5 inhibitors (22). Though both sildenafil and tadalafil were of the same onset, the latter has a longer acting duration than the former (23). In experimental studies conducted with PDE5 inhibitor, it was displayed that the substance decreased significantly ischemia area

after myocardial infarction. In addition, it was found that the size of infarction area was lower in tadalafil group than in either sildenafil or vardenafil (22,23). In addition, in an experimental study using sildenafil, which was a short acting PDE5 inhibitor, the administration with sildenafil before ischemia could decrease the occurrence of renal damages resulting from renal I/R injury (23).

The result of the present research revealed that administration PDE5 inhibitor (Tadalafil) improves significantly the condition of renal I/R damage in term of a parameter of serum marker (cystatin C). This is in agreement with the results of earlier studies showing that PDE5 inhibitor has a protective effect on renal I/R injury.

The present study revealed that a pre-administration with tadalafil can reduce renal damage by a protective effect in renal tubular cells and inhibition of leucocyte infiltration into renal tissues. Therefore, it needs further researches on the effects of long-acting PDE5 inhibitor drugs with a larger sample so as to find more significant results.

6. Conclusions

The results of this study showed that the use of PDE5 inhibitor (tadalafil) improves kidney I/R injury. Although it did not decrease the level of cystatin C, it significantly reduced tubular necrosis.

Authors' contribution

HS provided technical assistance, collection and preparation of the manuscript. TJ designed, supervised the study, and prepared the final draft of the article.

Conflicts of interest

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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