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# ENDOTHELIN-1 AND ENDOTHELIN-3 PLAY DIFFERENT ROLES IN ACUTE AND CHRONIC ALTERATIONS OF BLOOD PRESSURE IN PATIENTS WITH CHRONIC HEMODIALYSIS

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SUMMARY: We measured plasma concentrations of endothelin-1 (ET-1), ET-3 and big ET-1 by sandwich-enzyme immunoassays in patients (Pt) with chronic hemodialysis (HD) (Pt-HD, n=23) and age-matched normal subjects (NS, n=17). In Pt-HD, plasma levels (before HD) of ET-1, ET-3 and big ET-1 were significantly higher than those in NS. Reverse-phase HPLC analysis indicated that plasma concentrations of ET-1, ET-3 and big ET-1 in both Pt-HD and NS can be precisely measured by these sandwich-enzyme immunoassays. In Pt-HD, although the plasma ET-3 or big ET-1 levels did not significantly correlate with blood pressure (BP), plasma ET-1 levels significantly ($p<0.01$) correlated with both the levels of systolic ($r=0.63$) and diastolic ($r=0.54$) BP. After 4-hour HD, the plasma level of ET-3, but not ET-1 or big ET-1, was significantly elevated and BP was significantly lowered. The present findings indicate that ET-1 and ET-3 play different roles in acute and chronic alterations of BP in Pt-HD. 

Endothelin-1 (ET-1) has potent and extremely long-lasting vasoconstrictor effects (1). Several studies have demonstrated that humans and other mammals produce three distinct members of this peptide family, ET-1, ET-2 and ET-3, which may have different profiles of biological activity on vascular and non-vascular tissues (2,3). Although ET-1 is produced by vascular endothelial cells, these cells do not produce

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Abbreviations: ET-1, endothelin-1; ET-2, endothelin-2; ET-3, endothelin-3; HPLC, high performance liquid chromatography; sandwich-EIA, sandwich-enzyme immunoassay.

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ET-3 (2-4). The actual presence of ET-3 in porcine brain has indicated that ET-3 may be a novel neuropeptide (5). Thus, ET-1 and ET-3 are considered to possess different physiological roles.

Although increased plasma endothelin-like immunoreactivity was reported in hemodialysis patients (6), the constitution of these endothelins in the plasma of hemodialysis patients is unclear. We have recently established sandwich-enzyme immunoassays (sandwich-EIAs) for ET-1 (7,10), ET-3 (8) and big ET-1 (a precursor of ET-1) (9,10). In the present study, we measured the plasma levels of ET-1, ET-3 and big ET-1 in patients undergoing chronic hemodialysis and normal subjects by these sandwich-EIAs. Furthermore, the precision of detecting the immunoreactive ET-1, ET-3 and big ET-1 by these sandwich-EIAs in the plasma from both hemodialysis patients and normal subjects was verified by reverse-phase high performance liquid chromatography (HPLC).

## Materials and Methods

Blood samples Blood samples were collected from antecubital veins of male patients undergoing chronic hemodialysis (n=23, 46 ± 1.6 years old) and age-matched male healthy subjects (n=17, 45 ± 1.8 years old). Each blood sample was put in chilled tubes containing aprotinin (300 KIU/ml) and EDTA (2 mg/ml) and then centrifuged at 2000 x g for 15 min at 4 °C. The plasma was stored at -30 °C until used.

Extraction procedure Plasma (1 ml) was acidified with 3 ml of 4 % acetic acid, and immunoreactive ET-1, ET-3 and big ET-1 were extracted with a Sep-pac C-18 cartridge (Waters Associates, Milford, MA) as previously described (7-9). The elutes were reconstituted with 0.25 ml of assay buffer and subjected to the EIAs.

Sandwich-EIAs Sandwich-EIA for ET-1 was carried out as previously described using immobilized mouse monoclonal antibody AwETN40, which recognizes the N-terminal portion of ET-1, and peroxidase-labeled rabbit anti-ET-1 C-terminal peptide(15-21)Fab' (7,10). In the sandwich-EIA for ET-3, the monoclonal antibody AET-30, which recognizes N-terminal loop domain of ET-3, was used as an immobilized capture antibody (8). An antibody against the C-terminal heptapeptide of ET-3, the sequence common to other endothelins, was elicited in rabbits by immunizing them with ET-3(15-21)-BSA conjugates (8). The Fab' fragment of this rabbit antibody was used as an enzyme-labeled detector antibody after being coupled with horseradish peroxidase (8). Similarly, in the sandwich-EIA for big ET-1, immobilized AwETN40 and peroxidase-labeled rabbit anti-human big ET-1 C-terminal peptide(22-38)Fab' was used (9). The assay for ET-1 did not cross-react with ET-3 or big ET-1 (cross-reactivity: < 0.1%). The assay for ET-3 did not cross-react with ET-1, big ET-1 or big ET-3 (cross-reactivity: < 0.1%). The detection limits of these assays were 0.4 pg/ml for ET-1 and big ET-1 and 0.2 pg/ml for ET-3 (7-10).

Reverse-phase HPLC Plasma samples from both the hemodialysis patients (45 ml) and normal subjects (45 ml) were diluted twice with acetic acid/ethanol/H2O (= 6/20/74) and applied to a YMC-GEL, ODS-AM 120-S50 column (12 x 50 mm, Yamamura Chemical Labs., Ltd., Japan). The adsorbed materials were eluted with acetic acid/ethanol/H2O (= 4/86/10) and lyophilized. They were separated on a TSK ODS-80 column (4.6 x 250 mm, TOSOH, Co., Ltd., Japan) with increasing concentration of CH3CN at a flow rate of 1 ml/min. Each fraction (0.5 ml) was lyophilized, reconstituted with the EIA buffer, and subjected to the EIAs for ET-1, ET-3 and big ET-1.

Statistics Values are expressed as mean ± S.E.M. Statistical analysis was carried out by the Student's t-test for paired or unpaired values. The plasma endothelin concentrations and the levels of blood pressure were compared using least-squares regression analysis and Pearson's correlation coefficient. A p value of $< 0.05$ was accepted as the significance of the difference.

## Results

In patients with chronic hemodialysis, plasma levels (before hemodialysis) of ET-1, ET-3 and big ET-1 were significantly higher ($p < 0.01$) than those in normal subjects (Fig. 1). In patients undergoing hemodialysis, the plasma levels of ET-1, but not those of ET-3 (Fig. 2) or big ET-1 (data not shown), were significantly ($p < 0.01$) correlated with both the levels of systolic ($r=0.65$, Fig. 2) and diastolic ($r=0.58$) blood pressure. In normal subjects, no significant correlation was observed between plasma endothelin levels and blood pressure. After 4-hour hemodialysis, the plasma level of ET-3, but not ET-1 or big ET-1, was

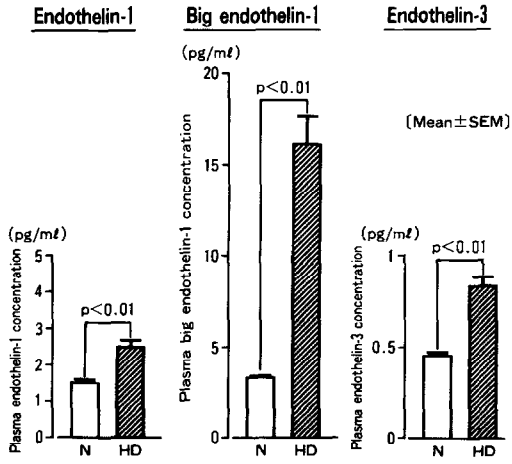


Fig. 1. Plasma concentrations of ET-1, big-ET-1 and ET-3 in normal subjects (open columns) and hemodialysis patients (hatched columns). N: Normal subjects HD: Patients with hemodialysis.

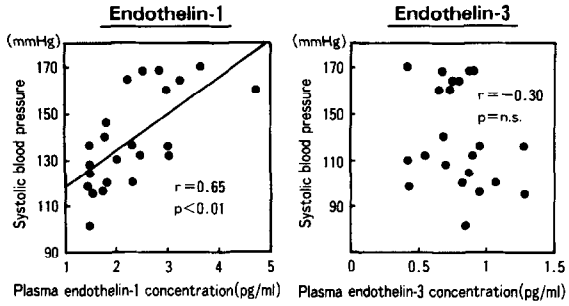


Fig. 2. Correlation between plasma ET-1 or ET-3 concentrations and the levels of systolic blood pressure in hemodialysis patients.

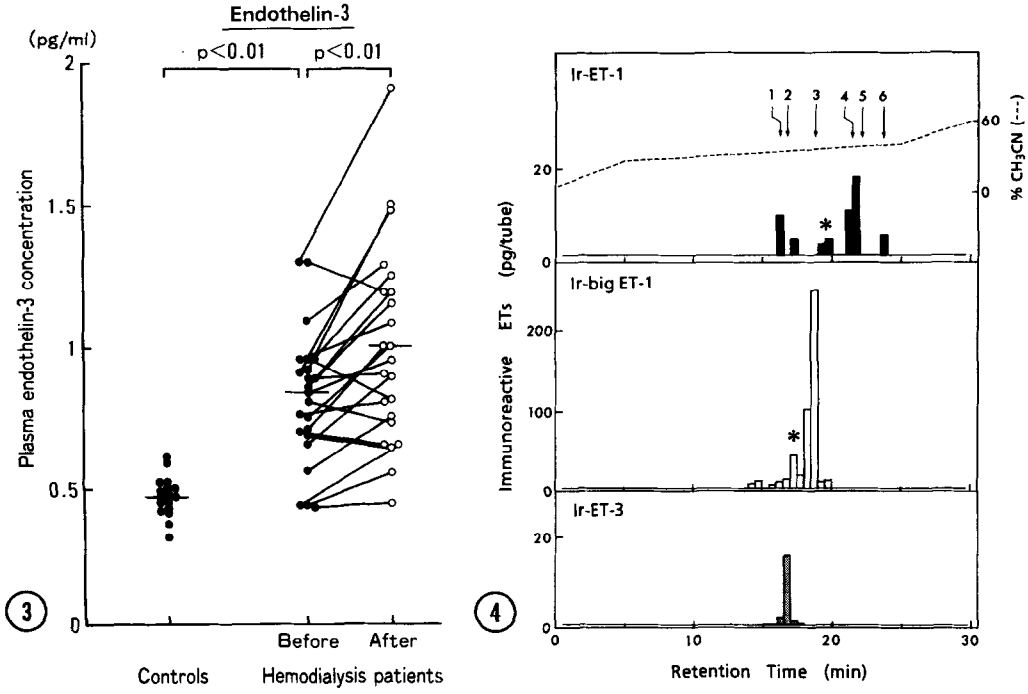


Fig. 3. Plasma ET-3 concentrations before and after 4-hour hemodialysis in hemodialysis patients and healthy subjects. The horizontal bar represents the mean of each group.

Fig. 4. Reverse-phase HPLC profiles of immunoreactive ET-1, big ET-1 and ET-3 extracted from the plasma of hemodialysis patients.

Major immunoreactivities appeared at the positions of authentic ET-1, big ET-1 and ET-3.

Arrows indicate the elution positions of the respective authentic endothelins. 1 = big ET-3, 2 = ET-3, 3 = big ET-1, 4 = ET-1, 5 = big ET-2, 6 = ET-2.

Asterisks show the elution positions of Met-sulfoxide forms of ET-1 (upper figure) and big ET-1 (middle figure).

Ir-ET-1: immunoreactive ET-1
Ir-big ET-1: immunoreactive big ET-1
Ir-ET-3: immunoreactive ET-3
ETs: endothelins

significantly elevated (1.10 ± 0.08 pg/ml, $p < 0.01$, Fig. 3) and blood pressure was significantly reduced (reduction in systolic blood pressure = 25.3 ± 3.2 mmHg, n=23, $p < 0.01$). Reverse-phase HPLC analysis indicated that these sandwich-EIAs certainly measured ET-1, ET-3 and big ET-1 in the plasma of both normal subjects (data not shown) and hemodialysis patients (Figure 4).

## Discussion

The elevation in plasma levels of ET-1, ET-3 and big ET-1 in hemodialysis patients might be partly attributed to the decreased clearance of these endothelins due to renal insufficiency. Since the vascular endothelial cells do not produce ET-3 (2-4), the significant correlation between levels of blood pressure and those of ET-1, but not ET-3, may suggest that the production of ET-1 in endothelial cells might be increased in the state of high blood pressure in hemodialysis patients. Since there was no significant correlation between plasma ET-1 levels and blood pressure in normotensive healthy subjects, the function of endothelial cells in the production of ET-1 in hemodialysis patients may be different from that in normal individuals. Inasmuch as ET-3 may be a neuropeptide (5), the acute elevation in plasma level just after the hemodialysis may be partly due to the neuronal response, e.g. a reflex caused by acute reduction in blood pressure.

Although the exact origin of each of the circulating endothelins has yet to be elucidated, the different alterations of plasma levels suggest that ET-1 and ET-3 may play different roles in acute and chronic alterations of blood pressure in hemodialysis patients. Since plasma endothelin-like immunoreactivity has been reported to be elevated in various states in humans (10-12), the present findings indicate that it is important to measure separately the concentration of each endothelin when studying the physiological/pathophysiological roles of endothelins in human diseases.

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