Blood endothelin-1 levels before and after carotid endoarterectomy for atherosclerotic stenosis

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Abstract

Background: Elevated plasma levels of endothelin-1 (ET-1) have been reported in advanced atherosclerosis. Further in vivo demonstration of cause-effect relationship between atherosclerotic lesion and high levels of ET-1 needs to be carried out. The aim of this study was to determine whether circulating levels of ET-1 are influenced by removing haemodynamically significant atherosclerotic stenosis in selected patients with mono or bilateral carotid atherosclerotic stenosis.

Methods: Cubital venous ET-1-immunoreactive (IR) levels were measured in 20 patients: 11 (mean age ± S.D. 63.1 ± 5.36 years; range 53–70 years) were affected by monolateral, and nine patients (mean age ± S.D. 64.7 ± 9.8 years; range 52–78 years) by bilateral extracranial carotid artery atherosclerotic stenosis. ET-1-IR levels were evaluated before and 7 days after monolateral surgical endoarterectomy. Pre-surgery levels of ET-1-IR were compared with those obtained from 18 healthy younger volunteers (mean age ± S.D. 27.8 ± 2.7 years; range 20–50 years).

Findings: The mean cubital venous levels of ET-1-IR in the atherosclerotic patients before endoarterectomy (mean ± S.D. 4.50 ± 3.35 pg/ml; range 1.28–10.66 pg/ml) were significantly higher than those observed in healthy subjects (mean ± S.D. 0.641 ± 0.137 pg/ml; range 0.36–1.02 pg/ml) (P = 0.000). The mean ET-1-IR level decreased significantly after endoarterectomy in the group of patients with monolateral stenosis (pre-surgery: mean ± S.D. 4.35 ± 3.11 pg/ml; range 1.28–10.66 pg/ml; post-surgery: mean ± S.D. 3.05 ± 2.94 pg/ml; range 0.28–8.86 pg/ml) (P = 0.005), but not in patients with bilateral extracranial carotid stenosis submitted to monolateral endoarterectomy (pre-surgery: mean ± S.D. 4.77 ± 3.79 pg/ml; range 2.18–10.3 pg/ml; post-surgery: mean ± S.D. 4.60 ± 3.70 pg/ml; range 2.20–11.10 pg/ml).

Interpretation: The removal of a haemodynamically significant atherosclerotic vascular stenosis is associated with a decrease in the circulating ET-1-IR levels 7 days after surgery when haemodynamically significant atherosclerotic lesions are absent. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Endothelin-1; Atherosclerosis; Carotid stenosis

1. Introduction

Endothelin-1 (ET-1) is a 21-amino acid peptide produced by endothelial cells (EC) [1]. Released predominantly in a polar fashion directed toward the underlying intimal smooth muscle cells (VSMC) [2], ET-1 binds to specific receptors and causes vasoconstriction by increasing the cytosol free calcium concentration, due to phospholipase C activation [3]. The demonstration of a promotogenic effect on VSMC through the activation of c-fos and c-myc gene expression [4–6], and the induction of fibroblast proliferation and collagen synthesis [7], is supported by a possible involvement of ET-1 in atherogenesis. Moreover, several potential contributors to atherogenesis, such as transforming growth factor-1 [8], insulin-like growth factor-1 [9–11], as well as atherogenic substances accumulating inside the plaque such as oxidized LDL [12], stimulate ET-1 release by EC. Recently, enhanced expression of endothelin-A receptors (ET-A) has been shown in the tunica media of porcine saphenous vein-
carotid artery grafts [13] and in carotid arteries of rat after balloon injury. This suggests a significant role of ET-1 in injury-induced-VSMC proliferation and neointima formation [14]. Taken together, these in vitro observations suggest that ET-1, released in excess by endothelial cells during atherosclerosis, might contribute to the development of atherosclerotic lesions.

Increased circulating ET-1 levels were reported in patients with advanced atherosclerosis [15–18] and a positive correlation has been found between levels of ET-1 and thickness of the intima-media complex in the common and internal carotid artery [17]; moreover, the increased venous levels of ET-1 are suggested to predict an increased cerebrovascular morbidity in patients with internal carotid stenosis [18]. The mentioned studies in men affected by atherosclerotic diseases agree with a possible role of ET-1 in the progression of carotid atherosclerosis.

While increased circulating ET-1 levels are associated with advanced clinical atherosclerosis [16–18], it is not known whether circulating levels of ET-1 in patients affected by isolated carotid artery stenosis are modified by removing a vascular atherosclerotic stenosis. Recently Cacoub et al. [16] reported a local decrease of ET-1 in internal jugular vein and only a small decrease in external and internal carotid arteries after carotid cross-clamping, during human carotid revascularization. To investigate the effect of endoarterectomy on cubital vein blood levels of the peptide, we measured plasmatic levels of ET-1-IR in a selected group of patients, affected by extracranial carotid artery atherosclerotic vascular stenosis, before and after surgical endoarterectomy.

2. Materials and methods

2.1. Study patients

Informed consent was obtained from each subject enrolled. A total of 20 patients (16 males and four females), aged 63.9 ± 7.5 years (mean ± S.D.; range 52–78 years) undergoing endoarterectomy for symptomatic extracranial carotid artery stenosis due to atherosclerosis, were studied. No patients were affected by any other symptomatic vascular stenosis. Echo-colour-Doppler, NMR angiography and carotid angiography were performed to localise the vascular lesion and the degree of carotid stenosis, ranging between 70 and 90%, was recorded. Any significant stenosis in other vascular areas was excluded by non-invasive diagnostic procedures [19]. Patients were divided into two groups: the first group consisted of 11 patients (mean age ± S.D. 63.1 ± 5.36 years; range 53–70 years) affected by monolateral carotid stenosis while the second group consisted of nine patients (mean age ± S.D. 64.7 ± 9.8 years; range 52–78 years) affected by bilateral carotid stenosis.

Patients were evaluated for occurrence of hypertension, coronary disease, peripheral vasculopathy, neurovascular disease, diabetes, and hyperlipemia. Moderate hypertension and/or diabetes were diagnosed only in a minority of cases equally distributed within the two groups (3/11 in group I and 2/9 in group II). Cardiovascular medications were discontinued at least 12 h before blood collection for the measurement of plasma ET-1.

2.2. Test procedures

ET-1-IR levels were evaluated in the cubital venous blood before surgery and 7 days after monoendoarterectomy. Blood samples were drawn from the patients after overnight bed rest. Blood was collected in tubes containing chilled potassium EDTA (1 mg/ml of blood) at 0°C. The blood was separated at 3000 × g for 10 min at 4°C and plasma was stored at −20°C until assay. A total of 1 ml of plasma was passed through C18 octyloecylsilane columns (Amersham, UK), previously activated with 0.1% trifluoroacetic acid. Each eluate was then analysed by reverse-phase high-pressure liquid chromatography over 70 min, using a linear gradient of 15–75% acetonitrile/0.1% trifluoroacetic acid in water. Fractions were collected each minute and dried under vacuum by a centrifugal evaporator system (Gyrovap, Hove, London, UK) and then reconstituted in 1 ml buffer (50 mmol/l phosphate buffer, pH 7.4 containing 0.9% sodium chloride, 0.05% nitrosamine and 0.5% bovine serum albumin). The chromatographic separation of plasma eluates indicated a single peak of immunoreactive ET, perfectly corresponding to the elution position of human ET-1 standard and 125I E-1. ET-1-IR was then assayed on reconstituted fraction by a sensitive radioimmunoassay (Peninsula Laboratories, Belmont, CA). Human ET-1 (Peptide Institute, Osaka, Japan) was used as standard. Interassay and intra-assay variation was <10%. Mean recovery of our methods for human ET-1 standard was 85%. Cross reactivity of ET-1 antibody with ET-2 and ET-3 was <7%, according to the supplier. Circulating levels of ET-1-IR collected before surgery in the two groups were compared to those obtained from 18 healthy volunteers (13 males and five females) with a mean age ± S.D. of 27.8 ± 2.7 years; range 20–50 years) after overnight bed rest.

2.3. Statistics

The Mann–Whitney U-test and Wilcoxon matched pairs test were applied as appropriate. A P-value of less than 0.05 was considered statistically significant. The Spearman rank order correlation test was applied to compare the relationship between the results of ra-

Fig. 1. Pre-endoarterectomy circulating ET-1-IR levels in patients with atherosclerotic carotid stenosis (○) and in healthy subjects (●) (P = 0.000, Mann–Whitney U-test). ET-1-IR, endothelin-1-like immunoreactivity.

Fig. 2. Circulating levels of ET-1-IR before and 7 days after monolateral endoarterectomy in patients with monolateral (A) and bilateral (B) carotid stenosis (P = 0.005, Wilcoxon matched pairs test). ET-1-IR, endothelin-1-IR-like immunoreactivity.

3. Results

Atherosclerotic patients before surgery had circulating cubital venous plasma levels of ET-1-IR significantly higher than healthy subjects (mean ± S.D. 4.50 ± 3.35 pg/ml; range 1.28–10.6 vs. 0.64 ± 0.137 pg/ml; range 0.36–1.02 pg/ml) (Fig. 1). Pre-endoarterectomy mean levels of ET-IR were not different in patients with monolateral and bilateral stenosis (mean ± S.D. 4.35 ± 3.11 pg/ml; range 1.28–10.66; and 4.77 ± 3.79 pg/ml; range 2.18–10.3 pg/ml, respectively).

However, 7 days after monolateral endoarterectomy, a significant decrease of the mean venous ET-1-IR level was found in the group of patients with monolateral stenosis (mean ± S.D. 3.05 ± 2.94 pg/ml; range 0.82–8.86 pg/ml) (P = 0.005) (Fig. 2A). In contrast no significant difference, compared with pre-endoarterectomy ET-1 levels, was observed in the group of patients with bilateral stenosis, who underwent monolateral endoarterectomy (mean ± S.D. 4.60 ± 3.70 pg/ml; range 2.20–11.1 pg/ml) (P = 0.34) (Fig. 2B).

No correlations were observed between pre-endoarterectomy plasma ET-1-IR levels and age (r = −0.42; P = 0.06), or the degree of stenosis (r = −0.16; P = 0.52. Spearman rank order correlation test).

4. Discussion

In the present study, pre-endoarterectomy serum levels of ET-1-IR, observed in patients with atherosclerotic extracranial carotid stenosis and no other haemodynamically significant atherosclerotic lesions, decreased significantly 7 days after surgical endoarterectomy.

So far there is no agreement on the effect of the revascularization procedures on the ET-1 circulating levels. An ET-1 increase has been reported immediately after performing percutaneous transluminal coronary angioplasty [20], after balloon vascular dilatation [21], in patients undergoing coronary bypass after induction of cardiopulmonary bypass [22] and during infrarenal aortic clamping and nifedipine infusion [23]. In contrast, other authors reported a decrease in the levels of ET-1 in internal jugular vein after carotid cross-clamping during human carotid revascularization procedures [17], or in patients after coronary revascularization [24].

In the present study, ET-1 was measured 7 days after endoarterectomy, to avoid the potential effect of surgery stress on ET-1 release. The reduction in circulating levels of ET-1-IR following endoarterectomy could be due to the improvement of the haemodynamic consequences related to the stenosis [25–28]. No significant decrease in plasma levels of ET-1 was found in patients with bilateral carotid stenosis submitted to monolateral endoarterectomy. We speculate that the persistence of a haemodynamically significant carotid stenosis after monolateral endoarterectomy could prevent the decrease of ET-1 circulating levels.

Patients with monolateral stenosis showed a significant reduction of ET-1 levels after endoarterectomy, although they were still significantly higher than in healthy subjects. This could be due to the older age of patients. A different possibility is that the carotid stenosis was associated with other, although not documented by non-invasive diagnostic procedures, atherosclerotic changes. Previous observations suggested that the functional balance between endothelium-mediated vasodilatation and vasoconstriction is deranged in early stage atherosclerosis, leading to a reduced release of endothelium-derived relaxing and antimitogenic factor (EDRF) and to an increased release of the vasoconstrictor and mitogenic peptide endothelin-1 [28,29]. Since ET-1 may participate, as a mitogen or as a comitogen peptide in the atherogenic process [4–7], we speculate that endoarterectomy, by removing the haemodynamic shear stress, a major contributing factor to the release of
endothelin-1 [24–26], may contribute to reducing the progression of atherosclerotic disease.

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References