Effects of Endothelin-1 and Endothelin-1 Receptor Blockade on Cardiac Output, Aortic Pressure, and Pulse Wave Velocity in Humans

Tycho J.L. Vuurmans, Peter Boer, Hein A. Koomans

Abstract—Endothelin-1 (ET-1) is a potent vasoconstrictor. Its effect on arterial wave reflections and central pressure augmentation is unknown. We studied whether ET-1, in plasma concentrations present in disease, increases pulse wave velocity (PWV) and augmentation index (AIx) and therefore compromises cardiac output, and whether the ET-1 receptor blocker VML-588 (previously AXV-034343 and Ro 61–1790) prevents such effects. Nine healthy men received a 2-hour infusion with ET-1 (2.5 ng·kg⁻¹·min⁻¹) superimposed on vehicle or VML-588 (0.05, 0.20, or 0.40 mg·kg⁻¹·h⁻¹) (randomized order). Arterial tonometry and pulse wave contour analysis were used to assess aortic PWV and central aortic pressures and impedence cardiography for cardiac output. ET-1 slightly increased mean arterial pressure and peripheral resistance but had no significant effect on systolic blood pressure and pulse pressure. PWV increased from 5.4±0.2 to 5.7±0.3 m/s (P<0.05), AIx from 9.9±3.3 to 17.2±3.8 (P<0.05), central systolic blood pressure by 8.7±1.7 mm Hg (P<0.05), and central pulse pressure by 5.1±1.9 mm Hg (P<0.05). This was associated with a fall in cardiac output by ≈18% (P<0.05). VML-588 caused a slight decrease in brachial mean arterial pressure, PWV, and AIx, and prevented the effects of ET-1 on central hemodynamics without a clear dose-response effect. In summary, ET-1 in plasma concentrations as found in renal failure and heart failure accelerates PWV, causes a disproportionate increase in central aortic systolic blood pressure and pulse pressure, and decreases cardiac output. These effects can be prevented with an ET-1 receptor blocker such as VML-588. This makes it worthwhile to focus on endothelin as a target to prevent ventricular hypertrophy and to maintain cardiac function in diseases associated with high ET-1. (Hypertension. 2003;41:1253-1258.)

Key Words: endothelin ■ vasoconstriction ■ pressure ■ cardiac output

Endothelin (ET)-1 is a potent vasoconstrictor. The effects in the kidney are particularly strong and include vasoconstriction and sodium retention. Although ET-1 has a positive inotropic effect on the heart, it decreases cardiac output probably by increasing systemic vascular resistance and afterload, and coronary vasoconstriction. Theoretically, vasoconstriction by ET-1 could also lead to decreased arterial compliance and, subsequently, enhanced arterial pulse wave reflection and central pressure augmentation, such as was shown recently for caffeine. This would further compromise cardiac function. Nonselective ET receptor blockade induces a mild decrease in blood pressure, suggesting resting ET-1 activity contributes modestly to circulatory control. However, in pathological conditions such as renal failure, heart failure, and salt-sensitive hypertension, ET-1 levels are elevated. In such conditions, ET-1 may suppress cardiac function, and ET receptor blockers may be valuable.

Most studies on the effects of ET in humans have been focused on kidney and peripheral circulation, but little is known on the effects on heart function and arterial wave conduction. The latter can be studied indirectly in humans by means of tonometry of peripheral arteries, which gives information on pulse wave velocity (PWV), arterial wave reflections, and aortic pressure augmentation (AIx). This method is noninvasive and highly reproducible. We presently studied in healthy humans the effects of ET-1 on the systemic circulation, without and with simultaneous administration of the ET-1 receptor blocker VML 588. This drug has a 1000-fold higher affinity for ETA receptors than for ETB receptors, but studies of pharmacological effects in humans are not available. We had 3 questions: (1) does VML 588 affect resting systemic circulation, (2) does ET-1 in a dose sufficient to cause vasoconstriction also increase aortic PWV and AIx, and (3) does VML 588 block these effects? Because the preference of VML 588 for vascular ET receptors has not been studied in humans, we applied various dosages of this drug.

Methods

Subjects and Study Design

The study was performed in 9 healthy men (ages 18 to 34 years) on a normal sodium intake. The protocol was approved by the institutional committee on ethics for study in humans, and the subjects gave

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written informed consent. Each subject underwent 4 experimental sessions with intervals of 7 days in a randomized order. The studies were done after an overnight fast, with the subjects in supine position. Venous cannulas were placed bilaterally in the cubital fossa for infusion and blood sampling. Each study consisted of 3 periods: baseline (90 minutes); vehicle (placebo study) or VML 588 infusion at a rate of 0.05, 0.2, or 0.4 mg · kg⁻¹ · h⁻¹ (90 minutes); and ET-1 infusion at a rate of 0.15 mg · kg⁻¹ · h⁻¹ while vehicle or VML 588 infusion was continued (120 minutes). VML 588 (Vanguard Medical Ltd) was dissolved in 0.9% saline; ET-1 (Cilag AG), in a plasma substitute (Gelofusine, B. Braun AG). Blood samples for determination of plasma ET-1 and VML 588 were collected at the end of each period. Blood pressure was recorded at 5-minute intervals with a Dinamap Compact T blood pressure monitor (Criticon). Cardiac output measurement (toracic impedance plethysmography; Bomed Medical Manufacturing Ltd), tonometry, and pulse wave analysis were performed at the end of each period.

VML 588
VML 588 (3-methyl-pyridine-2-sulfonic acid 6-[2-hydroxy-ethoxy]-5-[2-methoxy-phenoxyl]-2-[2 to 1H-tetrazol-5-y]pyrimidin-4-yl)-pyrimidin-4-ylamide, formerly known as AXV-034343 and Ro 61–1790) is a competitive ET receptor antagonist with an ~10-fold higher affinity for ET, receptors than for ET₂ receptors. The drug is cleared by the liver with a short half-life (<1 hour), and steady-state plasma concentrations are reached after ~1 hour of constant infusion.

Tonometry and Pulse Wave Analysis
The SphygmoCor system (BPAS, PWV Inc) was used for applana-
tometry and pulse wave analysis. Pulse contours were obtained at the carotid, radial, and femoral arteries, the artery gently being pressed against a bone with a pencil-type probe that incorporates a strain-gauge transducer at the tip with a small pressure-sensitive ceramic sensor area (Millar Instruments). To determine aortic PWV, pressure waves were recorded sequentially at the carotid and the femoral arteries. Wave transit time was calculated as the height of the late systolic peak above the inflection point divided by the aortic pulse pressure.16 The augmentation index was calculated as the height of the late systolic peak above the inflection point divided by the aortic pulse pressure.16,17

Chemical Analyses
Blood samples for determination of immunoreactive ET-1 and VML 588 were collected in prechilled di-K-EDTA containing tubes, centrifuged at 4°C and stored at ~70°C. ET-1 was extracted in duplicate from 1-mL plasma samples by using Sep-Pak C18 solid phase extraction chromatography cartridges (Waters, Etten-Leur) and measured by radioimmunoassay (Nichols Institute). VML 588 was measured by high performance liquid chromatography with mass spectrometry detection.

Calculations and Statistics
Data are presented as mean±SE. Statistical analysis was performed by 2-way ANOVA for repeated measures. If statistical significant differences were present, the Student-Newman-Keuls test for multiple comparisons was used as post-hoc test. Because changes in plasma ET-1 were not normally distributed, plasma ET-1 levels are presented as geometric means, and analysis was performed on logarithmically transformed data. The Student paired t test was used to compare changes in central aortic pressures to changes in peripheral pressures. A probability value ≤0.05 was considered to be statistically significant.

Results
Plasma ET-1 and VML 588 Concentration
Infusion of ET-1 alone increased plasma ET-1 levels to ~5-fold. The lowest dose of VML 588 had no significant effect on resting plasma ET-1, but ~30% and 50% increments were found after VML 588 infused at, respectively, 0.2 and 0.4 mg · kg⁻¹ · h⁻¹. VML 588 progressively enhanced the increase in plasma ET-1 levels obtained during ET-1 infusion (Figure 1). Steady-state VML 588 concentrations were obtained after 1 hour of VML 588 infusion and amounted to 91±5, 383±16, and 752±29 ng/mL during, respectively, 0.05, 0.2, and 0.4 mg · kg⁻¹ · h⁻¹ infusion rates.

Systemic Circulation
Infusion of ET-1 alone increased arterial pressure but had no effect on pulse pressure (Figure 2). Cardiac output, stroke index, and heart rate decreased, and peripheral resistance increased (Figure 3). Central aortic systolic pressure and pulse pressure increased significantly too (Figure 4). The AIx increased from 9.9±3.3 to 17.2±3.8 (P<0.05); the aortic PWV, from 5.4±0.2 to 5.7±0.3 m/s (P<0.05). The increase in central aortic systolic pressure (8.7±1.7 mm Hg) was significantly different (P<0.05) from the (nonsignificant) change in brachial artery systolic blood pressure (2.1±1.4 mm Hg). Likewise, the increase in central aortic pulse pressure (5.1±1.9 mm Hg) was significantly different (P<0.05) from the (nonsignificant) change in brachial artery pulse pressure (~1.0±2.1 mm Hg).

VML 588 alone decreased arterial pressure slightly but significantly, without a clear dose response effect (Figure 2). VML 588 also increased heart rate slightly but consistently. Nonetheless, we found no significant changes in cardiac output and total peripheral resistance (Figure 3). VML 588 also caused a consistent decrease in central aortic systolic pressure and AIx but had no significant effect on central aortic pulse pressure (Figure 4). The aortic PWV decreased consistently too.
VML 588 prevented the effects of ET-1 infusion on peripheral resistance, stroke index, cardiac output, heart rate, central aortic systolic and pulse pressure, Alx, and aortic PWV (Figures 3 and 4). Interestingly, during VML 588 treatment the ET-1 infusion tended to paradoxically decrease brachial arterial pressure (Figure 2).

**Discussion**

The present study in healthy humans shows that ET-1, in a dosage that induces vasoconstriction, also accelerates arterial pulse wave conduction, causes a disproportional increase in central aortic systolic pressure, and decreases cardiac output. ET-1 receptor blockade with VML 588 is able to prevent the effects of ET-1 on systemic circulation, central aortic pressures, and cardiac output. VML 588 has no effect on the basal systemic circulation except for a slight decrease in blood pressure.

**Plasma ET-1 Concentration**

ET-1 infusion increased plasma ET-1 ~5-fold. Such elevations in plasma ET-1 can be encountered in, eg, patients with end-stage renal disease, heart failure, and salt-sensitive hypertension. VML 588, in the middle and highest dosage, increased resting ET-1 concentration and dose-dependently enhanced the increase of plasma ET-1 caused by ET-1 infusion. Others have shown that ET₄ receptor blockade

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**Figure 2.** Changes in brachial arterial blood pressures. VML 0.05, 0.20, and 0.40 indicate VML 588 0.05, 0.20, and 0.40 mg · kg⁻¹ · h⁻¹, respectively. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; and PP, pulse pressure. aP<0.05 vs placebo or VML 588.

**Figure 3.** Changes in cardiac parameters and peripheral resistance. VML 0.05, 0.20, and 0.40 indicate VML 588 0.05, 0.20, and 0.40 mg · kg⁻¹ · h⁻¹, respectively. TPRI indicates total peripheral resistance index. aP<0.05 vs baseline; bP<0.05 vs placebo or VML 588.
effects on resting plasma ET-1, whereas blockade of the ET\textsubscript{A} receptor, or nonselective ET receptor blockade, increases plasma ET-1\textsuperscript{19–21}. It is therefore assumed that the clearance of ET-1 from the circulation depends on internalization through binding of the ET\textsubscript{B} receptor. The present observation therefore suggests that VML 588, in the dosages given, caused progressive blockade of the ET\textsubscript{B} receptor. This is in accordance with the observed plasma concentrations of VML 588 (90 to 750 ng/mL), its affinity for ET\textsubscript{B} receptor found in human placenta\textsuperscript{18} and the notion that the ET\textsubscript{B} receptors are readily reachable from the vascular lumen. This observation implies that VML 588, although its affinity for ET\textsubscript{B} receptors is 1000-fold less than that for ET\textsubscript{A} receptors, probably did not act as a selective blocker in the presently used dose range.

**Systemic Effects of ET-1 and VML 588**

ET-1 infusion increased mean arterial pressure and decreased cardiac output by \approx 18\%. Comparable effects were found previously by others during infusion of ET-1 or big-ET-1 in healthy humans\textsuperscript{4–6}. The decrease in cardiac output was caused by a decrease in stroke volume and in heart rate. Because the direct effect of ET-1 on the myocardium is positive inotropic\textsuperscript{3}, the fall in stroke volume is probably secondary to the increased afterload, whereas the decreased heart rate is probably baroreflex-induced. Indeed, ET-1 infusion caused a substantial increase in central aortic systolic pressure.

Novel elements in the present study concern the effects of VML 588, measurement of PWV, and the central aortic pressure. VML 588, in all applied dosages, effectively prevented the fall in cardiac output induced by ET-1 infusion. It has been shown that ET-1 receptor blockers can increase cardiac output of patients with heart failure\textsuperscript{13,22} or in animal models of heart failure\textsuperscript{23,24}. However, as far as we know, it has not been tried by others to prevent suppressant effects of ET-1 on cardiac output in healthy humans with an ET-1 receptor blocker. Whether this is indeed a good model to predict effects in patients with pathophysiological activity of ET-1 remains to be studied. We infused ET-1 to obtain concentrations as found in pathophysiological conditions. However, because endogenous ET-1 is mainly secreted from the abluminal side of endothelial cells\textsuperscript{25}, relevant tissue concentrations of ET-1 may differ in experimental and pathological conditions. VML 588 had no effect on resting cardiac output, although there was some decrease in arterial pressure. Others have found that high dosages of nonspecific or ET\textsubscript{A} receptor–specific antagonists increased cardiac index also in healthy subjects\textsuperscript{9,26}.

ET infusion increased AIx. This increase can be explained from the accelerated wave reflection (ie, the increased aortic PWV)\textsuperscript{27}. A reduction in heart rate also increases the AIx and central pulse pressure by prolonging ventricular ejection time. However, these effects are small, ie, \approx 3\% and 1 mm Hg per 6 bpm\textsuperscript{28}, and cannot have contributed much in the present setting (because heart rate decreased by only 2 bpm). As a result of the increased pressure augmentation, the central aortic systolic pressure and pulse pressure increased, although the peripheral systolic and pulse pressure did not. A comparable discrepancy has been described recently of the vasoconstrictor caffeine in patients with essential hypertension\textsuperscript{8}. Central aortic systolic pressure is the pressure that the heart has to confront and, therefore, is a predominant determinant of cardiac afterload. Intima-media thickening in carotid arteries correlates better with central aortic systolic pressure than with peripheral systolic pressure\textsuperscript{29}. Therefore, the impact of ET-1 on cardiac and vascular function may be stronger than can be derived from its effect on the peripheral blood pressure. Why ET-1 has this effect may be complex. On the one hand, the PWV will have increased as result from the increase in arterial distending pressure, which decreases aortic compliance\textsuperscript{30}. However, studies in isolated rat carotid arteries have shown that ET\textsubscript{A} receptor blockade increases.
cross-sectional compliance, indicating that ET-1 also has a direct effect on arterial compliance. In line with this observation, we found that VML 588 decreased PWV and AIx and prevented the increase in PWV and central aortic systolic pressure induced by ET-1 infusion.

**Perspectives**

The data on central pressures and augmentation index were based on analysis of the carotid artery pulse wave contour, which is, of course, an indirect approach. The validity of the applied transfer function has been tested successfully by Chen et al. Although the carotid artery–derived central pressure and AIx consistently underestimate the true aortic values, changes induced by vasomotor stimuli are quantified accurately. Another limitation concerns the intravenous administration of ET-1, which primarily induces increased plasma concentrations. Endogenous ET-1 as produced by endothelial cells is, for most part, secreted abluminally, and its distribution over (abluminal vasoconstrictor) ET, receptors and (luminal vasodilator) ETB receptors is probably different. Nonetheless, it is likely that the plasma concentrations obtained by ET-1 infusion will have mimicked the effect of the plasma concentrations as found in pathophysiologic situations.

Because the rising plasma ET-1 concentrations suggest that VML 588 also caused ETB receptor blockade, it remains speculative whether blockade of the central hemodynamic effects of ET-1 was due to blockade of ET, receptors, ETB receptors, or both. Although we administered 3 different dosages of VML 588, all dosages provided complete blockade of the hemodynamic effects caused by ET-1 infusion, without a dose-response effect. Instead, the blockade of the ET, receptor appeared progressive, because ET-1 levels rose with higher dose of VML 588. We presume, therefore, that blockade of the ET, receptors, which have a 1000-fold higher affinity for VML 588, was almost complete with all dosages of VML 588 and played an important role in blockade of the hemodynamic effects. Evidently, lower dosages of VML 588 will have to be tested to establish which dosage of VML 588 will effectively and selectively block ET, receptors in humans.

Increased central pressure augmentation as induced by ET-1 is detrimental for cardiac function, as it necessitates higher ventricular systolic pressures and metabolic demands, and predisposes to ventricular hypertrophy. This may be particularly important for patients with chronic renal failure, in whom plasma ET-1 may be elevated on long-term basis. Indeed, increased plasma ET-1 levels in chronic renal failure correlate with left ventricular hypertrophy. Although it is likely that ET-1 receptor blockade can help to prevent left ventricular hypertrophy in such patients, this has not been studied. In patients with heart failure, acute or short-term ET, receptor blockade or combined ET, ETB receptor blockade has been shown to improve cardiac function. Our finding of increased central aortic pressures after ET-1 infusion may help to explain this beneficial effect. However, it should be mentioned that preliminary results did not show a lasting benefit of ET-1 receptor blockade in heart failure.

In conclusion, ET-1 in plasma concentrations found in pathophysiological conditions such as renal failure and heart failure accelerates PWV and causes a disproportionate increase in central aortic systolic pressure and pulse pressure, and decreases cardiac output. Because these effects can be eliminated with ET receptor blockers such as VML 588, it becomes worthwhile to focus on ET as a target to prevent ventricular hypertrophy and to maintain cardiac function in disease states.

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