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Pharmacokinetic and pharmacodynamic properties of SOL1: A novel dual inhibitor of neutral endopeptidase and endothelin converting enzyme

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ABSTRACT

Aims: The pharmacological profile of the novel putative neutral endopeptidase (NEP) and endothelin converting enzyme (ECE) inhibitor SOL1 was examined.
Main methods: The enzyme inhibitory profile of SOL1 was established in vitro. The pharmacokinetic and pharmacodynamic profile was determined in rodents in vivo.
Key findings: In vitro, at neutral pH, 10 μM SOL1 inhibited NEP-1, NEP-2, and ECE-1 by 99%, 94% and 75%, respectively. The IC_{50}s were 25, 25 and 3200 nmol/L, respectively. In anesthetized rats, SOL1 inhibited blood pressure (BP) responses to big-ET-1 and ET-1(1–31) with ED_{50}s of 1.9 and 0.03 mg/kg, corresponding to plasma EC_{50}s of 4.6 and 0.1 μmol/L, respectively. Pharmacokinetics of SOL1 were examined after single injections in mice and rats. In these species, the estimated clearance of SOL1 varied between 5 and 9 ml/kg.min and $T_{1/2}$ between 20 and 60 min. Steady state kinetics of SOL1 were examined after continuous s.c. infusions of SOL1 for 3 weeks at 50 mg/kg.day in DOCA-salt hypertensive rats. This treatment lowered BP by 22 mmHg. Steady state concentrations of SOL1 in plasma were 3.9 μmol/L. In heart, lung, and kidney the concentrations of SOL1 were 0.4, 1.8, and 20.5 μmol/kg, respectively. About 63% of the daily dose was retrieved unaltered in the urine.
Significance: These data indicate that SOL1 is primarily a NEP inhibitor in vitro as well as in vivo. Given the preferential renal accumulation and renal clearance of SOL1 additional ECE-1 inhibition in the kidney may have contributed to its chronic BP lowering effects in the DOCA-salt hypertensive rat model.

Introduction

Endothelin (ET-1) is a pluripotent peptide that affects virtually all organ systems that regulate blood pressure (BP) and sodium homeostasis (Kohan et al., 2011). Soon after the discovery of ET-1 and its ET_A- and ET_B-receptors the potential therapeutic benefit of inhibiting the ET-axis was proven in several experimental animal models and clinical trials. $ET_{A/B}$ receptor antagonists have been developed to antagonize ET-1 related effects downstream of its synthesis. While these receptor antagonists are potentially effective in the treatment of several cardiovascular diseases, their clinical application is currently limited because of the high incidence of renal side effects leading to hemodilution and edema formation (Kirkby et al., 2008). This may be explained by blockade of the ET-1 mediated pro-natriuretic effects. Recent observations suggest that in addition to ET_B receptors also ET_A receptors may mediate sodium excretion (Kohan et al., 2011). One approach to overcome the side effects of $ET_{A/B}$ receptor blockade is to further unravel the complexity of the intra-, auto- and paracrine functions of ET-1. Alternatively, as addressed in the present study, one may also target the formation of ET-1.

ET-1 can be formed by proteolytic cleavage of its inactive precursor big-ET-1 (ET-1(1–38)) by endothelin converting enzymes (ECE, Fig. 1). These metalloproteases are structurally related to neutral endopeptidase (NEP), an enzyme that converts not only chymase derived ET-1(1–31) to ET-1 but also degrades well-known vasodilators such atrial natriuretic peptide (ANP), bradykinin (BK) and calcitonin gene-related peptide (CGRP). Dual inhibitors of ECE-1 and NEP have been synthesized by a number of groups (Jeng et al., 2002) with varying selectivity for ECE-1 and NEP. These enzyme inhibitors are less advanced in clinical development than ET-receptor antagonists. In preclinical studies dual NEP/ECE inhibitors appear not as potent as ET_A -antagonists in reducing elevated BP. Two recent studies (Kalk et al., 2011; Wengenmayer et al., 2011) suggest that their potential benefit may depend on their ability to alter the bioavailability of vasoactive peptides in an organ-specific manner rather than on their BP-lowering effect (Dhaun and Webb, 2011).

Here we report the pharmacokinetic and pharmacodynamic properties of a novel dual NEP/ECE inhibitor named SOL1. Our preclinical studies support the concept that the efficacy of SOL1 depends on the renal specific accumulation of the compound and hence varying local degrees of NEP/ECE inhibition.

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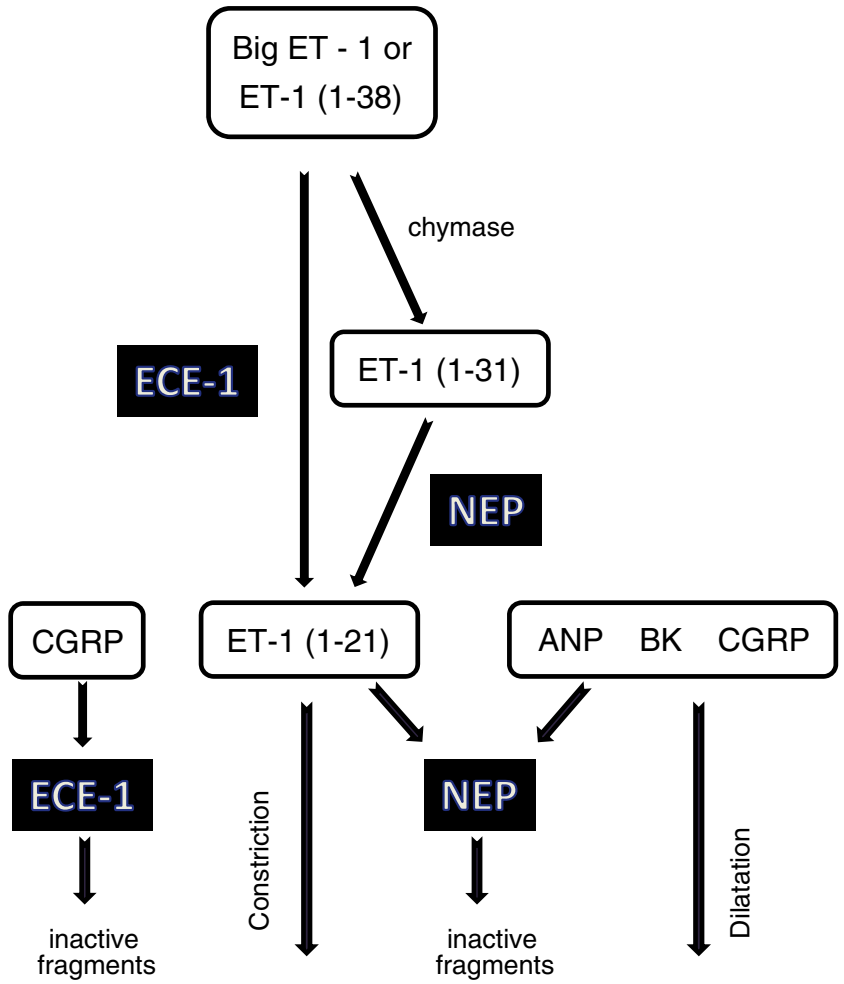

Fig. 1. Schematic overview of the potential role of endothelin (ET-1) converting enzyme (ECE-1) and neutral endopeptidase (NEP) in the synthesis and breakdown of ET-1. Big-ET-1 and ET-1(1–31) were administered in rats to assess the potential of SOL1 to inhibit the contribution of ECE-1 and NEP to the ET-1 induced increase in blood pressure, respectively. Note that ECE-1 can break down calcitonin gene related peptide (CGRP), while NEP is active against all atrial natriuretic peptide (ANP) and bradykinin (BK), CGRP as well as ET-1.

Methods

Chemicals

Benzazepine acid derivatives are well known NEP inhibitors (Brands et al., 2006; Jeng et al., 2002). The chemical name of SOL1 is [2-{[1-({[(3*S*)-1-(carboxymethyl)-2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepin-3-yl]amino}carbonyl)cyclopentyl]methyl}-4-[[3-(methylamino)propyl](methyl)amino]-4-oxobutanoic acid]. The dual NEP/ECE inhibitory profile was obtained by coupling a cyclopentyl group to benzazepine using a retrosynthetic strategy (Fig. 2). Details of the synthesis of this compound are given in the supplementary data. In the present study sodium salt batches of SOL1 were used.

In vitro enzyme inhibition

The enzyme inhibitory profile of SOL1 was determined in vitro using recombinant sources of NEP and ECE proteins (Innogenetics,

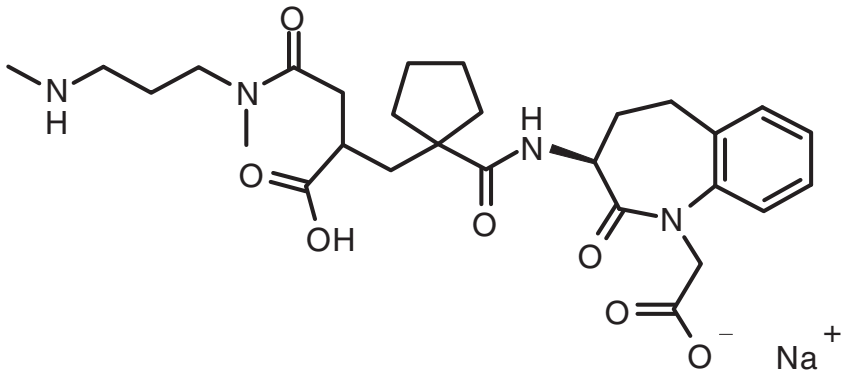

Fig. 2. Structure of SOL1. The chemical name is 2-{[1-({[(3*S*)-1-(carboxymethyl)-2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepin-3-yl]amino}carbonyl)cyclopentyl]methyl}-4-[[3-(methylamino)propyl](methyl)amino]-4-oxobutanoic acid. The molecular weight of SOL1 is 544.6.