Endothelin receptor blockade lowers plasma aldosterone levels via different mechanisms in primary aldosteronism and high-to-normal renin hypertension

Gian Paolo Rossi\textsuperscript{a,}:*, Chiara Ganzaroli\textsuperscript{a}, Maurizio Cesari\textsuperscript{a}, Andrea Maresca\textsuperscript{a}, Mario Plebani\textsuperscript{b}, Gastone G. Nussdorfer\textsuperscript{c}, Achille C. Pessina\textsuperscript{a}

\textsuperscript{a}Department of Clinical and Experimental Medicine, Clinica Medica 4, University Hospital, via Giustiniani 2, 35126 Padova, Italy
\textsuperscript{b}Department of Laboratory Medicine, University of Padova, Padova, Italy
\textsuperscript{c}Anatomy Section, Department of Human Anatomy and Physiology, University of Padova, Padova, Italy

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Abstract

Background: Endothelin (ET)-1 contributes to raising blood pressure (BP) and inducing cardiovascular disease by vasoconstriction and potent stimulation of aldosterone secretion. In the rat this latter effect occurs via ET\textsubscript{A} receptors; in humans in vitro studies implicated both ET\textsubscript{A} and ET\textsubscript{B} receptors, but there is no conclusive evidence in vivo. Methods: We recruited 13 consenting hypertensive patients: six with primary aldosteronism (PA) and seven with high-to-normal renin hypertension (HNRH). They were infused with a low dose (200 nmol/min for 5 min followed by 100 nmol/min for 10 min) of the ET-selective antagonist BQ-123 either alone or, on a different day, together with an identical dose of the ET\textsubscript{B}-selective antagonist BQ-788. Plasma aldosterone, cortisol and ACTH concentration and plasma renin activity (PRA) were measured with radioimmunoassay at 0, 15, 30, 60, 120, 240, 360 min, while BP was recorded non-invasively.

Results: BQ-123 alone and combined with BQ-788 significantly lowered mean BP in both PA and HNRH patients (by 6–10 mmHg at nadir; \(P<0.01\)). In PA patients, a short-lived decrease of aldosterone was elicited by combined BQ-123 and BQ-788 (214%; \(P<0.05\)), but not by BQ-123 alone; cortisol, ACTH, and PRA were unaffected by either treatment. In HNRH patients, BQ-123 both alone and combined with BQ-788 lowered aldosterone (239 and 228%, respectively) and PRA (243 and 216%, respectively), while cortisol and ACTH were unaffected.

Conclusions: Endogenous ET-1 contributes to maintaining the high BP values and the aldosterone secretion in both PA and HNRH patients. In the former patients, the aldosterone secretagogue effect of ET-1 is mediated via ET\textsubscript{A} receptors, while in the latter it occurs mainly via ET\textsubscript{B}-mediated stimulation of renin production.

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Keywords: Blood pressure; Endothelins; Hypertension; Receptors

1. Introduction

The potent vasoconstrictor endothelin (ET)-1 can play an important pathogenic role in arterial hypertension and its most ominous complications, including congestive heart failure (CHF) [1], by exerting multiple biologic effects via its type A (ET\textsubscript{A}) and B (ET\textsubscript{B}) receptors [2]. In patients with essential hypertension, an increased ET-1 vasoconstrictor tone [3,4], which seems to be dependent on decreased endothelial ET\textsubscript{B}-mediated NO production attributable to impaired NO availability, has been described [5]. Thus, endothelial ET\textsubscript{B}-induced vasodilatation could no longer compensate for the ET\textsubscript{A}-mediated vasoconstrictor effect of the peptide on vascular smooth muscle cells. This contention is supported by the demonstration that the dual (non-selective) ET\textsubscript{A} and ET\textsubscript{B} receptor antagonist bosentan was equipotent to a full dose of the ACE inhibitor enalapril in lowering blood pressure (BP), when given orally to patients with mild-to-moderate essential hypertension [6]. Activation of the ET-1 system, which can contribute to
adverse cardiovascular remodelling, has been reported in CHF patients who were in the New York Heart Association functional class III and IV [7], and in pulmonary hypertension, both primary [8] and secondary to CHF [9]. Thus, ET receptor blockade is being tested as a therapeutic strategy for CHF and several other cardiovascular indications [2]. Of interest, bosentan was shown to improve symptoms and functional class in patients with severe primary pulmonary hypertension [10] and therefore has been approved for the treatment of this dreadful disease in some countries.

Hyperaldosteronism appears to contribute substantively to the high mortality and morbidity of patients with severe CHF [1,11]. ET-1 could be involved in this poor outcome not only because of its haemodynamic effects, but also because of its potent secretagogue action on aldosterone, which occurs via a direct effect on the adrenocortical zona glomerulosa (ZG) [12–14]. The latter expresses the proET-1 and ET converting enzyme ECE-1 genes, along with ET\(_\alpha\) and ET\(_\beta\) receptor subtypes in several species, including humans [15–20] and therefore ET-1 is likely to be an important autocrine-paracrine regulator of ZG functions [21]. We found that in rat adrenocortical cells the ET\(_\beta\) receptor mediates the secretagogue effect of ET-1 [19], but in humans in vitro studies carried out on different adrenocortical cell phenotypes [12–14,22] did not exclude the participation also of ET\(_\alpha\) receptors. Thus, in humans the receptor subtype mediating the aldosterone secretagogue action of ET-1 [13], which can be relevant in pathophysiological conditions, such as CHF, malignant hypertension, primary and secondary aldosteronism, is controversial. Moreover, it remains unclear whether the haemodynamic effects of ET-1 and the relative relevance of ET\(_\alpha\) and ET\(_\beta\) selective receptor blockade differ according to the degree of activation of the renin-angiotensin system (RAS), which deeply interacts with the ET system (for review, see Ref. [23]).

The development of ET\(_\alpha\) and ET\(_\beta\) selective receptor antagonists (for review, see Ref. [2]) which can be infused into human subjects, has made it possible to directly investigate this issue in vivo.

Thus, in this study we investigated the acute haemodynamic effects of an infusion of the ET\(_\alpha\)-selective receptor antagonist BQ-123, either alone or associated with the ET\(_\beta\)-selective antagonist BQ-788, in hypertensive patients with different degrees of RAS activation. We also sought to identify the receptor subtype mediating the aldosterone secretagogue effect of ET-1 and therefore selected patients with either primary aldosteronism (PA) or high-to-normal renin hypertension (HNRH).

### 2. Methods

We enrolled for this study 13 hypertensive patients: six had PA, which was due to an aldosterone-producing tumour in five patients and was idiopathic in one while seven patients had HNRH. Patients with concomitant sicknesses that might affect the results of this investigation were carefully excluded. PA was identified based on a screening strategy with a logistic discriminant analysis [24]; the underlying adrenal pathology was diagnosed with both CT or MR imaging and with adrenal vein sampling as described in detail [25]. Of the PA patients, four were untreated and two were allowed to take a long-acting calcium entry blocker because this treatment does not affect aldosterone and plasma renin activity (PRA) under chronic conditions [26]. The predefined criterion for selecting patients with HNRH was a PRA value greater than the upper limit of the normal range (0.51–2.62 ng Ang I/ml per h); three of these patients were on oral chronic diuretic treatment with hydrochlorothiazide (25 mg/day). These patients were selected based on the assumption that they have high-to-normal plasma aldosterone levels.

The Ethics Committee of our University approved the study protocol and all patients gave written consent.

To avoid any bias due to the circadian rhythm of aldosterone all tests were performed at 8.30 a.m., after overnight fasting and at least 2 h of quiet lying in the supine position. An indwelling cannula was placed into the brachial vein and a slow drip saline infusion was started and continued for 30 min to establish baseline values before the first blood sampling. For each sample 10 ml of whole blood with 100 μl 6% Na\(_2\)EDTA were immediately put on ice and centrifuged at 3000×g (at 4 °C for 10 min). After centrifugation, aliquots of plasma were stored at −20 °C until assayed. Plasma levels of sodium, potassium, and creatinine and daily urinary excretion rate of sodium and potassium were measured using conventional methods.

Each patient received, on different days, in random order and in single-blinded fashion, an infusion of either BQ-123 (200 nmol/min for 5 min, followed by a 100-nmol/min infusion for 10 min, i.v.) plus placebo, or of an identical dose of both BQ-123 and BQ-788. These low doses were selected based on previous studies [27–29], in order to transiently antagonize endogenous ET-1. BQ-123 and BQ-788 were purchased from Clinalfa (Läufelfingen, Switzerland) as sterile solution approved for injection into human beings. Since plasma potassium levels are known to affect aldosterone secretion [30], hypokalemia was corrected in PA patients with i.v. potassium supplementation on the day before each test, whenever required.

Blood sampling was performed at −15, 0, 30, 60, 120, 240, 360 min for measurement of the plasma concentration of aldosterone (PAC), cortisol, ACTH and PRA, while arterial blood pressure (BP) was monitored by non-invasive ambulatory BP recording (Spacelab™, mod. 90207, Redmond) set to take readings every 6 min for the first 2 h and every 30 min for the last 4 h of the experiments.

We used radioimmunoassay and commercially available kits to measure PAC (Technogenetics, Milan, Italy), cortisol (Biochem Immunosystem, Casalcchio di Reno, Italy), and aldosterone (Biochem Immunosystem, Casalcchio di Reno, Italy).
Table 1
Clinical characteristics of the patients of the study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Primary aldosteronism</th>
<th>High-to-normal renin hypertension</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52.8±5.4</td>
<td>48.9±7.7</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>5/1</td>
<td>5/2</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>71±5</td>
<td>76±8</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>153±7</td>
<td>159±6</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>97±4</td>
<td>99±2</td>
<td>NS</td>
</tr>
<tr>
<td>Mean BP (mmHg)</td>
<td>116±5</td>
<td>119±3</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>86±10</td>
<td>93±5</td>
<td>NS</td>
</tr>
<tr>
<td>Serum K⁺ (mmol/l)</td>
<td>3.2±0.3</td>
<td>3.6±0.1</td>
<td>P=0.03</td>
</tr>
<tr>
<td>Na⁺V (mmol/24 h)</td>
<td>183±96</td>
<td>161±54</td>
<td>NS</td>
</tr>
<tr>
<td>K⁺V (mmol/24 h)</td>
<td>56±25</td>
<td>59±12</td>
<td>NS</td>
</tr>
<tr>
<td>Supine PRA (ng Ang I/ml per h) [n.v. 0.51–2.62]</td>
<td>0.59±0.13</td>
<td>2.63±0.95</td>
<td>P=0.03</td>
</tr>
<tr>
<td>Plasma aldosterone (pg/ml) [n.v. 20–110]</td>
<td>215±3</td>
<td>89±4</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Plasma cortisol (ng/ml) [n.v. 50–250]</td>
<td>98±15</td>
<td>125±13</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma ACTH (pg/ml) [n.v. 10–50]</td>
<td>10±2</td>
<td>19±4</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as mean±S.E.M. The normal values for our laboratory for subjects with 100–300 mmol Na⁺/day and in supine position are shown in square brackets.

Italy), ACTH (Medical System, Genoa, Italy), and PRA (RADIM, Pomezia, Italy). The normal values for our laboratory are shown in Table 1.

2.1. Statistical analysis

Results are expressed as mean±S.E.M. Plasma levels of the different hormones and BP values were compared between ET antagonist infusions in each group of patients by a generalized linear model for repeated measures ANOVA. To adjust for baseline differences, baseline BP and hormonal values, along with age, were entered as covariates in the model. We decided a priori to use a multivariate test to detect significant effects since it does not require the sphericity assumption. A P<0.05 was considered statistically significant. The SPSS for Windows™ package (vers. 10.0, SPSS, Bologna, Italy) was used for statistical analysis.

3. Results

3.1. Demographic and clinical features of the patients

The main features of the patients in the two groups during clinical evaluation before entering the study are shown in Table 1. The groups did not differ in terms of heart rate, systolic and diastolic blood pressure and serum creatinine levels. The PA patients had lower serum potassium levels and PRA, and higher PAC as compared to HNRH patients. No difference in the plasma levels of cortisol and ACTH between groups was observed.

3.2. Effects of ET receptor antagonists on BP

The patients selected for this study had moderately elevated BP values (Fig. 1), as compared to the upper normal values for our laboratory for non-invasively recorded ambulatory BP (119.8/73.9 mmHg) [31], which are lower than upper normal values (135/85 mmHg) of the JNC VI [32].
There were no significant changes in BP or in plasma hormones (not shown) during the placebo infusion from time −15 to time 0 on each of the 2 days of the test.

Both BQ-123 alone and combined with BQ-788 significantly \((P<0.01)\) lowered mean BP in both PA and HNRH patients (Fig. 1). In PA patients, the nadir was reached between 51 and 81 min after the start of the infusion and was −6 and −7 mmHg for BQ-123 and BQ-123+BQ-788, respectively. In HNRH patients, it was reached after 51–111 min and was −10 and −6 mmHg for BQ-123 and BQ-123+BQ-788, respectively. Repeated measures ANOVA showed no differences in BP responses between BQ-123 and BQ-123+BQ-788 and no differences between PA and HNRH patients.

3.3. Effects of ET receptor antagonists on plasma hormone concentrations

In PA patients, a short-lived decrease of PAC \((P<0.05)\) was induced by combined BQ-123 and BQ-788, but not by BQ-123 alone (Fig. 2). With combined BQ-123 and BQ-788 the nadir was attained 60 min after the start of the infusion (Table 2). PRA (Fig. 2), ACTH and cortisol were not significantly affected by either treatment (Table 2). In HNRH patients, both BQ-123 alone and combined with BQ-788 lowered \((P<0.02)\) aldosterone \((-39\) and \(-28\%,\) respectively), the nadir being attained 30–120 min after the start of the infusion. Both treatments also lowered PRA significantly \((-43\) and \(-16\%,\) respectively; \(P<0.02\)) (Fig. 2), but did not change significantly ACTH and cortisol plasma concentrations (Table 2).

4. Discussion

The detrimental effects of excess aldosterone in CHF [11] might be related to its capability to induce cardiac fibrosis [33], which can be enhanced by ET-1 [34]. In rats with CHF ET receptor blockade has been shown to improve survival [35,36] and to prevent cardiac fibrosis and foetal gene expression more effectively than ACE inhibitors [37]. In patients with CHF short-term treatment with ET receptor antagonists was consistently found to exert favourable haemodynamic effects [38,39], but to date...
the results of clinical trials have not fulfilled the promises of this therapeutic strategy (for review, see Ref. [40]).

The variable effects of selective and non-selective ET receptor blockade on neuro-hormonal activation in the different species might account for these results. Accordingly, in this study we sought to identify the effects on BP and the ET receptor subtype that mediates aldosterone secretion in hypertensive patients by administering for a short period of time a low dose of the ET\textsubscript{A}-selective antagonist BQ-123, either alone or together with the ET\textsubscript{B}-selective antagonist BQ-788. These low doses were chosen based on previous studies [27,28] in order to antagonize endogenous ET-1. We used the strategy of comparing ET\textsubscript{A}-selective and combined ET\textsubscript{A} and ET\textsubscript{B} blockade, instead of investigating the direct effect of isolated ET\textsubscript{B} blockade in a head-to-head comparison of BQ-123 and BQ-788, because experience with both angiotensin II type 1 (AT-1) and type 2 (AT-2) receptor antagonists and with ET receptor antagonists consistently showed that blockade of a single receptor subtype resulted in enhanced activation of the other. In fact, in human healthy volunteers the infusion of ET-1 on top of BQ-123 resulted in paradoxical vasodilatation in the forearm vascular bed, which indicated enhanced activation of the ET\textsubscript{B}-mediated NO production [41]. Furthermore, the intracoronary infusion of BQ-123 was associated with enhanced clearance of endogenous ET-1 through binding to ET\textsubscript{B} receptors in patients with coronary atherosclerosis [42]. Thus, had a head-to-head comparison of ET\textsubscript{A} and ET\textsubscript{B} blockade been undertaken, a less straightforward interpretation of results would have been derived.

We selected hypertensive patients with contrasting levels of PRA to verify whether the receptor subtype mediating this important biological effect of ET-1 differed depending on the prevailing degree of activation of the RAS. We found that the ET\textsubscript{A}-selective blockade, either alone or combined with the ET\textsubscript{B}-selective blockade, resulted in a lowering of BP that is consistent with the previous reports of vasodilatation in hypertensive patients [3,4].

More importantly, our results showed for the first time that this anti-hypertensive effect of ET receptor antagonists was associated with changes in PAC, which occurred with different mechanisms in patients with PA and HNRH. Thus, endogenous ET-1 does play a role in regulating BP and the plasma level of aldosterone in hypertensive patients with a suppressed RAS due to excess autonomous aldosterone secretion and with HNRH.

To our knowledge this lowering of BP with acute ET receptor antagonist administration is the first ever obtained in hypertensive patients with aldosteronism. The similar anti-hypertensive effect observed with both the ET\textsubscript{A} and the combined ET\textsubscript{A}/ET\textsubscript{B} blockade is consistent with the contention that the ET\textsubscript{A} is the receptor subtype primarily involved in vasoconstriction. It accords well with previous results with bosentan in mild-to-moderate essential hypertensive patients [6] and in different models of hypertension, as well as with findings in normotensive subjects [41]. Furthermore, it supports the contention that the ET-1 system is activated in mineralocorticoid-dependent forms of hypertension, both in humans [43] and in animals [44].

By selecting hypertensive patients with different degrees of activation of the RAS we could distinguish the effects of ET-1 resulting from an interaction of the peptide with the RAS from those occurring directly at the adrenal cortex level. In PA patients PAC was lowered by the combined administration of BQ123 and BQ-788 and not by BQ-123 alone, thus suggesting that the ET\textsubscript{B} subtype mediates the secretagogue effect of ET-1 on aldosterone when the RAS is suppressed. At variance with this, in patients with an activated RAS, the PAC were decreased by both BQ-123 alone and combined with BQ-788, thereby indicating that when the RAS is up-regulated, the lowering of aldosterone may occur through an ET\textsubscript{A}-mediated blunting of renin secretion. This contention is supported by studies in dogs, which indicated that exogenous ET-1 increases PRA [45] and that this effect might be blunted by the ET\textsubscript{A}-selective agent LU-135252 [46].

These conclusions have relevant implications for the development of ET antagonists in the treatment of other conditions, including CHF. In CHF patients, as well as in

<table>
<thead>
<tr>
<th>Primary aldosteronism</th>
<th>High-to-normal renin hypertension</th>
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<tbody>
<tr>
<td><strong>Mean blood pressure</strong>&lt;br&gt;(mmHg)</td>
<td>ET\textsubscript{A} blockade</td>
</tr>
<tr>
<td>Supine PRA&lt;br&gt;(ng Ang I/ml per h)</td>
<td>-5.9 (−5%)</td>
</tr>
<tr>
<td>Plasma aldosterone&lt;br&gt;(pg/ml)</td>
<td>-0.20 (−36%)</td>
</tr>
<tr>
<td>Plasma cortisol&lt;br&gt;(ng/ml)</td>
<td>+4.0 (+19%)</td>
</tr>
<tr>
<td>Plasma ACTH&lt;br&gt;(pg/ml)</td>
<td>-13 (−13%)</td>
</tr>
</tbody>
</table>

Table 2 Hormonal changes (maximum absolute difference and % change from baseline) after administration of BQ-123 (ET\textsubscript{A} blockade) and BQ-123+BQ-788 (ET\textsubscript{A}/ET\textsubscript{B} blockade)
those with hypertension and an activated RAS, blunting of aldosterone secretion could be achieved with selective ET₄ blockade, while in patients with hypertension due to primary aldosteronism only combined ET₄/ET₆ blockade would attain this goal.

It has to be stressed that our study has some limitations, which we wish to mention and briefly discuss. First, the changes in PAC could be seen as rather small and transient. However, we used very low doses of ET receptor antagonists, although the dosage of BQ-123 was four-fold higher than that found to increase forearm blood flow in healthy volunteers [27], and similar to that which induced systemic vasodilatation in chronic CHF patients, who have enhanced synthesis of ET-1 [28]. In addition, this dose of BQ-788 was shown to increase vascular resistance in humans [29]. Thus, it is conceivable that more marked changes of PAC might be accomplished with higher doses of ET antagonists such as those that are being use for systemic and pulmonary hypertension. Second, the fact that the PAC of our HNRH patients was on average not overtly elevated despite the elevation of PRA, might have contributed to diminish its responses to the ET antagonists in these patients. Third, we cannot exclude that the decrease of PAC after ET receptor blockade might be due to concomitant changes of aldosterone excretion and/or of hepatic metabolism, which were not measured in this study. However, these possibilities seem unlikely because the systemic haemodynamic changes were rather small and short-lived. Finally, larger and longer lasting studies in patients with secondary forms of hypertension, such as those with primary aldosteronism and those with renal artery stenosis, are needed in order to clarify the potential usefulness and safety of endothelin receptor antagonists for these conditions.

References


