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Binding kinetics and duration of in vivo action of novel prolyl oligopeptidase inhibitors

Jarkko I. Venäläinen [a,*], J. Arturo Garcia-Horsman [a], Markus M. Forsberg [a], Aaro Jalkanen [a], Erik A.A. Wallén [b], Elina M. Jarho [b], Johannes A.M. Christiaans [b,1], Jukka Gynther [b], Pekka T. Männistö [a,2]

[a] Department of Pharmacology and Toxicology, University of Kuopio, P.O. Box 1627, Kuopio FI-70211, Finland
[b] Department of Pharmaceutical Chemistry, University of Kuopio, P.O. Box 1627, Kuopio FI-70211, Finland

ARTICLE INFO

Article history:
Received 1 September 2005
Accepted 29 November 2005

Keywords:
Prolyl oligopeptidase
Inhibitor
Tight binding inhibition
Slow binding inhibition
Pharmacokinetics

ABSTRACT

Prolyl oligopeptidase (POP) is a serine protease that specifically hydrolyses small peptides at the carboxyl end of the proline residue. POP has gained pharmaceutical interest, since its inhibitors have been shown to have antiamnesic properties in rat. We examined the effect of the 2(*S*)-substituents CN and $COCH_2OH$ at the P1 site of the parent inhibitors isophthalic acid 2(*S*)-(cyclopentanecarbonyl)pyrrolidine-L-prolyl-pyrrolidine amide and 4-phenylbutanoyl-L-prolyl-pyrrolidine and bulky 5-*t*-butyl group at the P2 site L-prolyl residue of the parent inhibitor 4-phenylbutanoyl-L-prolyl-pyrrolidine on the binding kinetics to the enzyme. In addition, we studied the duration of POP inhibition in the rat tissues in vivo after i.p. administration. CN and $COCH_2OH$ substituents at the P1 site pyrrolidine group were found to greatly increase the affinity of the inhibitor and the enzyme–inhibitor complex half-life. In addition, 5-*t*-butyl group at the P2 site L-prolyl residue increased the dissociation half-life of the enzyme–inhibitor complex, without much affecting the inhibitory potency. The duration of the inhibition in the rat tissues followed the inhibition kinetic properties in that the compounds with fast dissociation produced shorter inhibition in the rat tissues than the compounds with slow dissociation. The duration of POP inhibition of compounds was evidently not governed by their serum clearance. The fact that the in vivo pharmacodynamic behaviour of POP inhibitors can be predicted by their in vitro-properties may be of importance when designing therapeutically useful POP inhibitors.

1. Introduction

Prolyl oligopeptidase (POP) (EC 3.4.21.26) is an 80 kDa enzyme that belongs to a POP family of serine proteases. The POP family includes POP, dipeptidyl peptidase IV (EC 3.4.14.5), oligopeptidase B (EC 3.4.21.83) and acylaminoacyl peptidase (EC 3.4.19.1). This enzyme family is of ancient origin and it is different from the classical trypsin- and subtilisin-like serine proteases in their selectivity for small peptide subtrates and in the order of catalytic triad residues [1–3].

POP preferentially hydrolyses small (<30 aa) peptides at the carboxyl side of a proline residue. POP degrades several proline

* *Corresponding author*. Tel.: +358 17163773; fax: +358 17162424.
E-mail address: Jarkko.Venalainen@uku.fi (J.I. Venäläinen).
[1] Present address: Altana Pharma bv, P.O. Box 31, 2130 AA Hoofddorp, The Netherlands.
[2] Present address: Division of Pharmacology and Toxicology, University of Helsinki, P.O. Box 56, FI-00014 Helsinki, Finland.
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2005.11.029

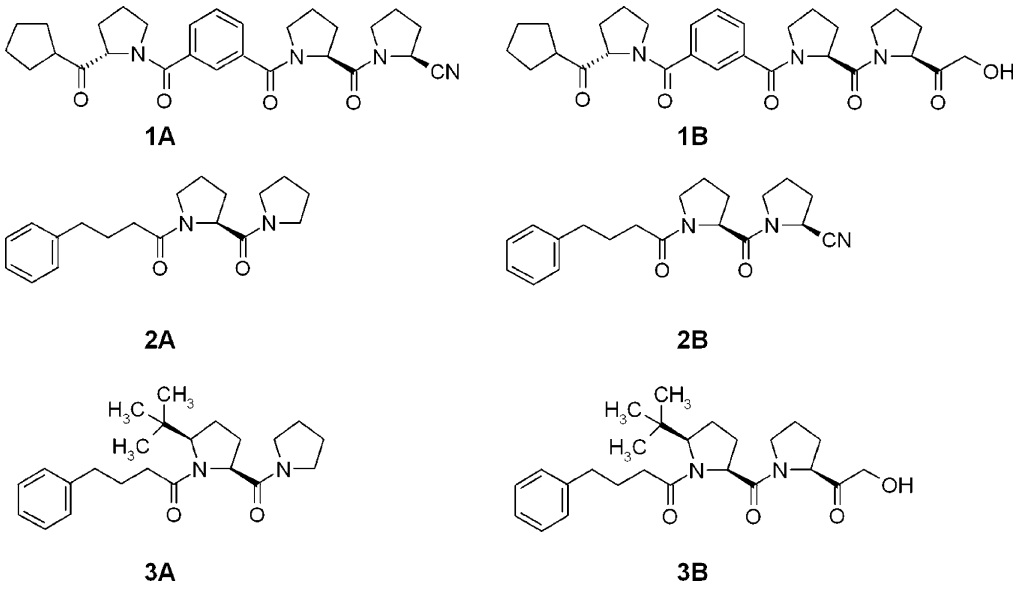

Fig. 1 – The prolyl oligopeptidase inhibitors used in this study: (1A) isophthalic acid 2(*S*)-(cyclopentanecarbonyl)pyrrolidine-L-prolyl-2(*S*)-cyanopyrrolidine amide; (1B) isophthalic acid 2(*S*)-(cyclopentanecarbonyl)pyrrolidine-L-prolyl-2(*S*)-(hydroxyacetyl)pyrrolidine amide; (2A) 4-phenylbutanoyl-L-prolyl-pyrrolidine (SUAM-1221); (2B) 4-phenylbutanoyl-L-prolyl-2(*S*)-cyanopyrrolidine; (3A) 4-phenylbutanoyl-5(*R*)-*tert*-butyl-L-prolyl-pyrrolidine; (3B) 4-phenylbutanoyl-5(*R*)-*tert*-butyl-L-prolyl-2(*S*)-(hydroxyacetyl)pyrrolidine.

containing neuropeptides in the central nervous system and several of its substrates, such as substance P, vasopressin, neurotensin and thyroliberin, are involved in learning and memory [4]. In addition, low levels of substance P are commonly found in the brains of Alzheimer's patients and administration of substance P has been reported to block β-amyloid-induced neurotoxicity [5,6]. Confusingly, both increased and decreased POP activity levels have been reported from post mortem brain tissues of Alzheimer patients [7–9]. It was recently reported that POP gene transcription was decreased when mice were exposed to an enriched environment and the transcription was increased many-fold in hypothalamus and cortex from aged mice [10,11]. As a summary, centrally acting POP inhibitors might theoretically be beneficial in patients with cognitive disturbances. Indeed, POP inhibitors have been shown to increase the brain levels of several neuropeptides, to reverse scopolamine-induced amnesia in rats and to improve cognition in old rats and MPTP-treated monkeys [12–15]. POP inhibitors have also been reported to prevent β-amyloid deposition in a neuroblastoma cell line and in senescence-accelerated mouse [16,17]. The possible cognition enhancing properties of POP inhibitors has gained pharmaceutical interest and several potent compounds have been synthesized. To date, however, none of the compounds has entered into the market.

Recently we have studied the effect of different 2(*S*)-substituents at pyrrolidine ring at the P1 site of the parent inhibitor dicarboxylic acid bis(L-prolyl-pyrrolidine) amide on the binding kinetics to the active site of POP [18]. We found that the addition of 2-(*S*)-substituents CN, $COCH_2OH$ or CHO at the inhibitor P1 site pyrrolidine ring results in slow binding inhibition kinetics and slow dissociation from the enzyme active site. It has been shown that Z-prolylprolinal with 2(*S*)-CHO substituent at the P1 site pyrrolidine ring forms a hemiacetal adduct with the Ser554 residue at the active site of POP [19]. It is probable that the CN and $COCH_2OH$ groups act similarly and form imino ether and hemiketal adducts with the active site Ser554 residue and these covalent adducts cause the slow binding and dissociation. Slow binding inhibition is a phenomenon in which inhibition of enzyme activity occurs relatively slowly and not at diffusion controlled rates. Analysis of slow binding inhibition allows the calculation of enzyme–inhibitor association and dissociation rates [20]. In this study, we determined the slow binding inhibition kinetic parameters and ex vivo inhibition profiles of six potent POP inhibitors with 2-(*S*)-substituents CN or $COCH_2OH$ at the P1 site pyrrolidine ring and 5-*t*-butyl substituent at the P2 site prolyl residue (see Fig. 1). K_i, the association and dissociation rate constants and the dissociation half-lives of the enzyme–inhibitor complex were measured using purified pig POP. In animal experiments, rats were given single i.p. injections of the inhibitors and the liver and brain POP activities were measured ex vivo at different time points. The correlation between the duration of POP inhibition and the in vitro dissociation half-life of the inhibitor was examined. In some cases also serum clearance of the compounds was measured.

2. Materials and methods

2.1. Compounds and reagents

Compounds **1A**, **1B**, **2B**, **3A** and **3B** were synthesized in the Department of Pharmaceutical Chemistry, University of Kuopio as described earlier [21–24]. The reference compound SUAM-1221 [25] (compound **2A**) was synthesized using slightly