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THE ROLE OF ARGININE IN THE BINDING OF LTD_4 ANTAGONISTS TO $cysLT_1$ RECEPTORS OF GUINEA PIG LUNG

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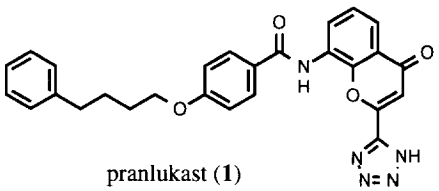
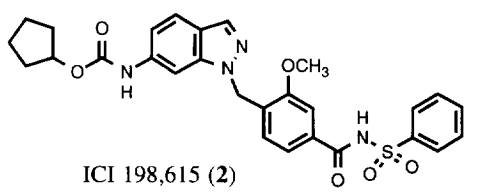
Abstract: Using the chemical masking agent phenylglyoxal, we have selectively blocked the arginine residues in leukotriene $cysLT_1$ receptors of guinea pig lung preparations. This treatment resulted in the markedly decreased affinity of LTD_4 antagonists to the receptor without affecting the affinity of LTD_4 itself. Our results indicate that LTD_4 and its antagonists may have different modes of interaction with the $cysLT_1$ receptor.

Cysteinyl leukotriene (cysLT) receptor antagonists are the most promising anti-asthmatic agents discovered in the last 20 years[1-3]. Several members of this class including pranlukast (**1**), zafirlukast, and montelukast have recently become available in clinical practice. In the near future, the therapeutic potential of these anti-leukotriene agents in asthma as well as other inflammatory and allergic diseases will be evident. Readers are directed to the excellent reviews cited herewith as references 1-3 for the state of the art in this field. A recent monograph[3] containing several fascinating case reports on the development of the well-known anti-leukotriene agents should be of great interest to medicinal chemists.

There are currently two types of membrane receptors that have been identified to specifically bind cysteinyl leukotrienes[4]: $cysLT_1$ selective for LTD_4 and LTE_4, and $cysLT_2$ selective for LTC_4. Selective blockade of the $cysLT_1$ receptor leads to the inhibition of cysLT-induced bronchoconstriction and inflammatory changes in asthma, whereas the consequences of selective blockade of the $cysLT_2$ receptor have yet to be defined.



pranlukast (**1**)



ICI 198,615 (**2**)

Most of $cysLT_1$ receptor antagonists (also known as LTD_4 antagonists) are designed consciously or unconsciously to mimic at least some of the structural features of the agonist LTD_4[2a,2i] (Figure 1A). These include 1) a lipophilic anchor that might occupy a lipophilic pocket of the $cysLT_1$ receptor; 2) a central flat unit

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that mimics the triene system of LTD_4; 3) one or two acidic groups that could bind to the same site as the peptide units or the C1-carboxylic acid of LTD_4; and 4) connecting units for these elements. Several molecular modelling studies[5] have actually been made to match the structures of LTD_4 and various antagonists. Thus the modes of interaction of LTD_4 and its antagonists with the $cysLT_1$ receptor are critical to the design and modification of future anti-leukotrienes.

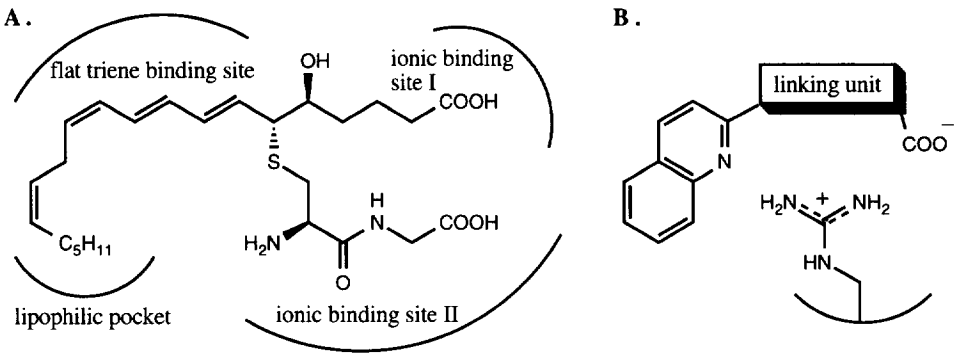


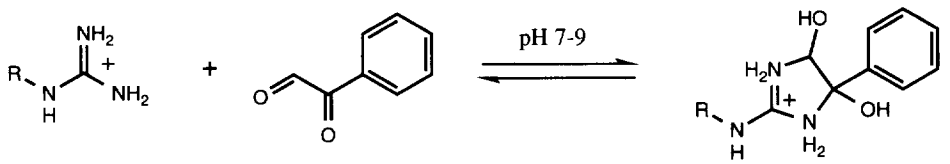
Figure 1. *Leukotriene $cysLT_1$ antagonist models. **A**: conceptualized model based on the structural features of LTD_4[5,9]; **B**: antagonist model incorporating an arginine residue from the receptor[8].*

The assumption that $cysLT_1$ receptor antagonists may bind to the same site as the agonist LTD_4 is based on the observation that minor structural changes of LTD_4, e.g. deletion of one methylene group to 2-nor-LTD_4[6], result in competitive antagonists. Aharony *et al*[7] however showed that the potent antagonist ICI 198,615 (**2**) bound to two different sites of $cysLT_1$ receptors whereas LTD_4 bound to a single site. The total density of antagonist binding sites (2660 fmol/mg protein) was more than twice the density of LTD_4 sites (1014 fmol/mg protein). The authors attributed these differences to the interaction of ICI 198,615 with two affinity states of the same receptor. In our attempt to rationalize the molecular interaction between the $cysLT_1$ receptor and its antagonists, we have built a pharmacophoric model[8] (Figure 1B) in which a basic residue of the receptor, i.e. an arginine, was used as the counterion to the common acidic function present in most of the known $cysLT_1$ antagonists. This arginine residue is also proposed to form a hydrogen bond with the western end of the antagonists. Compared to other models reported[9], our model is not only able to accommodate all known potent $cysLT_1$ antagonists geometrically, but also to explain the importance of an acidic function[10] and a hydrogen-bond acceptor function such as quinoline nitrogen[11] to the high receptor affinity of these agents.

In the present communication, we wish to report the first experimental proof for the involvement of an arginine residue in the interaction between $cysLT_1$ receptor and its antagonists as revealed by radioligand binding studies. The arginine residues were selectively masked by reaction with the widely used arginine-masking agent phenylglyoxal[12] (Scheme 1). Thus the crude guinea pig lung membrane fragments (± 170 μg protein/mL)[13] were allowed to react with phenylglyoxal (0.1 mM) in a PIPES buffer at 22 °C for 10 min. The cold ligands, ICI 198,615 (10^{-12} to 10^{-4} mol/L), pranlukast (10^{-11} to 10^{-4} mol/L) and LTD_4 (10^{-12} to 10^{-4} mol/L), were then added to the mixture, followed by [^{3}H]LTD_4 (0.2 nM, specific activity = 132 Ci/mmol). After incubation at 22 °C for 30 min, the mixture was filtered and the filter was washed twice with ice-cold TRIS buffer (10mM, pH

7.5). The receptor-[^{3}H]LTD_4 complex was measured by counting the radioactivity retained on the filter paper by a liquid scintilation counter after addition of 5 mL scintilation liquid. In the saturation experiments, 2 μM ICI 198,615 or LTD_4 was used to define the non-specific binding.

Scheme 1



As shown in Figure 2, after the blocking of arginine residues by phenylgloxal, the displacement curves of the antagonists ICI 198,615 and pranlukast were shifted dramatically to the right. The calculated dissociation constants (K_D) were increased 80 to 400 thousand times, with ICI 198,615 from $7.50 \pm 0.69 \times 10^{-11}$ mol/L to $3.01 \pm 0.85 \times 10^{-5}$ mol/L and pranlukast from $2.56 \pm 0.33 \times 10^{-9}$ mol/L to $2.14 \pm 0.47 \times 10^{-4}$ mol/L! In extreme contrast, the potency of LTD_4 to displace the radioligand was little influenced by this masking of arginine. In fact, the K_D value of LTD_4 was even slightly decreased after the treatment with phenylglyoxal, from $4.50 \pm 0.81 \times 10^{-10}$ mol/L to $2.09 \pm 0.25 \times 10^{-10}$ mol/L.

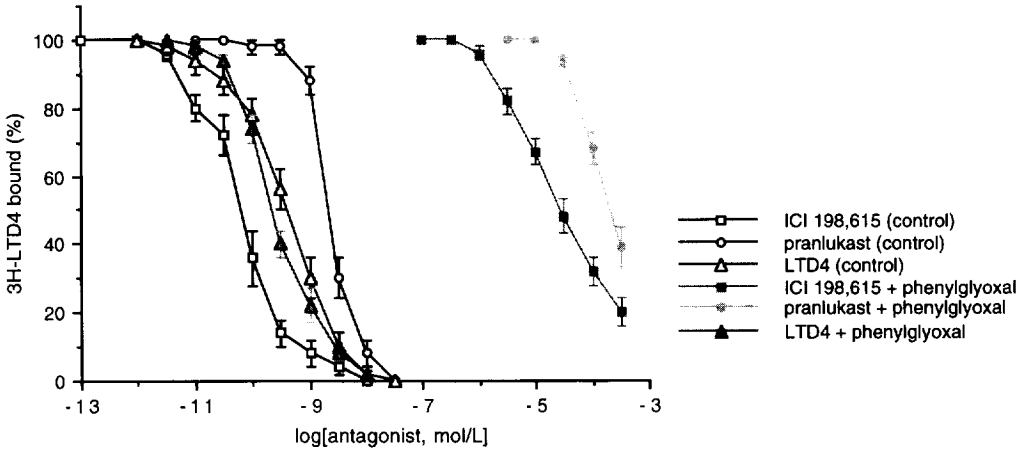


Figure 2. *Displacement of [^{3}H]LTD_4 bound in $cysLT_1$ receptors of guinea pig lung membranes with (filled spots) and without (open spots) the arginine-masking agent phenylglyoxal (0.1 mM). Data are means ± S.D. of three determinations.*

To check further whether the masking of arginine really influences the affinity of [^{3}H]LTD_4 to the receptor, we

performed saturation experiments with and without phenylglyoxal treatment. Scatchard analyses (Figure 3) showed that the affinity (K_D: 2.16 ± 0.10 x 10^{-10} *vs* 1.12 ± 0.45 x 10^{-10} mol/L) of [^{3}H]LTD_4 as well as the sites (B_{max}: 988 ± 74 *vs* 1081 ± 165 fmol/mg) reserved for the radioligand were little changed after the masking of arginine with phenylglyoxal.

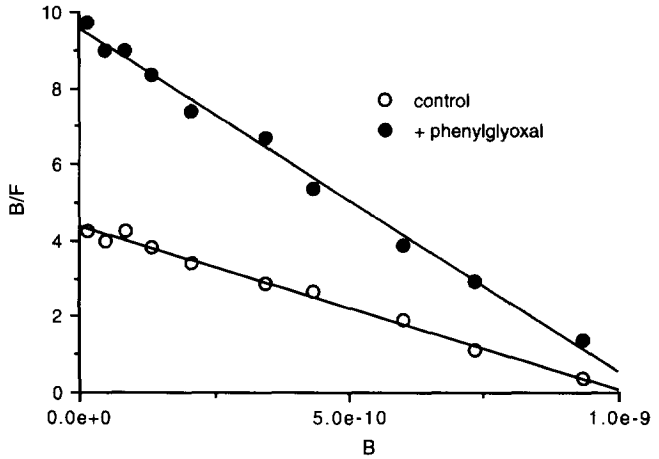


Figure 3. *Scatchard plots of [^{3}H]LTD_4 saturation experiments (n = 3). LTD_4 (2 μM) was used to define the non-specific binding. When ICI 198,615 (2 μM) was used to define the non-specific binding, in the control experiments (without phenylglyoxal) very similar values of K_D and B_{max} were obtained, but in the masking experiments (with phenylglyoxal) little displacement of [^{3}H]LTD_4 was observed. In this later case, the saturation curves with and without ICI 198,615 looked very similar (data not shown).*

Taken together our results indicate that an arginine residue is crucial for the binding of leukotriene antagonists, e.g. ICI 198,615 and pranlukast, to the $cysLT_1$ receptor but not for LTD_4 itself. We therefore propose the following model (Figure 4) for the binding site of leukotriene $cysLT_1$ receptors, in which LTD_4 and its antagonists have different modes of interaction, with the antagonists involving the interaction with an arginine residue and LTD_4 not.

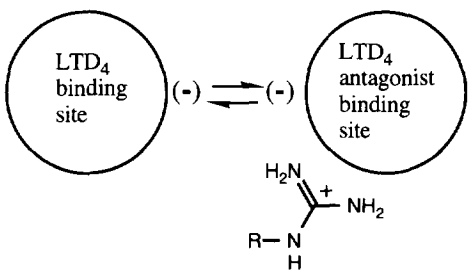


Figure 4. *A proposed binding model of LTD_4 and LTD_4 antagonists at the $cysLT_1$ receptor. Binding of LTD_4 and its antagonists negatively influence the affinity of each other. An arginine residue is involved in the binding of LTD_4 antagonists but not in that of LTD_4 itself.*

In conclusion, our results demonstrated the first time that LTD_4 and LTD_4 antagonists have different modes of interaction with the leukotriene $cysLT_1$ receptor. This information is important for the characterization of the receptor as well as for the design of new $cysLT_1$ receptor antagonists.

Acknowledgment

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