Sildenafil attenuates renal ischemia reperfusion injury by decreasing leukocyte infiltration

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Renal ischemia leads to depletion of cellular energy, accumulation of intracellular sodium, calcium and reactive oxygen species, activation of multiple enzyme systems including proteases, nitric oxide synthetase, phospholipase and endonuclease, resulting in cell damage and death [1]. Reestablishing blood flow to ischemic organs is vital to prevent tissue death. However reperfusion itself causes local injury secondary to an acute inflammatory response that involves tissue infiltration by activated polymorphonuclear leukocytes and platelets. Tissue damage is mediated by cytokines, local imbalance in nitric oxide levels, endothelial-cell adhesion molecules, platelet activating factors, and free radicals.

PDE5 enzyme is localized in the vasculature, platelets, kidneys such as glomeruli, mesangial cells, cortical tubules, inner medullary duct cells. Sildenafil citrate is a potent inhibitor of PDE5 and widely used to treat male erectile dysfunction. Studies have shown that apart from in erectile dysfunction, sildenafil citrate (SC) is also effective in pulmonary hypertension and esophageal motor disorders [2]. Nitric oxide (NO) is a key molecule involved in a variety of physiological and pathological conditions. NO has been shown to act via a variety of second-messenger cascades, although most of its effects are mediated by cyclic guanosine monophosphate (cGMP) [3]. NO stimulates generation of cGMP, not only in the vasculature, but also in the renal tubules, including the proximal tubules, thick ascending limb, and collecting ducts. cGMP has an important role in regulation of intracellular calcium levels and modulation of the platelet function in I/R injury. Although sildenafil does not increase renal blood flow in healthy individuals and in patients with liver cirrhosis and ascite, its effects on renal blood flow after ischemia remain unknown [4]. Sildenafil was shown to improve immediate post-transplant parameters in warm-ischemic kidney transplants without systemic secondary effects [5].

Although many factors contribute to the I/R injury, which is a complex interrelated sequence of events, we questioned whether increasing cGMP levels by inhibiting degradation by phosphodiesterase type 5 (PDE5) inhibitors can lead to a decrease in I/R injury.

Male Wistar albino rats were randomly divided into six groups each with six animals. In sham group, only right nephrectomy was performed. In ischemia group, rats were subjected to right nephrectomy, and occlusion of the left renal pedicle for 45 min with microvascular clamps. In I/R group rats were subjected to right nephrectomy, occlusion of left renal. Group SC+Sham; Group SC+Ischemia; and Group SC+I/R. Tissue samples were rapidly fixed in 10% buffer formalin and Zenker's fluid then stained with hematoxylin and eosin, Masson's trichrome, periodic acid-Schiff reaction (PAS). Renal tubular histopathologic score was also calculated for each rat by including all four regions. Moreover in independent four areas of the sections, neutrophil infiltration was evaluated as 4-point scoring system.

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MPO enzyme concentration as protein in supernatant of tissue homogenate was determined with an ELISA. Thiobarbituric acid reactive substances (TBARS) were also measured as an index of lipid peroxidation. Statistical analyses were used to determine the differences among the four groups.

In the ischemia group sclerosis of glomeruli and enlargement of Bowman's space indicated degenerative changes. The loss of microvilli in the epithelium of proximal tubules and cells with pyknotic nuclei were examined. In the I/R group, renal injury was evident with prominent leukocyte infiltration, glomerular and tubular degeneration. Necrosis in the tubular epithelium of cortex and hyaline degeneration in some areas were observed. In the ischemia group pretreated with sildenafil citrate, stasis was prominent in blood vessels of the inner cortex. Tubular damage in this group was less than that in the ischemia group. However, the difference between ischemia and SC+ischemia groups was not significant (p=0.150). SC+I/R group preserved the normal morphology of the kidney, and showed normal morphology of glomeruli and tubular cells. Stasis, congestion and in some sections hemorrhage were examined in medulla. While some epithelial cells lining the proximal tubules lost microvilli, the remaining epithelial cells lining the same tubule preserved their microvilli. However, tubular damage in the sildenafil pretreated I/R group was significantly decreased in all regions except for medullary rays and was not different from those in sham group in the outer cortex, inner cortex and medullary rays (p=0.125, p= 0.198 and p=0.106 respectively). Infiltration was significantly increased in ischemia and I/R group as compared to the sham group (p=0.03 and p=0.003, respectively). Sildenafil pretreatment resulted in a significant decrease in neutrophil infiltration in the SC+I/R group (p=0.003). The MPO activities or MPO enzyme levels were significantly higher in the I/R group compared to those in the sham (p=0.004 and p=0.037, respectively), and ischemia groups (p=0.004 and p=0.004, respectively), and the differences between the sham and ischemia group were insignificant (p=0.148 and p=0.521).TBARS levels were significantly higher in the ischemia and I/R group than those of the sham group (p=0.025 and p=0.013, respectively). Pretreatment with sildenafil did not change the TBARS levels in ischemia and I/R groups significantly (p>0.05).

Sildenafil pretreatment significantly decreased leukocyte infiltration in I/R group. As expected MPO activity, which is accepted as an indicator of neutrophil infiltration, was significantly higher in the kidney tissue of the I/R group As expected MPO activity, which is accepted as an indicator of neutrophil infiltration, was significantly higher in the kidney tissue of the I/R group than that of the sham group and furthermore this increase was inhibited by sildenafil treatment. In the present study, pretreatment with sildenafil in the I/R group did not result in any change in the lipid peroxidation level. Neutrophils are a potential source of oxygen free radicals however they are not the only source in ischemia I/R process and sildenafil has no documented antioxidant effect. The results of this study suggest that sildenafil citrate pretreatment reduce renal injury by inhibiting neutrophil infiltration in a rat model of renal ischemia-reperfusion. However, further studies are required to confirm this finding and reveal the exact mechanism of action before clinical applications.

1. Y.H. Lien et al., Life Sci. **74** (2003) p543.
2. A.J. Eherer et al., Gut **50** (2002) p 758.
3. A. Friebe et al. Circ. Res. **93** (2003) p 96.
4. B. Degirmenci et al., Urol Int **77** (2006) p170.
5. E. Lıedo-Garcia Transplant Proc. **39** (2007) p1354.