

UR-60427, a novel H₄ receptor-inverse agonist that shows good efficacy in a rat asthma model

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Introduction

Histamine H₄ receptor (H₄R) has an important role in immune and inflammatory diseases [1]. A pivotal study by Dunford et al. [2] has shown that treatment with the H₄R antagonist JNJ7777120 shows a similar pattern of efficacy in a mouse model of asthma compared to the H₄R knock-out mice. However, as this compound has impaired pharmacokinetic properties [3], its relevance as a pharmacological tool is limited. In our efforts to find new active H₄R substances, we have identified UR-60427 from the lead compounds investigated at Palau Pharma as a new ligand, and its pharmacological profile is described here.

Materials and methods

The affinity of UR-60427 to H₄R was determined in purified membranes over-expressing the human receptor.

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A displacement assay was employed using different [³H]-histamine concentrations equivalent to x times histamine K_d to H₄R (0.8, 1.7, 3.3 and 6.6), and 100 μ M unlabeled histamine to define nonspecific binding. K_i was calculated as the Y -intercept of the linear regression of [histamine]/ K_d versus IC_{50} . Binding displacement assays to H₁, H₂ and H₃ receptors were performed with [³H]pyrilamine, [¹²⁵I]APT, and [³H]N- α -methylhistamine respectively. An extended selectivity profile, including 43 additional off-targets was performed at Cerep. [³⁵S]GTPgammaS assay was performed with purified membranes prepared from a stable cell line over-expressing the human H₄R that was coupled to PVT-WGA beads. The effects of histamine and thioperamide (previously reported as full agonist and inverse agonist respectively [4]) were also compared.

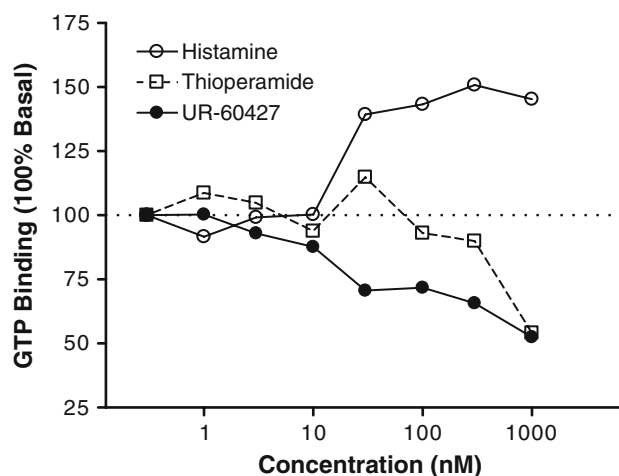


Fig. 1 [³⁵S]GTPgammaS binding assay and effects of UR-60427 compared with thioperamide and histamine. UR-60427 and thioperamide behaved as inverse agonists as shown by their reduced GTPgammaS binding, while histamine, an agonist, increased it

Table 1 Airway hyperreactivity (R_L) and cell (total and eosinophils) influx in BAL of OVA-sensitised and challenged rats

	Unsensitised	Sensitised	Dexa (1 mg/kg)	UR (30 mg/kg)	UR (10 mg/kg)	UR (3 mg/kg)	UR (1 mg/kg)
Pulmonary resistance (% baseline)							
5-HT (0 µg/kg)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
5-HT (5 µg/kg)	108 ± 3	110 ± 2	102 ± 2*	96 ± 11	110 ± 4	103 ± 2	105 ± 4
5-HT (10 µg/kg)	129 ± 7	150 ± 12	124 ± 6	136 ± 4	133 ± 4	122 ± 3	142 ± 6
5-HT (25 µg/kg)	165 ± 9	210 ± 13	182 ± 8	173 ± 7*	178 ± 4*	165 ± 5*	174 ± 9*
5-HT (50 µg/kg)	198 ± 11	293 ± 17	238 ± 12*	205 ± 8*	223 ± 11*	211 ± 14*	213 ± 8*
Cells in BAL ($\times 10^6$ /ml)							
Total	9.7 ± 0.8	75 ± 10	13 ± 1.3*	21 ± 2.5*	12 ± 1.2*	18 ± 3*	24 ± 4*
Eosinophils	0.1 ± 0.1	10 ± 1.2	0.1 ± 0.1*	1.1 ± 0.3*	0.8 ± 0.3*	1.0 ± 0.3*	2.0 ± 0.6*

The effects of oral dexamethasone (*dexa*) and four doses of UR-60427 (*UR*) were evaluated. Reductions in pulmonary resistance and cell counts were detected at all doses (* $P < 0.05$ vs. sensitised animals, two-way ANOVA)

The efficacy of UR-60427 was evaluated in a rat asthma model. Asthma was induced in male Brown Norway rats by three daily i.p. injections with 1 mg OVA/100 mg Al(OH)₃ (unsensitised animals were injected with saline). Rats were exposed to a single 1% OVA exposure on day 21 and concomitantly treated twice with one of four doses of UR-60427 (30, 10, 3, 1 mg/kg, oral) or 1 mg/kg dexamethasone. The following day, animals were anaesthetised, intratracheally intubated and pulmonary resistance increase induced by 5-HT was determined. Lungs were lavaged with PBS and cell influx to the bronchoalveolar lavage (BAL) was determined after diff-quick staining. All animal procedures were approved by the local ethics committee.

Results and discussion

UR-60427 displayed an affinity value of $K_i = 9$ nM ($IC_{50} = 31$ nM) to human H₄R. Affinities for the histamine H₁, H₂ and H₃ receptors were determined at 10 µM UR-60427, showing inhibitions of competitor binding of 23, 20, and 5%. This represents in all cases selectivity values >300-fold for these receptors. Extended binding profile showed good selectivity ratios: 40 off-targets, including adenosine, adrenergic, GABA, bradykinin, dopamine, endothelin, muscarinic and neurokinin receptors, as well as dopamine and serotonin transporters showed inhibition values below 50% at 10 µM UR-60427. On the other hand, the lowest selectivity ratios were obtained for noradrenaline transporter, 62%; 5-HT_{2A}, 74%; and 5-HT_{2B}, 90%.

Functional properties in a CHO-transfected cell line with H₄R showed that incubation with increasing concentrations of UR-60427 reduced [³⁵S]GTPgammaS basal binding to H₄R membranes up to values similar to those achieved with thioperamide, while histamine increased

[³⁵S]GTPgammaS binding to membranes. Therefore it can be concluded that UR-60427 behaves as an inverse agonist (Fig. 1).

The rat asthma model showed an increased airway hyperreactivity (pulmonary resistance) in sensitised animals that was reduced by all doses of UR-60427. This reduction in airway hyperreactivity can be explained by the reduced total and differential cell counts that were found in BAL (Table 1) with the compound. These effects were similar to those with dexamethasone.

In summary, UR-60427 is a high affinity and selective H₄R inverse agonist showing high efficacy and potency in a rat asthma model. Therefore, this study provides further evidence that H₄R inverse agonists/antagonists are a promising strategy for the control of allergic airway diseases such as allergic rhinitis and asthma.

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