A Kinetic Binding Study to Evaluate the Pharmacological Profile of a Specific Leukotriene C4 Binding Site Not Coupled to Contraction in Human Lung Parenchyma

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ABSTRACT

We report the identification of a novel pharmacological profile for the leukotriene (LT)C4 binding site we previously identified in human lung parenchyma (HLP). We used a series of classic cysteinyl-LT (CysLT)1 receptor antagonists belonging to different chemical classes and the dual CysLT1-CysLT2 antagonist BAY u9773 for both binding and functional studies. Because the presence of (S)-decyl-glutathione interfered with cysteinyl-LT binding, with a kinetic protocol we avoided the use of this compound. By means of heterologous dissociation time courses, we demonstrated that zafirlukast, iralukast, and BAY u9773 selectively competed only for 3H-LTD4 binding sites, whereas pobilukast, pranlukast, and CGP 57698 dissociated both 3H-LTC4 and 3H-LTD4 from their binding sites. Thus, with binding studies, we have been able to identify a pharmacological profile for LTC4 distinct from that of LTD4 receptor (CysLT1) in HLP. On the contrary, in functional studies, all of the classic antagonists tested were able to revert both LTD4- and LTD4- induced contractions of isolated HLP strips. Thus, LTD4 and LTC4 contract isolated HLP strips through the same CysLT1 receptor. The results of kinetic binding studies, coupled to a sophisticated data analysis, confirm our hypothesis that HLP membranes contain two cysteinyl-LT high-affinity binding sites with different pharmacological profiles. In functional studies, however, LTD4- and LTC4-induced contractions are mediated by the same CysLT1 receptor. In conclusion, the specific LTC4 high-affinity binding site cannot be classified as one of the officially recognized CysLT receptors, and it is not implicated in LTC4-induced HLP strip contractions.

ABBREVIATIONS: Cysteinyl-LT, cysteine-containing leukotrienes; LT, leukotriene; (S)-decyl-GSH, (S)-decyl-glutathione; PG, prostaglandin; HLP, human lung parenchyma; Gpp(NH)p, guanosine-5’-[(β,γ-imido)triphosphate].
Experimental Procedures

Materials. \(^{3}\text{H}\)-LTC\(_4\) (164–173 Ci/mmol) and \(^{3}\text{H}\)-LTD\(_4\) (164–173 Ci/mmol) were purchased from DuPont NEN (Boston, MA). LTC\(_4\) and prostaglandin (PG)F\(_{2}\alpha\) were purchased from Cayman Chemical Co. (Ann Arbor, MI). Pobilukast (SKF 104353) was kindly provided by SmithKline and Beecham Laboratories (King of Prussia, PA). LTD\(_4\), zafirlukast (ICI 204,219), pranlukast (ONO 1078), iralukast (CGP 45715A), and CGP 57698 were a generous gift of Dr. A. von Sprecher (Novartis, Basel, Switzerland). Guanosine-5’-(β,γ-imido) triphosphate \([\text{Gpp(NH)}_3]\), \((S)-\text{decyl-GSH}, \text{cysteine, glycine, boric acid, serine, indomethacin, Tyrode’s salts, and HEPES were purchased from Sigma Chemical Co. (St Louis, MO). Filtercount was from Packard Instruments Co. (Meriden, CT). All the reagents used in HPLC analysis were of analytical grade and purchased from Carlo Erba (Milan, Italy), as were GF/C Whatman Fiberglas filters.

Preparation of HLP Membranes. Crude membranes were prepared from macroscopically normal specimens removed at thoracotomy for lung cancer as previously described (Rovati et al., 1985). Briefly, specimens were minced, homogenized at 4° in 10 mM HEPES buffer, pH 7.4 (1:24, w/v), and centrifuged at 770 g for 10 min, and the supernatant was centrifuged at 27,000 g for 20 min. The pellet was resuspended, centrifuged under the same condition, and finally resuspended in 5% of the homogenization volume. Membrane aliquots were frozen at −80° and stored for no longer than 3 months. Protein content was determined according to the Bradford dye-binding protein assay (Pierce Chemical Co., Rockford, IL). Before use, serine-borate complex (40 mM final concentration in the assay, prepared as an equimolar solution of serine and boric acid), cysteine (10 mM), and glycine (10 mM) were added to the membrane suspension to avoid cysteinyl-LT metabolism.

Reverse Phase HPLC. Labeled and unlabelled leukotriene purity was always assessed by reverse phase HPLC. Only leukotrienes with a purity grade greater than or equal to 90% were used. The Beckman HPLC system was equipped with a 110B Solvent Delivery Module, an ODS Ultrasphere C18 column, and a programmable detector module 166 set at 280 nm. Both labeled and unlabeled leukotrienes were eluted isocratically with a filtered and degassed mixture of CH\(_3\)OH:H\(_2\)O:CH\(_3\)COOH (65:35:0.02 v/v/v), adjusted at pH 5.8 with NH\(_4\)OH, at a flow rate of 1 ml/min. To check the purity of tritiated leukotrienes, fractions were collected every 30 s, and the radioactivity profile assessed by liquid scintillation counting (Ultima Gold; Packard).

Binding Studies. Equilibrium binding studies were performed at 25°C for 30 min with 0.5 nM \(^{3}\text{H}\)-LTD\(_4\) or \(^{3}\text{H}\)-LTC\(_4\) and unlabeled homologous or heterologous ligands at the indicated concentrations, in the absence and presence of 10 μM \((S)-\text{decyl-GSH}\).

Association time courses were performed at 25°C with 0.5 nM \(^{3}\text{H}\)-LTC\(_4\) or \(^{3}\text{H}\)-LTD\(_4\) to label the high-affinity binding sites and with a total ligand concentration of 0.1 μM (mixture of 1 nM labeled ligand plus 0.1 μM unlabeled homologous ligand) to also label the low-affinity sites. The experiments were conducted for 30 or 60 min for \(^{3}\text{H}\)-LTD\(_4\) and \(^{3}\text{H}\)-LTC\(_4\), respectively. Dissociation was induced by

Fig. 1. Chemical structures of LTC\(_4\), LTD\(_4\), and receptor antagonists.
the addition of 1 μM unlabeled leukotriene (homologous dissociation) or 10 μM unlabeled antagonist (heterologous dissociation). Gpp(NH)p was used at a concentration of 30 μM where indicated.

In both equilibrium and kinetic studies, HLP membranes (0.25 mg/sample), 10 mM HEPES-KOH, pH 7.4, and 1 mM CaCl₂ were added to the incubation mixture to achieve a final volume of 250 μl. Unbound ligand was separated by rapid vacuum filtration (Brandel Cell Harvester) onto glass-fiber GF/C filters (Whatman) soaked in 2.5% polyvinyl alcohol, and the filters were washed twice with 4 ml of HEPES buffer at 4°C. Radioactivity was measured in a liquid scintillation counter (Filter Count; Packard).

Isolated HLP Strip Preparation. Strips of HLP (1.5–2 cm) were prepared from macroscopically normal human lung specimens placed in cold (4°C) saline solution and studied within 120 min from resection. The HLP strips were suspended in 5-ml organ baths containing Tyrode's solution (composed of 140 mM NaCl, 3 mM KCl, 1 mM CaCl₂, 0.05 mM MgCl₂, 0.5 mM NaH₂PO₄, 8.4 mM glucose, and 12 mM NaHCO₃), maintained at 37°C, and bubbled with 95% O₂, 5% CO₂, pH 7.4. Contractions were measured with a Basile 7004 isometric force transducer and recorded on a Basile Gemini 7070 polygraph. HLP strips were set at an initial tension of 1 g, washed with fresh buffer every 15 min over a 60-min equilibration period, and then treated with 40 mM serine-borate complex and 3 mM l-cysteine to inhibit LTC₄ and LTD₄ metabolism. For antagonist studies, after 15 min, cumulative concentration-response curves were obtained with an increasing concentration of LTC₄ or LTD₄ (0.1 nM to 1 μM).

At 15 min later, either a concentration of 10 μM of each antagonist tested or the vehicle DMSO was added. Only one LTC₄ or LTD₄ concentration-response curve was obtained from each HLP strip. The contractile response to each concentration of LTC₄ or LTD₄ was expressed as percent of the maximal response to 300 nM PGF₂α.

Computer Analysis. Analysis of binding data of association and homologous dissociation time-courses was performed using the program KINFIT II (Rovati et al., 1996). The computerized analysis of the data through KINFIT II has several advantages, as it allows 1) simultaneous analysis of association and dissociation time courses; 2) fitting of the data to a single binding model; and 3) calculation of dissociation rate constants. The data were analyzed using the computer program KINFIT II, and the parameters were estimated by the least squares method. The results were expressed as mean ± S.E. of three replicates from at least three experiments.
Kinetic Binding Study of a Specific LTC₄ Binding Site

Results

Effect of (S)-Decyl-GSH on [³H]-LTC₄ and [³H]-LTD₄ Binding. The ability of a series of antagonists (10 µM) to compete for [³H]-LTC₄ binding was assessed at equilibrium (Fig. 2A). In the presence of (S)-decyl-GSH, only pobilukast retained the ability to displace [³H]-LTC₄ from its binding sites, whereas no appreciable effect was observed for agonist constant for the specified antagonist. No direct calculation of k.off was possible for the heterologous dissociation time courses. Biphasic dissociation time courses represent interaction with a heterologous population of sites, where the fast dissociation rate represents the low-affinity component and the low dissociation rate represents the high-affinity component. Antagonist competition is expressed as percent dissociation specific binding.

Statistical analysis of concentration-response curves was performed by using the computer program ALLFIT (De Lean et al., 1978), which calculates the lower and upper plateaus, the slope, and the EC₅₀ value.

Different models of increasing complexity were selected using the statistical principle of the "extra sum of squares" (Draper and Smith, 1966). Parameter errors are always expressed in percent coefficient of variation (% CV). A statistical level of significance of P < .05 was accepted. All of the curves shown were computer generated.

Shown are [³H]-LTD₄ and [³H]-LTC₄ (Figs. 4 and 5, insets, respectively) dissociation time courses performed in the absence and presence of 30 µM Gpp(NH)p, a nonhydrolyzable GTP analog. Gpp(NH)p was able to almost completely shift the high-affinity [³H]-LTD₄ binding to its low-affinity component from 89% low-affinity site in the absence and presence of (S)-decyl-GSH, the profile of antagonism was identical to that obtained versus [³H]-LTC₄ (Fig. 2B).

In Fig. 3 we tested the ability of (S)-decyl-GSH to interfere with [³H]-LTC₄ binding in kinetic studies. Dissociation time courses are biphasic (P < .05), representing interaction with a heterologous population of sites, where the fast dissociation rate represents the low-affinity component and the low dissociation rate represents the high-affinity component. (S)-Decyl-GSH did not affect the kinetic parameters but abolished 75% of [³H]-LTC₄ specific binding [ratio of the specific binding, C1 + C2, in the absence and presence of (S)-decyl-GSH; Fig. 3A]. Furthermore, (S)-decyl-GSH prevented pranlukast-induced dissociation of [³H]-LTC₄ from its high-affinity binding site (Fig. 3B). On the basis of these results, all of the subsequent experiments were performed using a kinetic protocol in the absence of (S)-decyl-GSH.

[³H]-LTD₄ and [³H]-LTC₄ Time Courses. [³H]-LTD₄ and [³H]-LTC₄ association time courses were performed at different concentrations of total ligand (see Experimental Procedures): 0.5 nM to prevalently label the high-affinity sites (Figs. 4 and 5, respectively, and Table 1) and 0.1 µM to also label the low-affinity sites (Table 1). Both dissociation curves are biphasic. Simultaneous computerized analysis of association and dissociation time courses performed at different total ligand concentrations confirmed the presence of two classes of binding sites for both LTC₄ and LTD₄ (P < .05). Parameters are reported in Table 1.

Shown are [³H]-LTD₄ and [³H]-LTC₄ (Figs. 4 and 5, insets, respectively) dissociation time courses performed in the absence and presence of 30 µM Gpp(NH)p, a nonhydrolyzable GTP analog. Gpp(NH)p was able to almost completely shift the high-affinity [³H]-LTD₄ binding to its low-affinity component from 38 to 89% low-affinity site in the absence and
presence of Gpp(NH)p, respectively. On the contrary, Gpp(NH)p had no effect on the high-affinity site labeled by \textsuperscript{3}H-LTC\textsubscript{4}.

**Antagonist Binding Studies.** Heterologous dissociation time courses were performed with a series of “classic” CysLT\textsubscript{1} antagonists (Brooks and Summers, 1996) and the dual antagonist BAY u9773 (Cuthbert et al., 1991).

All of the antagonists tested (Table 2) were able to dissociate \textsuperscript{3}H-LTD\textsubscript{4} from its binding sites. Figure 6A shows the curves for three of these antagonists (the others are not shown for the sake of clarity) in comparison with the homologous curve. All antagonist-induced \textsuperscript{3}H-LTD\textsubscript{4} dissociation curves were biphasic (P < .05). On the contrary, only pobilukast, pranlukast, and CGP 57698 dissociated \textsuperscript{3}H-LTC\textsubscript{4} from its high-affinity binding sites, whereas zafirlukast, iralukast, and BAY u9773 did not (Fig. 6B and Table 2). Again, only three curves are shown for the sake of clarity.

The apparent potencies of the different antagonists in displacing \textsuperscript{3}H-LTC\textsubscript{4} and \textsuperscript{3}H-LTD\textsubscript{4} are presented in Table 2 as percent dissociation at 60 min.

**Isometric Contraction of Isolated HLP Strips.** Figure 7 shows LTD\textsubscript{4} and LTC\textsubscript{4} cumulative concentration-response curves obtained from isometric contractions of HLP strips. The EC\textsubscript{50} values are 6.6 nM ± 46% CV and 91 nM ± 3.3% CV, and the maximal contractions (expressed as percent versus PGF\textsubscript{2\alpha}) are 190 ± 9.5% CV and 111 ± 3.5% CV for LTD\textsubscript{4} and LTC\textsubscript{4}, respectively.

All of the antagonists were tested up to a concentration of 10 μM and were able to completely reverse (>85%) both LTD\textsubscript{4}- and LTC\textsubscript{4}-induced contractions, with the only exception of BAY u9773. The dual antagonist was less active than the other compounds with a maximal inhibition lower than 50% (data not shown), and BAY u9773 presented a partial agonist activity in most of the experiments. Tracings from a typical experiment for pranlukast and iralukast versus LTC\textsubscript{4} are shown in Fig. 8, A and B, respectively.

**Discussion**

It is well known that LTC\textsubscript{4} predominantly binds to a number of nonreceptor sites in cellular membranes (Keppler,
1992; Metters et al., 1994). As we previously demonstrated (Capra et al., 1998), to unmask a specific high-affinity binding site for LTC4, \((S)\)-decyl-GSH must be routinely included in the \(^3\)H-LTC\(_4\) binding assay at equilibrium to inhibit the interaction with most of these lower-affinity nonreceptor sites. However, we observed that all of the antagonists tested, with the exception of pobrilukast, were unable to compete for \(^3\)H-LTC\(_4\) binding in the presence of \((S)\)-decyl-GSH. To elucidate whether \((S)\)-decyl-GSH might interfere with the antagonist binding, we selected one representative compound from each structural class of antagonists and tested them in HLP membranes, also against \(^3\)H-LTD\(_4\) in the absence and presence of \((S)\)-decyl-GSH. Surprisingly, in the presence of \((S)\)-decyl-GSH, the antagonists were unable to also compete for \(^3\)H-LTD\(_4\), indicating that this compound interferes with antagonist but not with agonist binding (Fig. 2). Interestingly, only the binding of pobrilukast, the antag-

### TABLE 2
Antagonist-induced dissociation at 60 min

<table>
<thead>
<tr>
<th>Parameters are expressed as mean ± % CV.</th>
<th>(^3)H-LTD(_4)</th>
<th>(^3)H-LTC(_4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% dissociation at 60 min</td>
<td>(^3)H-LTD(_4)</td>
<td>(^3)H-LTC(_4)</td>
</tr>
<tr>
<td>Pobilukast</td>
<td>47 ± 42</td>
<td>53 ± 12</td>
</tr>
<tr>
<td>Pranlukast</td>
<td>75 ± 10</td>
<td>36 ± 47</td>
</tr>
<tr>
<td>CGP 57698</td>
<td>69 ± 31</td>
<td>46 ± 21</td>
</tr>
<tr>
<td>Zafirlukast</td>
<td>62 ± 3</td>
<td>N.D.</td>
</tr>
<tr>
<td>Iralukast</td>
<td>52 ± 8</td>
<td>N.D.</td>
</tr>
<tr>
<td>BAY u9773</td>
<td>47 ± 12</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., not detectable.

![Fig. 6](https://www.molpharm.aspetjournals.org/doi/abs/10.1124/jpet.1187)

**Fig. 6.** Homologous and heterologous dissociation time courses. A, labeled ligand 0.5 nM \(^3\)H-LTD\(_4\); dissociation was induced by 1 \(\mu\)M LTD\(_4\) (●), 10 \(\mu\)M pobrilukast (▲), zafirlukast (○), and BAY u9773 (□). B, labeled ligand 0.5 nM \(^3\)H-LTC\(_4\); dissociation was induced by 1 \(\mu\)M LTC\(_4\) (●), 10 \(\mu\)M pobrilukast (▲), zafirlukast (○), and BAY u9773 (□). Data are mean values of three replicates from a single experiment, representative of at least two other experiments. S.E. values are not shown for the sake of clarity.

![Fig. 7](https://www.molpharm.aspetjournals.org/doi/abs/10.1124/jpet.1187)

**Fig. 7.** Cumulative concentration-response curves of LTD\(_4\) (●) and LTC\(_4\) (□) induced contraction of isolated HLP strips. Data are mean values of four to six replicates ± S.E.

![Fig. 8](https://www.molpharm.aspetjournals.org/doi/abs/10.1124/jpet.1187)

**Fig. 8.** Effect of pranlukast (A) and iralukast (B) on cumulative concentration-response curve of LTC\(_4\) in isolated HLP strips. Data are from a single experiment, representative of at least two other experiments.
nast with the structure closest to that of cysteinyI-LTs, was not affected by (S)-decyl-GSH.

Because the presence of (S)-decyl-GSH prevents the pharmacological characterization of $^{3}$H-LTC$_{4}$ binding at equilibrium, to avoid the use of (S)-decyl-GSH, we have performed the pharmacological characterization of the $^{3}$H-LTC$_{4}$ high-affinity site by means of kinetic binding studies. This protocol is rarely used for this purpose, yet in this specific case, with $K_{d1}$ far apart from $K_{d2}$ (1600-fold difference), it is possible to choose a concentration of $^{3}$H-LTC$_{4}$ to saturate the high-affinity/low-capacity site without saturating the low-affinity/high-capacity site in the association phase (Rovati et al., 1996; Rovati, 1998). Clearly, a portion of the low-affinity sites, due to their abundance, is also labeled (dissociation time courses are always biphasic). However, with this approach, there is no longer a need to inhibit the binding to the lower-affinity sites by means of (S)-decyl-GSH. The same also applies, in part, to $^{3}$H-LTD$_{4}$, despite the difference between $K_{d1}$ and $K_{d2}$ being only 340-fold.

Thus, having primarily labeled the high-affinity binding sites, one can perturb the equilibrium with the antagonists to assess their ability to dissociate $^{3}$H-LTD$_{4}$ and $^{3}$H-LTC$_{4}$ from both sites. This protocol is indeed a heterologous dissociation time course, which allows a study of the interaction of unlabeled ligands (i.e., the antagonists) with $^{3}$H-LTD$_{4}$ and $^{3}$H-LTC$_{4}$. The only limitation of this type of protocol is that no dissociation constants for the antagonist can be calculated, but only their apparent potency order (Table 2).

We observed that (S)-decyl-GSH interferes with antagonist-induced $^{3}$H-LTC$_{4}$ dissociation from its high-affinity sites without interfering with the kinetic parameters of the agonist (Eq. 3), confirming the data obtained at equilibrium. A possible explanation for these findings could reside in a non-total coincidence of agonist and antagonist sites on CysLT receptors and in the steric hindrance of (S)-decyl-GSH at the antagonist binding site.

The results obtained from the simultaneous computerized analysis of association and dissociation time courses for $^{3}$H-LTD$_{4}$ and $^{3}$H-LTC$_{4}$ confirmed the model and parameters (Table 1) for cysteinyl-LT binding sites in HLP (Capra et al., 1998), thus validating the kinetic approach in the absence of (S)-decyl-GSH. In fact, LTD$_{4}$ interacts with two interconvertible states of a G protein-coupled receptor, whereas LTC$_{4}$ displays a different kinetic profile, and both sites are GTP insensitive.

Heterologous dissociation time courses indicated that among all of the “classic” CysLT$_{1}$ antagonists we tested, only pobilukast, pranlukast, and CGP 57698 were able to dissociate both $^{3}$H-LTD$_{4}$ and $^{3}$H-LTC$_{4}$ from their high- and low-affinity binding sites. On the contrary, zafirlukast and iralukast were unable to interact with the $^{3}$H-LTC$_{4}$ high-affinity binding site (Fig. 6 and Table 2), whereas they retain the ability to dissociate the ligand from the nonreceptor sites (low-affinity component). Hence, $^{3}$H-LTC$_{4}$ high-affinity binding site has a unique pharmacological profile, suggesting the existence of a specific LTC$_{4}$ receptor different from that of LTD$_{4}$ (CysLT$_{1}$).

Among all of the cysteinyl-LT antagonists available, BAY u9773 is, until now, the only compound able to recognize both CysLT$_{1}$ and CysLT$_{2}$ receptors (Coleman et al., 1995). In HLP membranes, BAY u9773 is indeed able to dissociate $^{3}$H-LTD$_{4}$ from both of its sites but is unable to dissociate $^{3}$H-LTC$_{4}$ from its high-affinity sites, thus excluding that this LTC$_{4}$ specific site is a CysLT$_{2}$ receptor.

Taken together, these binding data confirm our hypothesis that HLP membranes contain two cysteinyl-LT high-affinity binding sites with different kinetic (sensitivity to GTP) and pharmacological profiles. LTD$_{4}$ binding sites can be classified as a CysLT$_{1}$ receptor (Lynch et al., 1999), whereas LTC$_{4}$ high-affinity binding site is neither a CysLT$_{1}$ nor a CysLT$_{2}$ receptor. Moreover, these results indicate that classic antagonists should no longer be considered a homogeneous class of compounds with respect to LTC$_{4}$ binding sites and that their specificity seems to be unrelated to the chemical structure, because antagonists of the same class (e.g., pobilukast, iralukast, and BAY u997) behave differently versus the two different receptors.

It is well known that in human airways, CysLT$_{1}$ receptors predominantly mediate the contraction of smooth muscle tissue, thus playing an important role in the acute phase of asthma. To evaluate whether LTD$_{4}$ and LTC$_{4}$ share the same effect and the same pharmacological profile in isolated HLP strips, all of the antagonists were also tested in a functional assay against LTD$_{4}$- and LTC$_{4}$-induced contractions. Despite the fact that LTD$_{4}$ and LTC$_{4}$ have different potencies (14-fold difference) and efficacies (1.7-fold difference; Fig. 7), all of the classic antagonists tested were able to reverse LTD$_{4}$- as well as LTC$_{4}$-induced contractions up to 85 to 100%. BAY u9773 showed a lower efficacy (60% inhibition of LTD$_{4}$- and LTC$_{4}$-induced contractions at the same time point), suggesting it could behave as a partial agonist, as already proposed both in this tissue (Wikstrom Jonsson et al., 1998) and in human pulmonary veins (Gardiner et al., 1994).

Thus, we can conclude that LTD$_{4}$ and LTC$_{4}$ contract isolated HLP strips through the same CysLT$_{1}$ receptor, as already suggested by Gardiner and Cuthbert (1988) on the basis of more limited data (only one antagonist, FPL 55712). The specific and characteristic LTC$_{4}$ high-affinity binding site cannot be classified among one of the officially recognized CysLT receptors, nor it is implicated in LTC$_{4}$-induced HLP strip contractions. The recent cloning of the CysLT$_{1}$ receptor (Lynch et al., 1999) will rapidly lead to the identification and characterization of the different classes and subclasses of CysLT receptors, but the LTC$_{4}$ specific binding site identified here is unlikely to be one of these. In fact, this binding site is not GTP sensitive (Fig. 5 and Capra et al., 1998) and thus should not belong to the superfamily of seven-transmembrane domain receptors.

It is tempting to speculate that this putative receptor is implicated in aspects of the asthmatic syndrome different from bronchoconstriction, such as smooth muscle hyperplasia and proliferation or mucus secretion. Indeed, there are data in the literature that indicate a proliferative role of cysteinyl-LTs in human airway epithelial (Leikauf et al., 1990) or smooth muscle (Panettieri et al., 1998) cells. These data not only suggest LTC$_{4}$ as a more potent mitogenic stimulus than LTD$_{4}$ (Leikauf et al., 1990) but also indicate LTD$_{4}$ to be a weak agonist with a different pharmacological profile compared with the classic contractile function mediated by the CysLT$_{1}$ receptor.

Although direct evidence to correlate proliferation with the putative LTC$_{4}$ receptor is still lacking, our findings might prompt a deeper investigation into the role of LTC$_{4}$ not only as a precursor of LTD$_{4}$/LTE$_{4}$ but also as an active indepen-
dent agonist per se. This in turn might contribute to the discovery and development of new and more active drugs with a wider spectrum of action to be used in the treatment of an overlooked disease such as asthma.

Finally, our data also suggest that the homologous kinetic protocol is a valid alternative to classic equilibrium binding studies when supported by sophisticated data analysis. The intrinsic complexity of these experiments can be easily offset by the advantages that this type of protocol presents in particular biological systems where equilibrium studies might fail for theoretical or practical reasons (e.g., when one deals with a high-affinity ligand with a low specific activity; Rovati, 1998). Moreover, heterologous dissociation time courses, albeit with the limitation previously discussed, appear to be a powerful tool for the study of the kinetic characteristics of compounds not available in the labeled form.

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References

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