

## Increased Plasma Endothelin-1 in Acute Ischemic Stroke

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**Background and Purpose:** Endothelins are a recently discovered group of most powerful vasoconstrictor peptides. Endothelin-1 is produced by endothelial cells, and endothelin-3 is derived from neuronal tissue. Theoretically, endothelin-mediated vasoconstriction may enhance ischemic neuronal damage. This study aimed to measure plasma levels of both endothelins in patients with acute nonhemorrhagic cerebral infarction.

**Summary of Report:** Plasma levels of endothelin-1 and endothelin-3 were measured by radioimmunoassay in 16 consecutive patients within the first 72 hours after the onset of nonhemorrhagic cerebral infarct, as diagnosed clinically and by computed tomography. There was a marked (fourfold) elevation in plasma endothelin-1 levels in the patients (median, 11.7 pg/ml; 25th and 75th centiles, 5.4 and 13.2 pg/ml) compared with those in a control group of 13 age-matched subjects (median, 2.56 pg/ml; 25th and 75th centiles, 2.4 and 3.0 pg/ml;  $p < 0.0001$ ). The first 24 hours after stroke onset were associated with higher endothelin-1 levels, and there was a trend to elevated levels with more severe neurological deficits. In all patients and controls endothelin-3 levels were below 0.5 pg/ml.

**Conclusions:** Ischemic stroke is associated with acute and marked increases in plasma levels of endothelin-1. This may reflect enhanced production by damaged endothelial cells within the infarcted tissue. Local leakage of endothelin-1 may induce severe and prolonged constriction of collateral vessels and may therefore have a deleterious role in the pathogenesis and final outcome of cerebral infarction. (*Stroke* 1992;23:1014-1016)

**KEY WORDS** • cerebral ischemia • endothelin

**E**ndothelin-1 (ET-1) is a recently discovered endothelium-derived peptide that seems to be the most potent vasoconstrictor yet identified.<sup>1</sup> Cerebral microvessels show marked sensitivity to ET-1<sup>2</sup> and produce this substance.<sup>3</sup> The ET-1 concentration is elevated in the cerebrospinal fluid of patients with subarachnoid hemorrhage, suggesting the involvement of this peptide in the generation of cerebral vasospasm.<sup>4</sup> Endothelin-3 (ET-3) is structurally and functionally similar to ET-1 but is mostly detected in, and probably produced by, neural tissue.<sup>5</sup> Theoretically, in acute cerebral ischemia there might be increased secretion of ET-1 and ET-3 from damaged endothelial and neuronal cells, respectively. The resultant severe and prolonged local vasoconstriction may play a role in the pathogenesis and outcome of the acute cerebral injury. We therefore measured plasma concentrations of both ET-1 and ET-3 in patients during the acute phase of ischemic stroke.

### Subjects and Methods

#### Patients

Sixteen consecutive patients with acute stroke were included in the study. There were eight men and eight women, mean age 70, range 40-90 years. All patients were admitted with the sudden onset of a focal neurological deficit due to acute nonhemorrhagic cerebral infarction, as verified by computed tomography (CT). Fifteen patients had hemiparesis or hemiplegia; in five patients right-sided weakness was associated with dysphasia, and one patient presented with homonymous hemianopsia. In seven patients the neurological deficit was defined as mild to moderate; these patients had an initial Canadian Neurological Scale<sup>6</sup> score of 6.5-8.0 and were characterized by significant improvement of their neurological status within the first 72 hours after onset. In the other nine patients the neurological deficit was defined as severe; the initial Canadian Neurological Scale score was 2.5-5.0, and there was no evidence of short-term improvement.

In all patients CT performed within 7 days after stroke onset ruled out hemorrhage. CT demonstrated a lacunar subcortical infarct in six patients and a cortical infarct in six patients. In the remaining four patients CT performed 1-4 days after onset did not demonstrate the acute infarction. The demonstrated cortical infarcts were in the territory of the middle cerebral artery (MCA); one infarct correlated with MCA stem occlusion, four were in the territory of the MCA superior

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division, and one was in the territory of the MCA inferior division.

Plasma endothelin levels were measured in all patients within 72 hours after onset of the neurological deficit. All patients were hemodynamically stable; there were signs of neither concomitant infection nor cardiac ischemia.

The control group consisted of 13 age-matched patients (mean age, 69; range, 43–89 years). All controls had nonvascular disorders; three had Parkinson's disease, two multiple sclerosis, two acute myelorradiculitis, two spastic quadriplegia due to cervical spondylosis, one motor neuron disease, two duodenal ulcer, and one an acute exacerbation of asthma.

In all subjects blood was drawn from the cubital vein after at least 30 minutes in recumbency, transferred to a chilled tube containing 500 units/ml edetic acid and aprotinin (Trasylol, Bayer Co.), and centrifuged at 3,000 rpm for 15 minutes at 4°C. The plasma was stored at –70°C until assayed.

#### Endothelin Extraction Procedure

Six milliliters of plasma was acidified by 12 ml of 4% acetic acid in water and applied to a C<sup>18</sup> Sep-Pak cartridge (Waters Chromatography Division, Milford, Mass.). Each cartridge was washed with 15 ml of 4% acetic acid, and endothelin was then eluted with 2.5 ml of 60% acetonitrile in 0.5% ammonium acetate. The samples were then dried by evaporation in a SpeedVac apparatus (Savant, Farmingdale, N.Y.). The dried fractions were reconstituted with 0.25 ml of the radioimmunoassay buffer.

Two experiments were performed to test the efficiency of the endothelin extraction procedure. First, iodine-125-labeled ET-1 (13,000 cpm) was mixed with increasing amounts of unlabeled ET-1 (128 pg in 3-ml fractions of 4% acetic acid). Each fraction was then applied to a C<sup>18</sup> Sep-Pak cartridge. The cartridge was then washed with the same buffer, and ET-1 was eluted with 2.5 ml of 60% acetonitrile containing 0.5% ammonium acetate. The mean±SD recovery of radioactive material was found to be 75±5%. In the second experiment 56 pg ET-1 was added to a sample of control plasma (6 ml) that was then processed by following a standard extraction procedure. After removal of the acetonitrile by evaporation, the sample was divided into two duplicates and the amount of ET-1 was determined. In a representative assay, 30 and 28 pg were detected in each tube. Assuming an endogenous level of 5 pg ET-1 per tube, the above figures represent a recovery of exogenous ET-1 of about 90%. The intra-assay coefficient of variation for the extraction was 8%.

#### Radioimmunoassay Procedure

Radioimmunoassay was performed with Peninsula Laboratories (Belmont, Calif.) ET-1 and ET-3 kits (RIK-6901). Duplicate samples of 0.1 ml were assayed according to the manufacturer's instructions. Cross-reactivities of the assay (according to the manufacturer's specifications) for ET-1, endothelin-2, ET-3, and big endothelin are 100%, 7%, 7%, and 17%, respectively.

#### Statistical Analysis

Endothelin levels are expressed as median±(25th, 75th centiles), and the significance of differences be-

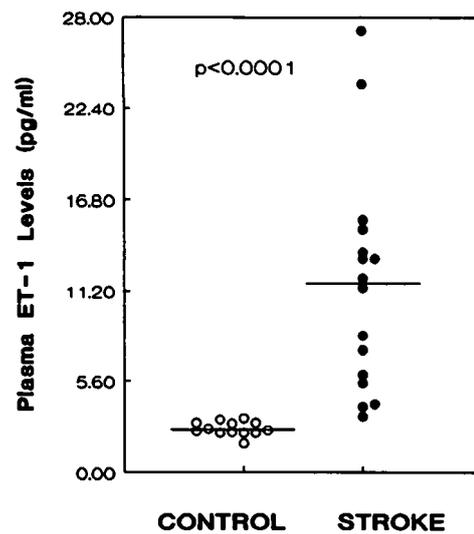


FIGURE 1. Scatterplot of peripheral venous plasma levels of endothelin-1 (ET-1) in controls (○) and stroke patients (●) during first 72 hours after onset of acute ischemic stroke. Horizontal lines mark medians.

tween groups was evaluated by using the Mann-Whitney *U* test. Analysis of blood pressure values revealed a normal distribution of the results, which are therefore expressed as mean±SD with evaluation of intergroup differences by using Student's *t* test.

#### Results

ET-1 levels in the controls were 2.56±(2.4, 3.0) pg/ml. There was a marked (fourfold) increase in plasma ET-1 levels in the patients, 11.7±(5.4, 13.2) pg/ml ( $p < 0.0001$ , Figure 1). The ET-1 levels measured during the first day after the stroke were significantly higher than those measured later, 14.3±(8.4, 23.6) versus 6.5±(4, 11.2) pg/ml, respectively ( $p = 0.01$ , Figure 2).

In the subgroup of nine patients with a severe neurological deficit CT demonstrated cortical infarction in

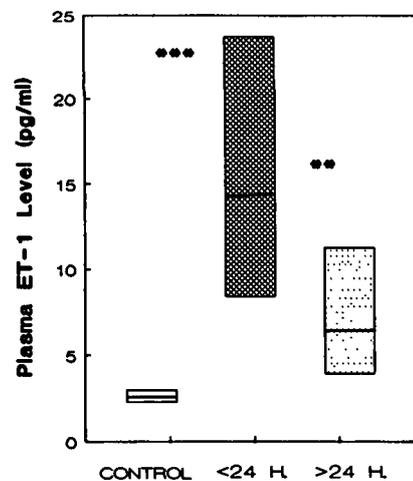


FIGURE 2. Bar graph of median±25th and 75th centiles of plasma endothelin-1 (ET-1) levels in controls ( $n = 13$ , open bars) and stroke patients during first 24 hours after stroke onset ( $n = 9$ , cross-hatched bars) and 24–72 hours after onset ( $n = 7$ , shaded bars). \*\* $p = 0.01$  different from <24 H. \*\*\* $p < 0.0001$  different from controls.

six (median ET-1 level, 13.0 pg/ml) and lacunar infarction in three (median ET-1 level, 14.2 pg/ml). In the seven patients with a mild to moderate neurological deficit the CT scan was normal in four (median ET-1 level, 8.4 pg/ml) and showed lacunar infarction in three (median ET-1 level, 10.1 pg/ml). ET-1 levels in the patients with more severe neurological impairment tended to be higher than levels in the patients with milder disorders. This trend, however, did not reach statistical significance. There was no significant difference between ET-1 levels in the patients with lacunar versus cortical infarction ( $12.2 \pm [7.7, 20.4]$  and  $11.3 \pm [6.1, 19.8]$  pg/ml, respectively).

Mean arterial blood pressure at the time of ET-1 measurement was  $115.0 \pm 11.1$  mm Hg in the patients and  $107.1 \pm 9.7$  mm Hg in the controls ( $p < 0.05$ ). There was no significant intragroup correlation between ET-1 levels and either age or arterial blood pressure of the patients.

In all subjects, plasma ET-3 levels were below the detection threshold of the assay ( $< 0.5$  pg/ml).

### Discussion

This study shows that acute ischemic stroke is associated with marked elevations of plasma ET-1 levels. These increases were more pronounced during the first 24 hours after stroke onset and tended to correlate with the severity of neurological deficit.

The cause for the stroke-induced increases in plasma ET-1 levels is yet unclear. The phenomenon may represent a nonspecific enhancement of ET-1 production by systemic vascular endothelium in response to general stress<sup>7</sup> associated with the acute cerebral infarction. For instance, epinephrine is known to induce ET-1 release.<sup>8</sup> The fact that lacunar infarcts were associated with ET-1 levels similar to those of cortical infarcts may support this possibility. Alternatively, the observed plasma ET-1 elevations may originate predominantly from the central nervous system. In the area of cerebral infarction there is damage not only to neuronal tissue but also to local blood vessels. The ischemic insult may cause increases in the production of ET-1 by as well as leakage of ET-1 from injured endothelial cells of the involved cerebral microvessels. Hypoxia is known to stimulate ET-1 synthesis.<sup>9</sup> In addition, reduction of perfusion pressure with resultant decreases in shear stress on the endothelial cells has also been shown to increase ET-1 production.<sup>1</sup> Elevated thrombin concentrations within the ischemic region may also contribute to the induction of ET-1 release.<sup>8</sup>

Elevations of ET-1 levels may be an important factor in the pathogenesis and final outcome of cerebral ischemia. ET-1 is a powerful vasoconstrictor substance.<sup>1</sup> Its potency exceeds 10 times that of angiotensin II, vasopressin, or neuropeptide Y.<sup>1</sup> Cerebral arteries are extremely sensitive to exposure to ET-1, which was found to be even more potent than a maximally depolarizing potassium solution.<sup>2</sup> Intracisternal injection of ET-1 in vivo causes severe and long-lasting contraction of the basilar artery.<sup>10</sup> The relatively high ET-1 levels that are normally found in the cerebrospinal fluid<sup>11</sup> rise further following subarachnoid hemorrhage<sup>4</sup> and may contribute to the development of vasospasm. It is also possible that the increased peripheral plasma ET-1

levels reflect much higher endothelin concentrations at the site of the cerebral infarct. ET-1 concentrations at the interface of the endothelium and smooth muscle cell layers are known to be higher than those in the bloodstream.<sup>8,12</sup> Circulating endothelin has been shown to be biologically active,<sup>13</sup> and endothelial injury greatly potentiates the susceptibility of blood vessels to the vasoconstrictor effect of ET-1.<sup>14,15</sup> Our finding of increased plasma levels of ET-1 despite its very rapid elimination from the blood (60% within 1 minute, mainly through the lung<sup>8</sup>) further suggests that substantial increases in ET-1 production may be involved.

Enhanced levels of ET-1, either produced locally or derived from the systemic circulation, within and at the boundaries of the area of cerebral ischemia may therefore be deleterious to the already-injured neuronal tissue. This situation is similar to that in myocardial infarction, in which elevated plasma ET-1 levels have been reported.<sup>7,15</sup> Mainly, ET-1 may cause constriction of collateral vessels and contribute to a vicious circle, with further reduction in regional blood flow, enhancement of the severity and size of the infarcted tissue, and worsening of the neurological outcome. If such a sequence of events proves valid, the development of pharmacological strategies to modify the production and activity of this potent vasoconstrictor peptide may be beneficial in improving the outcome of brain ischemia.

### References

1. Yanagisawa M, Kurihara S, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Goto K, Masaki T: A novel potent vasoconstrictor peptide produced by vascular endothelium. *Nature* 1988;332:411-415
2. Hardebo JE, Kahrstrom J, Owman C, Salford LG: Endothelin is a potent constrictor of human intracranial arteries and veins. *Blood Vessels* 1989;26:249-253
3. Yoshimoto S, Ishizaki Y, Sasaki T, Yoshizumi M, Yanagisawa M, Yazaki Y, Masaki T, Takakura K, Murota S: Cerebral microvessel endothelium is producing endothelin. *Brain Res* 1990;508:283-285
4. Suzuki H, Sato S, Suzuki Y, Takekoshi N, Ishihara S, Shimoda S: Increased endothelin concentration in the CSF of patients with subarachnoid hemorrhage. *Acta Neurol Scand* 1990;81:553-554
5. Inoue A, Yanagisawa M, Kimura S, Kasuya H, Miyauchi T, Goto K, Masaki T: The human endothelin family: Three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc Natl Acad Sci U S A* 1989;86:2863-2867
6. Côté R, Hachinski VC, Shurrvell BL, Norris JW, Wolfson C: The Canadian Neurological Scale: A preliminary study in acute stroke. *Stroke* 1986;17:731-737
7. Miyauchi T, Yanagisawa M, Tomizawa T: Increased plasma concentrations of endothelin-1 and big endothelin-1 in acute myocardial infarction. *Lancet* 1989;2:53-54
8. Vane JR, Anggard EE, Botting RM: Regulatory functions of the vascular endothelium. *N Engl J Med* 1990;323:27-36
9. Highsmith RF, Pang DC, Rappoport RM: Endothelial cell-derived vasoconstrictors: Mechanisms of action in vascular smooth muscle. (abstract) *J Cardiovasc Pharmacol* 1989;13(suppl 5):S36
10. Mima T, Yanagisawa M, Shigeno T, Saito A, Goto K, Takakura K, Masaki T: Endothelin acts in feline and canine cerebral arteries from the adventitial side. *Stroke* 1989;20:1553-1556
11. Hoffman A, Keiser HR, Grossman E, Goldstein DS, Gold PW, Kling M: Endothelin concentrations in cerebrospinal fluid in depressive patients. (letter) *Lancet* 1989;2:1519
12. Hartter E, Woloszczuk W: Radioimmunoassay of endothelin. (letter) *Lancet* 1989;1:909
13. Vierhapper H, Wagner O, Nowotny P, Waldhausl W: Effect of endothelin-1 in man. *Circulation* 1990;81:1415-1418
14. Stewart DJ, Baffour R: Functional state of the endothelium determines the response to endothelin in the coronary circulation. *Cardiovasc Res* 1990;24:7-12
15. Stewart DJ, Kubac G, Costello KB, Cernacek P: Increased plasma endothelin-1 in the early hours of acute myocardial infarction. *J Am Coll Cardiol* 1991;18:38-43

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