Clinical potential of endopeptidase-24.11 inhibitors in cardiovascular disease

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Introduction
Interest in the cardiovascular pharmacology of endopeptidase-24.11 (E-24.11) inhibitors arose from the observation that E-24.11 includes among its substrates a number of peptides that have cardiovascular actions, most notably the natriuretic-peptide family: atrial (ANP), brain (BNP) and C-type (CNP) natriuretic peptides, and urodilatin. The natriuretic peptides exhibit a common structural motif, a 17-amino-acid ring formed by a disulphide bond, and exert their biological actions through guanylyl-cyclase-linked receptors. ANP and BNP are synthesized by the heart and circulate in the plasma, CNP is found predominantly in the brain, and urodilatin is thought to have a paracrine role in the kidney. Of particular interest to the cardiovascular pharmacologist is the profile of biological activity of this family, which includes natriuresis-diuresis, vasorelaxation, inhibition of the renin-angiotensin system and inhibition of vascular-smooth-muscle hypertrophy. These are the properties that are sought after in drugs that are used to treat a range of cardiovascular disorders, such as heart failure and hypertension. However, use of synthesized peptides as pharmacological agents is constrained by their susceptibility to degradation in the gut and by their short plasma half-life. E-24.11 inhibitors offer an alternative approach, by inhibiting the metabolism and so increasing the levels of the endogenous peptides.

Role of E-24.11 in the metabolism of natriuretic peptides in vivo
Metabolism by E-24.11 is not the only mechanism that has been proposed for removing natriuretic peptides in vivo. A large sub-group of natriuretic-peptide receptors (C-receptors) are not linked to guanylyl cyclase and display less rigorous structural criteria for ligand binding. These receptors are thought to have a clearance function and to act in concert with E-24.11 in the regulation of natriuretic-peptide levels. The effect of inhibiting each clearance pathway on the pharmacokinetics of a bolus injection of 125I-labelled ANP has been examined in the anaesthetized rat. Co-administration of a specific C-receptor ligand reduces both the volume of distribution and the rate of clearance of the radioligand, while inhibition of E-24.11 affects only the plasma half-life. It appears, however, that the effect of inhibiting one pathway is partly offset by the activity of the other; thus, the combined inhibition of the two clearance mechanisms leads to a greater increase in plasma 125I-labelled ANP levels than the inhibition of either pathway alone.

The buffering capacity of the receptor-mediated pathway may explain the very modest (<2-fold) rises in plasma ANP levels that are seen in healthy volunteers after dosing with an E-24.11 inhibitor. Larger and more consistent increases in plasma ANP levels have been observed when these compounds have been given in the presence of plasma ANP levels that are already elevated. For example, 2–5-fold increases have been recorded in patients with cardiac failure. Presumably, in these circumstances, the C-receptors are more saturated, and the capacity of this system to compensate is reduced.

Further evidence that E-24.11 is involved in the metabolism of ANP in vivo comes from the measurement of ANP in urine. The efficiency of E-24.11 in the brush-border of the proximal tubule means that, even when plasma ANP levels are increased to pharmacological levels, very little filtered ANP is detected in the urine. Treatment with an E-24.11 inhibitor increases significantly the amount of immunoreactive ANP that is measured in urine. H.p.l.c. analysis suggests that the peptide is intact and the increased exposure of the renal tubule to potentially biologically active peptide may be relevant to the actions of E-24.11 inhibitors in vivo (see below).

Effect of E-24.11 inhibitors on the biological activity of natriuretic peptides
Consistent with their reducing the clearance of the natriuretic peptides in vivo, E-24.11 inhibitors enhance the biological actions of these peptides. Previous administration of an E-24.11 inhibitor
potentiates the increase in urinary sodium and cyclic-GMP excretion that is produced by ANP in the normal rat [15, 16] and, in addition, enhances the hypotensive response to this peptide in the spontaneously hypertensive rat (SHR) [13, 17]. More recently, similar observations have been made for BNP [18] and for urodilatin [19]. Data in man are fewer, but candoxatrilat has been shown to increase the natriuretic response to a low-dose infusion of ANP in healthy volunteers [20].

Response to E-24.11 inhibitors in animal models

Again consistent with an effect mediated by the natriuretic peptides, the renal response to E-24.11 inhibitors appears to be very dependent on intravascular volume status. Given on their own to euolemic animals, these compounds have little or no effect on sodium excretion [16]. When given at the start of acute volume expansion, a manoeuvre that is known to increase endogenous ANP and BNP levels, a 4-5-fold increase in sodium output over an appropriate control group is observed [21].

Heart failure is associated with the chronic elevation of plasma ANP and BNP levels, an attempt by the heart to reduce its work load by reducing intravascular volume and vascular resistance. However, the condition is also hyporesponsive to the effects of infused ANP [12] (and BNP). This resistance is produced by the increased activity of the renin-angiotensin system and by increased renal-sympathetic-nerve activity, together with reduced renal perfusion pressure and, perhaps, the downregulation of natriuretic-peptide receptors. Nonetheless, E-24.11 inhibitors are effective in stimulating a natriuretic response in animal models of this condition. In the aortovenocaval fistula rat, the bolus administration of thiopropan or of candoxatrilat induced a 4-fold rise in urinary sodium excretion from baseline [12, 16] while SQ 28603 produced an 8-fold rise in sodium output in the atrial-paced dog [22]. The renal response in each case occurred without a significant fall in blood pressure. Moreover, the response in these models was more pronounced than that produced by ANP infusions alone, and could not be explained on the basis of the rise in plasma ANP level.

In animal models of hypertension, E-24.11 inhibitors produce greater falls in blood pressure in the deoxycorticosterone acetate (DOCA)-salt rat, a volume-dependent model of hypertension, than the SHR, where blood pressure is genetically determined [13, 14, 23]. For example, SCH 34826 (90 mgkg⁻¹ subcutaneously) has been reported to reduce mean arterial pressure by 35 ± 12 mmHg in the DOCA-salt model but to have no effect on blood pressure in the SHR [24]. Again, this cannot be explained by a difference in circulating plasma ANP levels between the two models; rather the DOCA-salt rat appears to be more sensitive to the actions of ANP than does the SHR. The effect of E-24.11 inhibitors on blood pressure in the SHR (but not the DOCA-salt rat) can be enhanced by the addition of an angiotensin-converting enzyme (ACE) inhibitor [25].

Despite this lack of effect on blood pressure, Monopoli et al. [26] have provided some evidence of benefit from chronic E-24.11 inhibition in the SHR. In these studies, treatment with SCH 34826 (100 mgkg⁻¹ orally, twice daily) for 4 weeks was associated with a small but significant reduction in left-ventricular weight, when compared with vehicle-treated controls.

Clinical studies in cardiac failure and hypertension

Only a few E-24.11 inhibitors have been studied in humans; of these, only candoxatril and sinorphan have been studied in any detail (Table 1).

In an early study, Northridge et al. [7, 11, 27] randomly treated six patients with mild New York Heart Association (NYHA) 2 heart failure either with saline or with UK 69578 (a mixture of candoxatrilat enantiomers) in doses in the range 10–400 mg intravenously. The active compound doubled urinary sodium excretion in the period 4–6 h after dosing compared with saline, with no significant change in potassium excretion. There was no significant fall in blood pressure or change in heart rate, but small falls in right-atrial and pulmonary-capillary-wedge pressures were recorded.

These observations have been confirmed and extended by other groups [28–32]. Munzel et al. [31] have examined the effect of three doses of candoxatrilat (150 mg intravenously) over 24 h in nine patients with severe heart failure (NYHA 3/4). There was an impressive 6-fold increase in sodium excretion after the first dose and sodium excretion remained elevated above the baseline for the 24 h period. There was no significant change in glomerular-filtration rate or in potassium excretion. There was a sustained fall in right-atrial and pulmonary-capillary-wedge pressures, with no significant change in blood pressure or heart rate. Chronic dosing of patients with mild-to-moderate heart failure (NYHA 2/3) with the orally active pro-drug, candoxatril (150 mg twice daily), gives results that
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Table I
Effect of E-24.11 inhibitors in patients with cardiac failure

Abbreviations used: UNa, urinary sodium; Uk, urinary potassium; BP, blood pressure; HR, heart rate; RAP, right-atrial pressure; PCWP, pulmonary-capillary-wedge pressure; t, increase; ↓, decrease; +, no change.

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>ANP</th>
<th>renin</th>
<th>aldosterone</th>
<th>UNa</th>
<th>BP</th>
<th>HR</th>
<th>RAP</th>
<th>PCWP</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candoxatrilat</td>
<td>6</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>↓</td>
<td>↓</td>
<td>7, 11, 27</td>
</tr>
<tr>
<td>Sinorphan</td>
<td>12</td>
<td>↑</td>
<td>↓</td>
<td>↑</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>↓</td>
<td></td>
<td>28</td>
</tr>
<tr>
<td>Sinorphan</td>
<td>12</td>
<td>↑</td>
<td></td>
<td>↑</td>
<td>↓</td>
<td>→</td>
<td>→</td>
<td></td>
<td></td>
<td>29</td>
</tr>
<tr>
<td>Candoxatril</td>
<td>14</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>↓</td>
<td>↓</td>
<td>30</td>
</tr>
<tr>
<td>Candoxatril</td>
<td>12</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>↓</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>Candoxatrilat</td>
<td>9</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>→</td>
<td>→</td>
<td>→</td>
<td>↓</td>
<td></td>
<td>31</td>
</tr>
</tbody>
</table>

suggest that the beneficial haemodynamic effects last through 10 days of treatment [32].

E-24.11 inhibitors have been given for periods up to 28 days to patients with essential hypertension [33-36]. Small (<2-fold) increases in plasma ANP levels and urinary sodium excretion have been observed, but in general the effects on blood pressure have been disappointing. No significant effect on blood pressure has been observed with candoxatril either in single-dosing studies (using doses up to 200 mg) [33, 34] or during chronic treatment (200 mg twice daily, 28 days) [35]. An important exception is the study by Kosoglou et al. [36]. In this study, 24 black patients with mild hypertension (151/104 mmHg) received SCH 34826 (400 mg four times daily) or a placebo for 14 days. Mean supine and diastolic blood pressure fell by 16 and 12 mmHg respectively on active treatment, compared with falls of 3 and 2.6 mmHg on the placebo. The higher prevalence of 'low-renin hypertension' among black hypertensives may be pertinent to the greater hypotensive effect of E-24.11 inhibition in this study.

Mechanism of the cardiovascular and renal effects of E-24.11 inhibitors

Several observations support the thesis that the natriuretic peptides are the major mediators of the cardiovascular and renal actions of E-24.11 inhibitors in vivo. Firstly, these compounds increase plasma ANP and BNP levels and potentiate the activity of these peptides. Secondly, the renal and hypotensive actions of E-24.11 inhibitors are more pronounced when circulating ANP and BNP levels are elevated. Thirdly, E-24.11 inhibition is accompanied by increases in urinary cyclic GMP, a marker of natriuretic-peptide activity. Fourthly, several groups have shown that the effects of E-24.11 inhibitors can be blocked or significantly attenuated by antibodies that have been raised against ANP [14, 21].

Nonetheless, several authors have observed that the effects of E-24.11 inhibitors are more potent than can be explained by the rise in plasma ANP levels accompanying their actions [12, 13, 37]. Part of this discrepancy may be explained by BNP and by CNP. Moreover, E-24.11 inhibitors may enhance the local concentrations of these peptides, rather than simply increasing circulating levels. In this context, an interesting possibility is that the inhibition of E-24.11 in the proximal tubule unmasks a renal tubule-action of filtered ANP and BNP, by allowing these peptides access to tubule sites that are normally protected by the action of the enzyme in the brush border.

The range of substrates that are susceptible to degradation by E-24.11 is extensive, however, and the possibility that other peptides may contribute to the cardiovascular actions of E-24.11 inhibitors cannot be dismissed. Particular attention should be given to the kinins and to the endothelins. Although E-24.11 inhibitors do not potentiate the effects of co-administered bradykinin, bradykinin antagonists have been reported to reduce the renal response to combined treatment with ANP and thiorphan [38]. This suggests that kinins, perhaps locally released in the kidney, may have a permissive role in the effect of E-24.11 inhibitors on ANP-induced natriuresis. An interesting possibility is that the inhibition of endothelin synthesis may participate in the hypotensive response to some E-24.11 inhibitors. Phosphoramidon, SQ 28603 and, to a lesser extent, thiorphan have been reported to reduce the conversion of big endothelin to endothelin-1, and to reduce the pressor response to big endothelin in the rat [39-41].
The competition
As a novel approach to treating cardiac failure and hypertension, E-24.11 inhibitors have to be considered alongside diuretics and ACE inhibitors. These drugs form the cornerstone of therapy for cardiac failure and are widely used anti-hypertensive agents.

Diuretics are cheap, effective and well-tolerated, but have metabolic side effects that are of increasing clinical concern (Table 2). In particular, these drugs cause hypokalaemia, hyperuricaemia, and may raise serum lipids. Over-use can cause serious dehydration with renal impairment. In contrast, E-24.11 inhibitors are relatively potassium sparing, and do not affect uric-acid excretion. Another attractive attribute is that the risks of dehydration with these compounds should be minimal; ANP and BNP levels should fall with successful diuresis, checking the action of these drugs, as the need for them diminishes.

An important difference from conventional diuretics is that E-24.11 inhibitors act to suppress rather than activate the renin–angiotensin system. These compounds are in fact complementary to ACE inhibitors (Table 3). While ACE inhibitors reduce angiotensin II synthesis and aldosterone levels, E-24.11 inhibitors act to suppress the secondary activation of renin secretion and to further reduce aldosterone levels. Both inhibitors act to increase bradykinin levels. The renin–aldosterone system acts to antagonize the activity of the natriuretic peptides, and co-inhibition of E-24.11 and ACE would be expected to have greater effects on sodium excretion and on blood pressure than inhibition of E-24.11 alone. Support for this approach has come from studies in the rat and in the dog [25, 42] and there is current interest in the development of mixed-profile compounds that exhibit both E-24.11 and ACE inhibitory activity.

Conclusion
E-24.11 inhibitors reduce the clearance and enhance the biological actions of natriuretic peptides in vivo. Acute-dosing studies indicate that these compounds exhibit a favourable profile of renal and haemodynamic effects in patients with cardiac failure. There is still concern, however, about tolerance to these agents, and the results of long-term dosing studies are awaited. E-24.11 inhibitors have not proved to be potent hypotensive agents, but may be effective in a sub-group of patients with low-renin hypertension. They may also be useful in preventing the secondary activation of the renin–aldosterone system by other anti-

Table 2
Comparison of E-24.11 inhibitors with conventional diuretic agents

<table>
<thead>
<tr>
<th>Drug</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional diuretics</td>
<td>Cheap and effective</td>
<td>Can cause metabolic upset</td>
</tr>
<tr>
<td></td>
<td>Long safety record</td>
<td>Can over-dehydrate</td>
</tr>
<tr>
<td>E-24.11 inhibitors</td>
<td>Relatively K⁺ sparing</td>
<td>Activate renin–aldosterone system</td>
</tr>
<tr>
<td></td>
<td>May not cause metabolic upset</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Should not over-dehydrate</td>
<td>? Safety record</td>
</tr>
<tr>
<td></td>
<td>Do not activate renin–aldosterone system</td>
<td></td>
</tr>
</tbody>
</table>

Table 3
Comparison of effects of E-24.11 inhibitor and ACE inhibitor on renin–angiotensin–aldosterone system

<table>
<thead>
<tr>
<th>Hormone</th>
<th>E-24.11 inhibitor</th>
<th>ACE inhibitor</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin</td>
<td>↓ secretion</td>
<td>↑ secretion</td>
<td>↓ or → secretion</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>↓ action but ↑ levels</td>
<td>↑ production</td>
<td>↑ production</td>
</tr>
<tr>
<td>Aldosterone</td>
<td>↓ secretion</td>
<td>↓ secretion</td>
<td>↓ secretion</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>↑ levels</td>
<td>↑ levels</td>
<td>↑ levels</td>
</tr>
</tbody>
</table>
hypertensive agents. E-24.11 inhibitors are complementary and additive to ACE inhibitors, and studies with mixed-profile (combined E-24.11 and ACE inhibitory activity) will be of considerable interest. Meanwhile, if the anti-trophic property of ANP can be harnessed by E-24.11 inhibitors, this will further strengthen enthusiasm for pursuing this novel class of compounds.

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1993
Zinc metallopeptidases: Active site structure and design of selective and mixed inhibitors: New approaches in the search for analgesics and anti-hypertensives

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Introduction

A considerable interest has been devoted to ecto-enzymes (membrane-bound peptidases) since the discovery that angiotensin-converting enzyme (ACE), the enzyme implicated in the formation of angiotensin II from angiotensin I, had anti-hypertensive effects [1], and three years later the discovery that the inhibition of another membrane-bound Zn-metallopeptidase, involved in the inactivation of the opioid enkephalins in the brain, induced analgesic responses [2]. Due to its activity in the brain, the latter enzyme was designated ‘enkephalinase’, but soon after was shown to be identical to the neutral endopeptidase EC 24.11 (NEP), a well-characterized enzyme demonstrated to be present in brush-border cells of the kidney, intestine etc. [3]. Ecto-enzymes such as ACE or NEP are widely distributed and the latter was shown to participate in clearing the circulating atrial natriuretic peptide (ANP) from the plasma [4, 5]. Recently NEP was demonstrated to correspond with CD10 or common acute lymphoblastic antigen (CALLA) a lymphocyte marker.

Despite their relatively broad specificities, a certain in vivo specificity seems to be achieved for these peptidases, governed both by their distribution and by that of their potential substrates.

In contrast with classical neurotransmitters, the activation or the interruption of the responses that are induced by regulatory peptides is ensured by ecto-enzymes. Therefore, the pharmacological effects resulting from the inhibition of these enzymic processes will appear only in tissues where the peptide substrate is tonically or phasically released. This was expected to avoid, or at least to minimize, the side effects resulting from excessive and ubiquitous stimulation of peptide receptors by exogenously administered agonists or antagonists [6]. The results summarized here show that this hypothesis has been verified in the case of mixed inhibitors of NEP and aminopeptidase N, or NEP and ACE, leading to new analgesics and anti-hypertensive agents that are now under clinical trials. Furthermore, selective NEP inhibitors could be used in cardiac failure in the near future because of their ability to potentiate the diuretic, natriuretic and vasorelaxant effect of ANP. Moreover thiorphan, the first synthetic inhibitor of NEP [2] is now in the market under the trademark thiorfan®, as a novel anti-diarrhoeal agent, capable of potentiating the anti-secretory action of the enkephalins acting in intestinal cells on δ-opioid receptors, but not their effect on smooth-muscle contraction, thus avoiding the rebound constipation that is observed with non-selective opioids such as loperamide [7, 8].

Substrate specificity and mechanism of action of NEP

The Zn-metallopeptidases form a large group of enzymes that includes, apart from NEP, aminopeptidase N, carboxypeptidases A, B and E, ACE, collagenases and the bacterial endopeptidase, thermolysin (TLN; review in [9]). As shown from the crystallographic analyses of TLN, all the Zn-metallopeptidases have similarities in their active sites and in their respective mechanisms of action [10]. Crystallographic studies of TLN complexed with carboxyl or with hydroxamate inhibitors have

Abbreviations used: ACE, angiotensin-converting enzyme; ANP, atrial natriuretic peptide; HACBO-Gly, N-[(2RS)-4-(hydroxy amino)-1, 4-dioxo-2-(phenylmethyl)-butyl]-glycine; NEP, neutral endopeptidase EC-24.11; TLN, thermolysin.

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Further Reading


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