European Journal of Pharmacology 586 (2008) 306–312

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Novel triple neurokinin receptor antagonist CS-003 strongly inhibits neurokinin related responses

Hiroshi Tsuchida [a], Sakiko Takahashi [a], Emi Nosaka [a], Osamu Mukaiyama [b], Makoto Yamashita [c], Kiyoshi Morimoto [a,*]

[a] Biological Research Laboratories III, Daiichi Sankyo Co., Ltd., 1-16-13 Kitakasai, Edogawa-ku, Tokyo, 134-8630, Japan
[b] Global Project Management Department, Daiichi Sankyo Co., Ltd., Tokyo, Japan
[c] Biological Research Laboratories IV, Daiichi Sankyo Co., Ltd., Tokyo, Japan

ARTICLE INFO

Article history:
Received 28 August 2007
Received in revised form 20 February 2008
Accepted 20 February 2008
Available online 4 March 2008

Keywords:
CS-003
Neurokinin
Tachykinin
Triple neurokinin receptor antagonist
Capsaicin
Cough

ABSTRACT

Neurokinins are known to induce neurogenic inflammation related to respiratory diseases, though there is little information on triple neurokinin receptor antagonists. The pharmacological properties of the novel triple neurokinin 1, 2 and 3 receptor antagonist [1-{2-[(2*R*)-(3,4-dichlorophenyl)-4-(3,4,5-trimethoxybenzoyl) morpholin-2-yl]ethyl}spiro[benzo[*c*]thiophene-1(3*H*),4′-piperidine]-(2*S*)-oxide hydrochloride] (CS-003) were evaluated in this study. The binding affinities of CS-003 were evaluated with human and guinea pig neurokinin receptors. As well, the *in vivo* antagonism of CS-003 against exogenous neurokinins and effects on capsaicin-induced and citric acid-induced responses were investigated in guinea pigs. CS-003 exhibited high affinities for human neurokinin 1, neurokinin 2 and neurokinin 3 receptors with K_i values (mean±S.E.M.) of 2.3±0.52, 0.54±0.11 and 0.74±0.17 nM, respectively, and for the guinea pig receptors with K_i values of 5.2±1.4, 0.47±0.075 and 0.71±0.27 nM, respectively. Competitive antagonism was indicated in a Schild analysis of substance P-, neurokinin A- and neurokinin B-induced inositol phosphate formation with pA_2 values of 8.7, 9.4 and 9.5, respectively. CS-003 inhibited substance P-induced tracheal vascular hyperpermeability, neurokinin A- and neurokinin B-induced bronchoconstriction with ID_{50} values of 0.13, 0.040 and 0.063 mg/kg (i.v.), respectively. CS-003 also inhibited capsaicin-induced bronchoconstriction (ID_{50}: 0.27 mg/kg, i.v.), which is caused by endogenous neurokinins. CS-003 significantly inhibited citric acid-induced coughs and the effect was greater than those of other selective neurokinin receptor antagonists. CS-003 is a potent antagonist of triple neurokinin receptors and may achieve the best therapeutic efficacy on respiratory diseases associated with neurokinins compared to selective neurokinin receptor antagonists.

1. Introduction

Neurokinins (or tachykinins), which include substance P, neurokinin A and neurokinin B, are members of a family of small peptides with a common C-terminal sequence of Phe-X-Gly-Leu-Met-NH_2. Three types of G protein-coupled receptors, named neurokinin 1, neurokinin 2 and neurokinin 3, mediate the biological effects of neurokinins. Substance P interacts preferentially with neurokinin 1 receptor, neurokinin A with neurokinin 2 receptor and neurokinin B with neurokinin 3 receptor (Helke et al., 1990). Activation of the receptors induces stimulation of phospholipase C, resulting in phosphoinositide breakdown and the elevation of intracellular calcium (Maggi et al., 1993; Otsuka and Yoshioka, 1993).

Neurokinins are widely distributed in the whole body and in the airways they are found in unmyelinated pulmonary C fibers which are sensitive to capsaicin (Otsuka and Yoshioka, 1993; Maggi, 1995). Neurokinins released by mechanical and chemical stimuli elicit a wide range of biological actions in mammalian airways (Joos et al., 2003). Neurokinin 1 receptors are primarily distributed on the vascular endothelium and epithelial cells and within the mucus glands. Furthermore, various cells that have infiltrated the lungs in inflammatory diseases may also express neurokinin 1 receptors. Neurokinin 2 receptors are primarily associated with the airway smooth muscle. Epithelial cells and some inflammatory cells may also express neurokinin 2 receptors (Mazzone, 2004). Neurokinin 3 receptors have been shown to be present in airway parasympathetic ganglia, where they play an important role in regulating parasympathetic nerve excitability (Phillips et al., 2003; Myers et al., 2005). In recent years, it has become clear that immune cells are additional sources of neurokinins (Maggi, 1997) and that epithelial cells may also produce substance P (Chu et al., 2000).

Coinciding with these reports, all three neurokinin receptors are also reported to mediate respiratory responses. For example, they induce vascular hyperpermeability, bronchoconstriction, airway hyperresponsiveness, cough and mucus secretion (Chapman et al., 1998).

* Corresponding author. Tel.: +81 3 3680 0151; fax: +81 3 5696 8347.
E-mail address: morimoto.kiyoshi.f4@daiichisankyo.co.jp (K. Morimoto).

0014-2999/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.02.056

Since these effects are characteristic of patients suffering from respiratory diseases such as asthma, rhinitis and chronic obstructive pulmonary disease, it has been proposed that neurokinin receptor antagonists may be useful for the treatment of these respiratory diseases (Barnes, 1986; Mazzone, 2004). Furthermore, it is reported that neurokinins and their receptors are upregulated in several respiratory diseases (Nieber et al., 1992; Adcock et al., 1993; Heaney et al., 1998; Bardelli et al., 2005).

Although there is a great deal of information available on neurokinins, only two triple neurokinin receptor antagonists have been reported (Rumsey et al., 2001; Anthes et al., 2002). However, besides the contribution of all three receptors to respiratory diseases, all three receptor subtypes are known to interact with all of the endogenous neurokinins (Severini et al., 2002; Pennefather et al., 2004). Thus, antagonizing only one neurokinin receptor subtype may not be enough for the treatment of respiratory diseases and we have developed compounds with high affinities for all of three neurokinin receptors (Nishi et al., 2000). This study describes the pharmacological properties of CS-003, a novel, triple neurokinin 1, 2 and 3 receptor antagonist.

2. Materials and methods

2.1. Animals

Male Hartley guinea pigs (Japan SLC, Inc., Hamamatsu, Japan) were purchased (5–6 weeks old, 301–400 g) and housed in aluminum cages in a room set at a room temperature of 23±2 °C, humidity of 55±5% and with a 12 h lighting cycle (7:00–19:00). The animals were fasted for about 24 h before the experiments. The experimental procedures employed in this study were in accordance with the guidelines of the Institutional Animal Care and Use Committee at Sankyo Research Laboratories (Tokyo, Japan).

2.2. Reagents

CS-003 [1-{2-[(2*R*)-(3,4-dichlorophenyl)-4-(3,4,5-trimethoxybenzoyl)morpholin-2-yl]ethyl}spiro[benzo[*c*]thiophene-1(3*H*),4′-piperidine]-(2*S*)-oxide hydrochloride] was synthesized by Sankyo Co., Ltd. (Tokyo, Japan). The chemical structure of CS-003 is shown in Fig. 1. FK888 (neurokinin 1 receptor antagonist, N^2-[(4*R*)-4-hydroxy-1-(1-methyl-1*H*-indol-3-yl)carbonyl-L-prolyl]-*N*-methly-*N*-phenylmethyl-3-(2-naphthyl)-L-alaninamide) and SB 223956 (neurokinin 3 receptor antagonist, (−)-3-methoxy-2-phenyl-*N*-[(1*S*)-phenylpropyl]quinoline-4-carboxamide) were synthesized by Sankyo Co., Ltd. SR 48968 (neurokinin 2 receptor antagonist, (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) was synthesized by Chemtech Labo., Inc. (Tokyo, Japan). In the *in vivo* experiments, all the test compounds were intravenously injected (i.v.) to the animals 5 min before administration of the stimulating agents.

[^{3}H]Substance P, [^{3}H]SR 48968 and *myo*-[^{3}H]inositol were purchased from Amersham Pharmacia Biotech Ltd. (Tokyo, Japan). [^{3}H]Senktide was purchased from PerkinElmer Life Sciences, Inc. (Boston, MA, USA). Substance P, neurokinin A and neurokinin B were obtained from Peptide Institute Inc. (Osaka, Japan). Succinyl-[Asp^6, N-Me-Phe^8]-substance P fragment 6–11 (senktide), gallamine triethiodide, capsaicin and citric acid were obtained from Sigma Chemical Company (St. Louis, MO, USA) and Evans blue from Merck KGaA (Darmstadt, Germany). Pentobarbital sodium (Abbott Laboratories, Abbott Park, IL, USA) was used to anesthetize the animals.

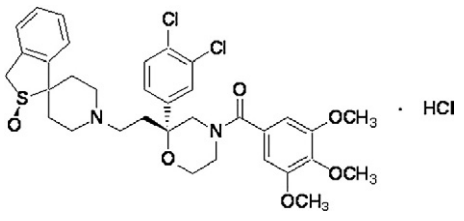

Fig. 1. Chemical structure of CS-003.

2.3. Receptor binding assay

Receptor binding assays were performed with [^{3}H]substance P, [^{3}H]SR 48968 and [^{3}H]senktide as ligands for neurokinin 1, neurokinin 2 and neurokinin 3 receptor binding assays, respectively, according to the method described by Kudlacz et al. (1996) with some modifications. For the human receptor assays, crude membranes prepared from 1.25×10^6 cells of COS cells stably expressing human neurokinin 1 receptor (GeneBank: M81797), human neurokinin 2 receptor (GeneBank: M57414) or human neurokinin 3 receptor (GeneBank: M89473) were used for each assay tube. For the guinea pig receptor assays, crude membranes containing 0.25 mg protein prepared from guinea pig lung, ileum or brain were used for the neurokinin 1, neurokinin 2 and neurokinin 3 receptor binding assays, respectively. The binding assay was initiated by incubating the membrane preparations with the radioactive ligand (0.050–15 nM) in 50 mM Tris–HCl buffer (pH 7.4). The reaction was terminated by the addition of ice cold buffer after 35 min incubation, and the radioactivity of the membrane preparation harvested on a GF/B glass microfiber filter (Whatman International Ltd., Maidstone, Kent, UK) was counted using a liquid scintillation counter (TRI-CARB 2300TR, PerkinElmer Inc., Wellesley, MA, USA). Non-specific binding was determined in the presence of 10 μM unlabeled ligand, and specific binding was calculated by subtracting the non-specific binding from the total binding. The inhibitory effects of the antagonists were evaluated with 1.0 nM of radioactive ligands. Dissociation constant (K_d) values were computed with KELL software (Biosoft, Great Shelford, Cambridge, UK) and the 50% inhibitory concentration (IC_{50}) values were determined by linear regression. The inhibition constant (K_i) values were calculated according to Cheng and Prusoff (1973) and expressed as the means of three independent experiments in triplicate.

2.4. Inositol phosphate formation

Measurement of inositol phosphate formation in cells expressing human neurokinin receptors was performed according to the method described by Kudlacz et al. (1996) with a slight modification. COS cells expressing human neurokinin receptors were cultured with Dulbecco's Modified Eagle Medium (D-MEM) containing 74 kBq/ml of *myo*-[^{3}H]inositol and incubated for 24 h before the assay. After the medium was replaced with inositol-free D-MEM, CS-003 was added and the cells were incubated for 15 min. Then they were stimulated with neurokinins and incubated for 60 min. The reaction was terminated by eluting the cell content with Triton and the cell content was then loaded onto a poly-prep prefilled chromatography column (AG 1-X8, Bio-Rad Laboratories Inc., Hercules, CA, USA). Inositol phosphate was eluted from the column with 0.1 M formic acid/0.4 M ammonium formate (1:1, v/v) and the radioactivity was counted using the liquid scintillation counter. Data are expressed as a percent of the agonist-induced maximum formation of [^{3}H]inositol phosphate over the basal levels. The pA_2 value, the negative logarithm of the molar concentration of an antagonist that produces a two-fold shift to the right of the agonist concentration curve, and the slope were determined by a regression analysis of Schild plots (Arunlakshana and Schild, 1959).

2.5. Tracheal vascular hyperpermeability in guinea pigs

The effects of the compounds on substance P-induced tracheal vascular hyperpermeability were measured according to the method of Rogers et al. (1988). Briefly, Evans blue dye (20 mg/kg) was injected