Experimental and Clinical Study of Endothelin-1 in Renal Failure

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Background: We evaluated the role of endothelin-1 in renal failure by an experimental study using rats (study 1), and a clinical study (study 2) that included patients undergoing hemodialysis and patients who had received renal transplantation.

Methods: In study 1, changes in plasma endothelin-1 level in renal function were evaluated in 2 groups of rats. Group 1 (control) received a sham operation on the left kidney and a right nephrectomy, and group 2 (warm ischemic) had their kidneys damaged by 60 minutes of warm ischemia, and a right nephrectomy. In study 2, a comparison was made between the endothelin-1 levels in healthy controls, and in patients with chronic renal failure treated by hemodialysis or by renal transplantation. The changes over time in plasma endothelin-1 levels after surgery were also studied in patients who had received renal transplantation.

Results: In study 1, plasma endothelin-1 levels in group 2 increased, followed by renal insufficiency. In study 2, plasma endothelin-1 levels in patients undergoing hemodialysis were significantly higher than those in patients who had received renal transplantation. The change in plasma endothelin-1 level corresponded to renal function, as measured by BUN and creatinine levels. In patients who had received a renal transplantation, plasma endothelin-1 levels decreased over time, as renal function improved.

Conclusions: The results of these studies suggest that the measurement of plasma endothelin-1 levels is useful as a marker of renal function and improved kidney function after transplantation.

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Endothelin-1 is a potent vasoconstrictor that was detected in cultures of swine aortic endothelial cells. The endothelin-1 molecule, as determined by using the recently developed techniques of advanced genetic engineering, is composed of peptide protein, made up of 21 amino acids. A number of subsequent studies have shown that endothelin-1 has more diverse biologic actions than had been expected. The secretion and metabolism of endothelin-1 were also found to be diverse in nature, and its receptors have been detected in various organs. The most important physiologic action of this compound is vasoconstriction. It acts temporarily or continuously on the smooth muscles of systemic blood vessels. The relationship of this substance to essential hypertension and other types of hypertension in humans has been reported. Its relationship to other vasoactive factors, such as the renin-angiotensin system and atrial natriuretic peptide, has also been studied. The relationship of endothelin-1 to arteriosclerosis has more recently been reported, and close attention has been paid to the possible role of this substance as an exacerbation factor for cardiovascular diseases.

Endothelin-1 has also been found to have various physiologic actions on the kidneys. It is thought to be a major factor involved in the onset of renal failure. However, since endothelin-1 is contained only in small amounts in living subjects, and because it exerts paracrine and autocrine effects, it is difficult to quantify this substance. Thus, the role of endothelin-1 in renal failure has not yet been fully clarified.

To study the relationship between renal failure and endothelin-1, we recently compared plasma endothelin-1 levels and renal function between normal rats and rats with acute renal failure, induced by warm ischemia as a model of renal failure, such as we have previously reported. We also compared plasma endothelin-1 levels between patients with transplanted kidneys and patients with chronic renal failure.
failure on maintenance hemodialysis. In addition, the relationship between plasma endothelin-1 levels and posttransplant renal function was analyzed over time in individuals with transplanted kidneys. Through these analyses, the relationship between renal failure and endothelin-1 was examined.

**MATERIALS AND METHODS**

**Study 1: Plasma Endothelin-1 in Rats with Renal Failure**

Renal failure was induced in rats using the method we previously reported. Sixteen 12-week-old male Fischer rats (Oriental Bio-service, Kyoto, Japan), weighing 200 to 300 g each, were raised in an air-conditioned room where the temperature was kept at 22°C. All animals were allowed free access to a diet of CRF-1 food (Oriental Bio-service) and tap water. Experiments were performed according to the principles of laboratory animal care of the NIH on the care and use of laboratory animals.

The animals were divided into 2 groups: a control group and a warm ischemic group. The control group consisted of 8 rats that underwent a median incision of the abdomen under general anesthesia with intraperitoneal pentobarbital (5 mg/kg). The left renal artery was freed, the right kidney was removed, and the wound was closed to complete the sham operation. In the warm ischemia group, under general anesthesia using the method used for the control group, the left renal artery of each of 8 animals was freed and clamped with a mini-clip for 60 minutes. After the clamp was released, the right kidney was removed, and the wound was closed to complete the operation.

After surgery, all rats in each group were observed for 14 days. During the follow-up period, animals were allowed free access to water and food. On the 14th day after surgery, renal function was assessed, accompanied by measurement of plasma endothelin-1 levels. As indicators of renal function, we measured inulin clearance, which reflects glomerular function, as well as p-hippuric acid clearance, which reflects renal blood flow.

**Measurement of inulin and p-hippuric acid clearance**

According to the method we previously described, polyethylene tubes were inserted into the external carotid artery and jugular vein and the left ureter. Inulin (Sigma Chemical, St. Louis, MO, USA) and p-hippuric acid (Dai-ichi Pharmaceutical, Tokyo, Japan) were introduced via the external jugular vein in amounts of 3 mg and 1.6 mg, respectively. These were given as a bolus injection, 30 minutes before the start of measurement. Subsequently, these 2 agents were infused continuously at a rate of 12 mg/100 g body weight per minute and 2.4 mg/100 g body weight per minute, respectively. Blood was sampled from the external carotid artery, and urine was collected from the ureter. During measurement, blood pressure was monitored using an arterial pressure monitor attached to the external carotid artery. Any time blood pressure decreased to below 80 mm Hg, a 0.9% saline solution was infused intravenously to increase blood pressure until it was under control.

**Inulin measurement**

The anthrone method was used to measure inulin levels in the blood and urine. Supernatant of the serum, centrifuged in 0.3 N perchloric acid to remove protein, urine diluted 100 to 1000 times, and reference inulin solution was combined with concentrated sulfuric acid and anthrone reagent (Wako Pure Chemical, Tokyo, Japan). This mixture was incubated at 56°C for 10 minutes and immediately cooled at 4°C, and measured by colorimetry at 636 nm, using a spectrometer (model 150-200; Hitachi Seisakusho, Tokyo, Japan). Inulin levels were calculated by referring to the reference curve of the standard sample of inulin.

**p-Hippuric acid measurement**

Serum was combined with trichloroacetic acid and left standing at room temperature for 10 minutes. Protein-free supernatant was harvested after 15 minutes' centrifugation. Each urine sample was diluted 100 to 1000 times before analysis. Each sample (urine and serum) or reference p-hippuric acid solution was combined with 2 N concentrated hydrochloric acid (HCl) and sodium nitrite. The mixture was incubated for 3 minutes at room temperature, then combined with urea and agitated adequately. The mixture was left standing for 30 minutes. After combining with Tsuda's reagent, 1-(β-diethylaminomethyl) α-naphthylamine oxalate (Wako Pure Chemical), it was left standing for an additional 30 minutes. The sample was then subjected to colorimetry at 570 nm to determine p-hippuric acid levels, using the reference curve.

**Biochemistry**

In study 1, serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, and lactate dehydrogenase were measured. Serum urea nitrogen and creatinine were also analyzed as indicators of renal function.

**Study 2: Plasma Endothelin-1 in Patients Undergoing Hemodialysis and Renal Transplant Patients**

Plasma endothelin-1 levels were determined in a prospective cohort study of patients undergoing hemodialysis and patients after renal transplantation, in comparison to healthy adult volunteers.