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Iloprost Pretreatment Before Unilateral Nephrectomy: An Experimental Study in Rats

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OBJECTIVE: To evaluate the effects of iloprost administration before unilateral nephrectomy on postoperative interleukin-6 (IL-6), nitric oxide (NO), and oxidant/antioxidant status.
METHODS: Malondialdehyde, glutathione, catalase and Cu-Zn superoxide dismutase were measured in the blood and remnant kidney of Adult male Wistar albino rats to asses oxidant and antioxidant status. The rats were divided into three experimental groups: sham group (S) ($n = 12$); unilateral nephrectomized group (N) ($n = 12$); an hour before unilateral nephrectomy iloprost (1 ng/mL/kg, intraperitoneal) administered group (IN) ($n = 12$).
RESULTS: Iloprost administration before unilateral nephrectomy lowered oxidant parameters and IL-6 levels significantly. NO levels were increased in both N and IN groups.
CONCLUSION: Iloprost pretreatment before unilateral nephrectomy can reduce oxidative stress and IL-6, which increases due to anaesthesia and surgery and causes organ damage during surgery and in the short-term postoperative period. [*Asian J Surg* 2008;31(2):69–74]

Key Words: iloprost, interleukin-6, nitric oxide, oxidative stress, unilateral nephrectomy

Introduction

Many drugs and different techniques are being examined to reduce organ damage due to surgery and to inhibit the stress responses to surgical procedures.[1,2] As an example, in abdominal aortic surgery, the effects of intravenous N-acetylcysteine for the prevention of renal injury in patients with no previously documented renal dysfunction was evaluated and it was found that it did not offer any significant protection from renal injury during elective aortic operation in patients with normal preoperative renal function.[3] Iloprost is a chemically stable derivative of a naturally-occurring prostacyclin. Prostacyclin, which is derived from the endothelium, is a potent vasodilator. Prostacyclin has antithrombotic effects and inhibits activation of neutrophil leucocytes, activates fibrinolysis and impairs erythrocyte flexibility.[4] Several studies have demonstrated protective effects of iloprost.[1]

Unilateral nephrectomy is a surgical procedure that is performed for the treatment of many kidney pathologies, such as renal tumours and trauma or for living kidney donation. Preservation of functional kidney tissue is important for the maintenance of homeostasis.[5] Unilateral nephrectomy could be risky for metabolism and especially for the remaining kidney which has to adapt to the new conditions after unilateral nephrectomy.[6] Removal of one kidney induces some short- and long-term adaptation mechanisms that lead to biochemical and physical changes.

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Some hormonal neuronal functions can take place in this adaptive response.[7] During the adaptation period, some unfavourable consequences can occur. Infection, bleeding and other postoperative complications can be observed.[8]

Oxidant and antioxidant status is vital for regulation of homeostasis. Reactive oxygen species (ROS), namely superoxide and hydroxyl free radicals, together with hydrogen peroxide, are believed to be directly toxic, and ROS can initiate a free-radical-mediated chain reaction that causes additional organ damage.[9] A number of changes follow tissue injury stimulated by cytokines, particularly interleukin-6 (IL-6), which is primarily involved in the regulation of immune and inflammatory responses.[10] It has been demonstrated that plasma IL-6 increases in surgical stress, which is the name given to the hormonal and metabolic changes that occur following surgery such as laparotomy.[11] IL-6 which is a fundamental cytokine function in regulating acute phase response is produced by activated T and B lymphocytes, monocytes/macrophages, endothelial cells, fibroblasts, vascular smooth muscle cells, endothelial cells and locally at the sites of tissue damage, and induces the hepatic synthesis of acute phase plasma proteins.[10–12] Nitric oxide (NO) can mediate a number of physiological and pathological reactions.[13] NO has an important role in kidney function such as the suppression of smooth muscle cell proliferation, renal macrovascular and microvascular dilatation, modulation renal medullary blood flow, simulation of fluid, regulation of mitochondrial respiration and electrolyte balance.[6]

Increased oxidative stress is one of the important factors that causes organ dysfunction during surgery and in the short-term postoperative period.[14] We prefer unilateral nephrectomy to induce surgical stress. The kidney is one of the most sensitive organs whose functions could diminish due to anaesthesia and surgery. The effects of iloprost pretreatment in unilateral nephrectomy on oxidative stress and proinflammatory cytokines are unknown. This study was performed to detect the effects of iloprost administration before unilateral nephrectomy on postoperative oxidative stress, NO and IL-6, which is a proinflammatory cytokine. We evaluated if iloprost pretreatment could reduce the oxidative stress and inflammation that can cause organ damage during surgery. Whole blood and remaining kidney tissue were assed to evaluate systemic and tissue oxidative stress after unilateral nephrectomy. Malondialdehyde (MDA), glutathione (GSH) and Cu-Zn superoxide dismutase (Cu-Zn SOD) were measured in both blood and remnant kidney. Urea, creatinine and plasma IL-6 levels were measured only in blood, and catalase and NO levels were measured only in the remaining kidney.

Methods

This study was performed after approval from the Ethics Committee of the Animal Care Review Board of Istanbul University's Cerrahpasa Medical Faculty, and conducted according to the *Guide for the Care and Use of Laboratory Animals*. Adult male Wistar albino rats obtained from the Experimental Animal Research and Production Laboratory of Cerrahpasa Medical Faculty, weighing 200–250 g, were used. Animals were housed in cages in a regulated environment (23 ± 2°C, 55 ± 15% relative humidity) under a 12-hour light/dark cycle (on 8:00 to 20:00), cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* and permitted *ad libitum* access to standard lab chow and tap water during all experimental procedures (Committee on Care and Use of Laboratory Animals. *Guide for the Care and Use of Laboratory Animals.* Washington, DC: Institute of Laboratory Animal Resources, National Research Council, 1985, page 83). All experiments were performed at the Cerrahpasa Medical Faculty Experimental Animal Research and Production Laboratory. The rats were divided into three weight-matched groups: sham group (S) ($n = 12$); unilateral nephrectomized group (N) ($n = 12$); an hour before unilateral nephrectomy iloprost (1 ng/mL/kg, intraperitoneal) administered group (IN) ($n = 12$).

Surgical procedure

The operations were performed in groups of rats between 9 a.m. and 12 p.m. to standardize the effects of diurnal changes. The left posterior and lateral abdominal wall was cleansed with batticon solution after shaving. Under ketamine chlorohydrate (40 mg/kg, intraperitoneal) anaesthesia, left posterior intercostal incision was performed between the 11th and 12th ribs and the left kidney removed. In the S group, only posterior incision was performed and the kidney was not removed. All of the operations were performed in sterile conditions. The incision was closed with 2/0 silk continuous sutures as a single layer. The rats were sacrificed by decapitation, blood samples were collected by intracardiac puncture, and remnant kidneys

were collected for biochemical analyses after 24 hours from operation. Remnant kidneys were stored at −70°C until analysis.

Biochemical procedure
Blood samples were collected in heparinized vacutainer tubes and immediately transported in a cooler with ice to the laboratory. Serum urea and creatinine levels were measured immediately after collection of the blood samples before plasma separation. On arrival at the laboratory, plasma was separated by centrifugation (+4°C, 3,000 rpm, 10 min) and divided into 0.5–1.0 mL aliquots, placed in cryovials and stored at −70°C until analysis.

Assay of serum urea and creatinine
All animals, rat serum urea and creatinine levels were determined with a commercially available kit (Diasis; Teco Diagnostics, Anaheim, CA, USA).

Assay of catalase
Catalase activity was measured by the breakdown of hydrogen peroxide catalysed by the catalase enzyme.[15]

Assay of MDA
Lipoperoxidation was ascertained by the formation of MDA, which was estimated by the modified thiobarbituric acid method, described by Buege and Aust.[16] Thiobarbituric acid-reactive substance (TBARS) concentration was calculated using 1.56×10^{-5} M cm^{-1} as mol/L extinction coefficient.

Assay of Cu-Zn SOD activity
Cu-Zn SOD activity was determined by the method of Sun et al.[17] The assay involves inhibition of nitroblue tetrazolium (NBT) (Sigma Chemical Co., St Louis, MO, USA) and reduction with xanthine–xanthine oxidase (Sigma Chemical Co.) that is used as a superoxide generator. One unit of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50%.

Assay of tissue GSH
Tissue GSH concentration was determined according to the method of Beutler et al using metaphosphoric acid for protein precipitation and 50-50-dithiobis-2-nitrobenzoic acid for colour development.[18] The total protein concentration of tissues was measured by the method of Lowry et al.[19]

Assay of NO
NO was measured as its stable metabolites nitrate (NO_3^-) and nitrite (NO_2^-). Nitrate was first reduced by nitrate reductase to nitrite and then nitrite was determined spectrophotometrically by the Griess reaction[20] (Roche, Cat No 1 756 281). Plasma and tissue NO concentrations were expressed as μmol/L and μmol/g wet tissue, respectively.

Assay of intraerythrocytic GSH
Reduced GSH concentration was determined according to the method of Beutler et al using metaphosphoric acid for protein precipitation and 5-5′-ditiobis-2-nitrobenzoic acid for colour development.[21] Erythrocyte and tissue GSH concentrations were expressed as mg/g haemoglobin and mg/g protein in kidney tissue, respectively. Haemoglobin concentration was determined by the cyanamethaemoglobin method.[22]

Measurement of IL-6 by ELISA
Rat serum IL-6 levels were analysed by a commercially available ELISA kit (R&D Systems, Abingdon, Oxon, UK). Analyses of all samples, standards and controls were run in duplicate. The principal methodology for this immunoassay was the same for all cytokines. The coefficients of intra- and interassay variations for IL-6 were 4.6% ($n = 7$), 7.4% ($n = 7$), 4.3% ($n = 7$) and 7.1% ($n = 7$), respectively.

Data analysis
The data are presented as mean ± standard deviation. SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Analysis of variance and independent samples *t* test were employed to determine the *p* values between groups. The level of statistical significance was taken as $p < 0.05$.

Results

The serum urea and creatinine levels, plasma IL-6, MDA and intraerythrocytic GSH, and SOD levels are summarized in Table 1. There were no significant differences between the experimental groups' serum urea and creatinine levels. N group serum urea and creatinine levels were increased but were not significant. N group plasma IL-6 levels were significantly higher than S group levels ($p < 0.001$). IN group plasma IL-6 levels were significantly lower than N group levels ($p < 0.05$). There was no significant difference between S and IN groups' plasma IL-6 levels. The plasma MDA levels

Table 1. Urea, creatinine, interleukin (IL)-6, malondialdehyde (MDA), glutathione (GSH) and Cu-Zn superoxide dismutase (SOD) levels

	S ($n=12$)	N ($n=12$)	IN ($n=12$)
Serum urea (mg/dL)	45.00 ± 7.00	46.67 ± 5.03	45.34 ± 5.24
Serum creatinine (mg/dL)	0.53 ± 0.05	0.60 ± 0.001	0.55 ± 0.06
Plasma IL-6 (pg/mL)	749.50 ± 31.65	834.00 ± 41.55*	808.50 ± 33.17†‡§
Plasma MDA (nmol/mg protein)	1.42 ± 0.16	2.09 ± 0.21*‖	1.58 ± 0.23*§
Intraerythrocytic GSH (g/mg protein)	3.57 ± 0.29	2.82 ± 0.15*‖	3.09 ± 0.25†§
Intraerythrocytic Cu-Zn SOD (U/mg protein)	24.23 ± 1.28	24.01 ± 1.61	

*$p < 0.001$; †$p < 0.05$; ‡significant different between S and IN groups ($p < 0.05$); §significant difference between N and IN groups ($p < 0.05$); ‖significant difference between S and N groups ($p < 0.05$). S = sham group; N = unilateral nephrectomized group; IN = an hour before unilateral nephrectomy iloprost administered group.

Table 2. Remnant kidney tissue malondialdehyde (MDA), glutathione (GSH), Cu-Zn superoxide dismutase (SOD), catalase (CAT) and nitric oxide (NO) levels

	S ($n=12$)	N ($n=12$)	IN ($n=12$)
MDA (nmol/mg protein)	0.31 ± 0.05	0.48 ± 0.07*‡	0.29 ± 0.06*§
GSH (g/mg protein)	30.09 ± 6.50	22.23 ± 7.39	32.09 ± 6.50†§
SOD (U/mg protein)	0.97 ± 0.11	0.79 ± 0.12†‡	1.04 ± 0.15†§
CAT (U/mg protein)	18.78 ± 3.96	30.37 ± 4.83*‡	15.10 ± 3.38*§
NO (μmol/g wet tissue)	0.145 ± 0.01	0.156 ± 0.01	0.175 ± 0.01†§

*$p < 0.001$; †$p < 0.05$; ‡significant difference between S and N groups ($p < 0.05$); §significant difference between N and IN groups ($p < 0.05$). There was no significant difference between S and IN groups.

in the N group were significantly higher than in the S group. IN group MDA levels were significantly lower than N group levels. Intraerythrocytic GSH levels in the N group were significantly lower than in the S and IN groups. There were no significant differences between the experimental groups' intraerythrocytic Cu-Zn SOD levels.

Remnant kidney tissue MDA, GSH, SOD, catalase and NO levels are summarized in Table 2. In remnant kidney, N group MDA and catalase levels were significantly higher than in the S and IN groups. IN group NO and GSH levels were higher than in the N group. N group Cu-Zn SOD levels were lower than in the S and IN groups.

Discussion

Cytokine production reflects the degree of tissue trauma; cytokine release is lowest with the least invasive and traumatic procedures. Increased plasma IL-6 levels are observed as a result of tissue damage and high stress conditions.[23,24] It has been suggested that elevated endogenous IL-6 could attenuate renal injury, dysfunction and inflammation after ischaemia-reperfusion injury.[25] It has been documented that high IL-6 level is closely associated with duration of hospitalization after surgery.[23] Increased plasma IL-6 levels were observed as a result of tissue damage and high stress conditions. Czeslick et al suggested that iloprost may down-regulate the intracellular expression of IL-6 and tumour necrosis factor-α in human monocytes.[26] It has been shown that iloprost reduces oxidative damage in various tissues.[4,27] Iloprost pretreatment reduces IL-6 and lipid peroxidation level, which are increased after unilateral nephrectomy.

Compensatory hypertrophy of the remaining kidney occurs after unilateral nephrectomy. The remaining kidney adapts by a compensatory increase in the glomerular filtration rate and renal mass.[28] Increased renal blood flow and glomerular pressure due to unilateral nephrectomy are suggested as the mechanical triggers of compensatory hypertrophy of the remaining kidney.[29] Flow-mediated renal vasodilatation in the remnant kidney that occurs after unilateral nephrectomy is suggested to be NO dependent.[6] NO and prostacyclin are released from endothelium. Endothelial functions play a key role in functional adaptation mechanisms after nephrectomy. Iloprost may protect the endothelium by reducing lipid peroxidation and

support antioxidant response.[30] NO levels in remnant kidney increased after unilateral nephrectomy and iloprost pretreatment in our study. It has been demonstrated that NO release can be evoked by a stable analogue of prostacyclin.[31] The role of NO seems to be controversial because in some models of inflammation, it has been shown that tissue dysfunction or injury could occur after inhibition of NO.[32] However, high production of NO is suggested to be a cause of tissue injury, perhaps through the generation of potent radicals. Studies are not in consensus as to whether NO is cytotoxic or cytoprotective. It may act both as a cytotoxic agent and a cytoprotective agent, the main determinants being its concentration and the environment.[33,34]

Oxidative stress and IL-6 levels increase due to anaesthesia and surgery and cause organ damage during surgery and in the short-term postoperative period. Iloprost pretreatment before unilateral nephrectomy could reduce tissue damage and support the healing process by reducing oxidative stress and inflammation. According to this data, we speculate that iloprost pretreatment could reduce complications during and after surgery in high risk patients who have diabetes, end-stage renal disease or renal tumours, but further studies are needed to clarify this hypothesis. By doing this current study, we showed that iloprost pretreatment before unilateral nephrectomy reduces oxidative stress, forming a sound basis for our further studies.

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