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Antagonism of leukotriene responses in human airways by BAY x7195

Isabelle Gorenne [c], Jose Luis Ortiz [b], Carlos Labat [c], Trevor Abram [a], Stephen Tudhop [a], Nigel Cuthbert [a], Peter Norman [a], Phillip Gardiner [a], Esteban Morcillo [b], Charles Brink [c,*]

[a] *Bayer plc, Pharmaceutical Business Group, Research Department, Stoke Court, Stoke Poges, UK*
[b] *Departamenta de Farmacologia, Universitat de Valencia, Valencia, Spain*
[c] *CNRS URA 1159, Centre Chirurgical Marie Lannelongue, 133 Avenue de la Resistance, 92350 Le Plessis Robinson, France*

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Abstract

Contractions induced by leukotriene and anti-IgE (sheep antiserum to human IgE) were antagonized by pretreatment of human airways with the cysteinyl leukotriene receptor antagonist BAY x7195 ((4*S*)-[4-carboxyphenylthio]-7-[4-(4-phenoxybutoxy)-phenyl]-hept-5-(*z*)-enoic acid). However, this receptor antagonist did not inhibit either leukotriene D_4- or leukotriene C_4-induced contractions in human pulmonary veins. The pA_2 value for BAY x7195 in human airways against leukotriene D_4 was 7.83 ± 0.16 with a slope of 1.07 ± 0.15 (means $\pm$ S.E.M; $n = 11$). The IC_{50} value for BAY x7195 in human airways contracted with anti-IgE was 0.31 ± 0.08 μM ($n = 11$). These results were comparable to those obtained with ICI 204,219 (4-(5-cyclopentyl-oxycarbonylamino-1-methylindol-3-ylmethyl)-3-methoxy-*N*-otolyl-sulfonylbenzamide). These data demonstrate that BAY x7195 is a potent selective leukotriene receptor antagonist which may block allergic reactions in the lung.

Keywords: Airway human; Leukotriene; Anti-immunoglobulin E; Contraction; Cysteinyl leukotriene receptor antagonist

1. Introduction

Leukotrienes are 5-lipoxygenase pathway metabolites of arachidonic acid and have been shown to be potent putative mediators in allergy and inflammation (Samuelsson, 1983). Their formation can be triggered by immunological or chemical stimuli. The interaction of an antigen with a receptor in the airways probably occurs at the level of the mast cell. Stimulation provokes a local release of cysteinyl-leukotrienes causing airway smooth muscle contraction (Dahlén et al., 1983), mucus secretion (Marom et al., 1982) and inflammatory cell infiltration (Ford-Hutchinson et al., 1980).

Pharmacological studies using animal tissues demonstrated that leukotrienes activate different types of receptors (Fleisch et al., 1982; Krell et al., 1983). In the human respiratory tract, Buckner et al. (1986) showed the potent inhibitory actions of several receptor antagonists against the contractile effects of leukotrienes in airway smooth muscle. These data suggested the presence of a single leukotriene receptor in human airways. However, in the human pulmonary vascular bed the leukotriene receptor is resistant to classical receptor antagonists which block leukotriene contractions in airways (Labat et al., 1992). In addition, Björck and Dahlén (1993) demonstrated that leukotriene receptor antagonists inhibit anti-IgE-induced contractions in human airways. These data, together with those of previous studies suggested indirectly that anti-IgE stimulation of airways released leukotrienes and that these mediators were involved in the airway muscle contraction (Jones et al., 1989; Muccitelli et al., 1987). Such results suggest that compounds which act as leukotriene receptor antagonists on human airways should be selective for the cys-LT-1 versus the cys-LT-2 receptor as well as inhibit the anti-IgE-induced contractions produced in these tissues.

The aim of this study was to examine the potency of BAY x7195 against anti-IgE-induced contractions in isolated human airways and to provide information on the selectivity of this compound for the leukotriene receptor (cys-LT-1) described in human airways.

* Corresponding author.

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2. Materials and methods

2.1. Isolated preparations

Human lung samples were obtained from 17 male and 2 female patients who had undergone surgery for lung carcinoma. The age was 59 ± 4 years (means ± S.E.M.). Following resection the bronchus and pulmonary veins were dissected free from parenchymal lung tissue and placed in Tyrode's solution at 4°C for 12 h. Experiments were performed on 180 subsegmental bronchial preparations derived from 17 lung samples (n). The bronchial ring preparations were 2–5 mm internal diameter. The human pulmonary veins were 2–4 mm internal diameter ($n = 4$). Values are means ± S.E.M.

2.2. Leukotriene-induced contractions

Bronchial ring preparations were set up in 10 ml organ baths containing Tyrode's solution (concentration mM): NaCl, 139.2; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 0.49; $NaHCO_3$, 11.9; NaH_2PO_4, 0.4 and glucose, 5.5; pH 7.4. The rings were mounted under initial loads of 2–3 g; maintained at 37°C and gassed with 5% CO_2-95% O_2. Changes in force were monitored on Linseis recorders by using isometric force-displacement transducers (Narco F-60). The preparations were allowed to equilibrate for 90 min and the medium was replaced every 15 min with fresh Tyrode's solution. Subsequent to the 90 min equilibration period bronchial rings were contracted with histamine (50 μM) and pulmonary veins were contracted with 5-hydroxytryptamine (5-HT; 10 μM). When the response reached a plateau the tissues were washed every 10 min by exchanging the medium until the basal tone was re-established. The bronchial tissues were then incubated for 30 min with Tyrode's solution or Tyrode's solution containing BAY x7195 (0.01–10 μM). Pulmonary veins were incubated with either Tyrode's solution or Tyrode's solution containing BAY x7195 (1 μM; 30 min). Leukotriene cumulative concentration-effect curves were produced following the drug treatments.

2.3. Anti-human IgE-induced contractions

Following the equilibration period, the bronchial tissues were contracted with acetylcholine (100 μM). When the acetylcholine response reached a plateau, the rings were washed until the basal tone was re-established. The preparations were then incubated for 30 min with either Tyrode's solution (control), Tyrode's solution containing a drug combination (atropine, 1 μM; indomethacin, 3 μM and chlorpheniramine, 1 μM) or Tyrode's solution containing this drug combination and one of the following antagonists (ICI 198,615, ICI 204,219 or BAY x7195) at different concentrations (0.01–10 μM). At the end of the incubation period the tissues were stimulated with anti-IgE (1:1000). The tissues used were not passively sensitized.

2.4. Calculations

The changes in force were determined from recordings and expressed in g. The anti-human IgE-induced contractions are expressed as a percentage of the acetylcholine (100 μM) contractions. The EC_{50} values for each leukotriene curve were obtained by linear regression analysis of data points in g or as percentage of the maximal response produced by the leukotriene verses log concentration above and below the 50% response level. These values were transformed into pD_2 values (−log EC_{50} value). The pK_B values were calculated by using the following equation: $K_B = [B]/(CR-1)$, where [B] is the concentration of the antagonist and CR (concentration ratio) is the ratio of EC_{50} of agonist in the presence and absence of antagonist. The K_B value was transformed into the pK_B value, that is, the negative logarithm of the K_B value. The pA_2 value and slope were derived from linear least squares regression analysis (Schild plot analysis). Results from other protocols were evaluated using the ANOVA followed by Student's t-test.

2.5. Drugs

The drugs and their sources were: acetylcholine chloride, atropine sulfate, chlorpheniramine maleate, indomethacin, histamine dihydrochloride, 5-HT (Sigma Chemical Co., St. Louis, MO, USA); sheep antiserum to human IgE (anti-IgE; Nordic Immunological Laboratories, Tilburg, Netherlands); BAY x7195 ((4*S*)[4-carboxyphenylthio)-7-[4-(4-phenoxybutoxy)phenyl]hept-5-(*z*)-enoic acid; Fig. 1), ICI 198,615 (1-[[2-methoxy-4-[[phenylsulfonyl)amino]carbonyl]-phenyl]methyl]-1*H*-indazol-6-yl]carbamic acid cyclopentyl ester), ICI 204,219 (4-(5-cyclopentyloxycarbonylamino-1-methylindol-3-ylmethyl)-3-methoxy-*N*-*o*-tolylsulfonylbenzamide), leukotriene C_4 and leukotriene D_4 were synthesized by Bayer (Stoke Court, UK). The receptor antag-

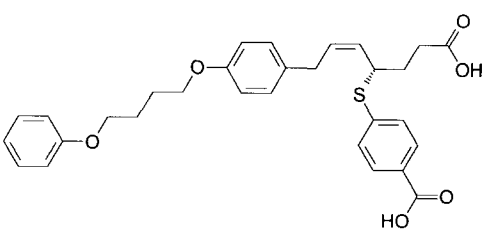

Fig. 1. The chemical structure of BAY x7195: (4*S*)-4-[4-carboxyphenylthio]-7-[4-(4-phenoxybutoxy)-phenyl]-hept-5-(*z*)-enoic acid.

onists and indomethacin were dissolved in DMSO and subsequent dilutions were made in Tyrode's solution. The leukotrienes were shipped on dry ice from Bayer in vials containing a stock solution of leukotriene C_4 or leukotriene D_4 in 20% ethanol and physiological salt solution at pH 7.2.

3. Results

3.1. Leukotriene-induced contractions

During the 30 min incubation period, BAY x7195 (0.1, 1 and 10 μM) induced a dose-dependent relaxation of the resting tone in human bronchial preparations. The relaxations were 0.23 ± 0.05 g, 0.43 ± 0.18 g and 0.98 ± 0.44 g, respectively. These values were significantly different ($P < 0.05$) from the spontaneous relaxations observed in control conditions (0.04 ± 0.05 g). In human pulmonary venous preparations BAY x7195 did not significantly modify the resting tone. The effects of BAY x7195 on leukotriene D_4 curves for isolated human bronchial preparations are presented in Fig. 2. BAY x7195 caused a significant ($P < 0.05$) rightward shift of the leukotriene D_4 curves in human bronchial muscle preparations (Fig. 2 and Table 1). In isolated human pulmonary venous preparations BAY x7195 did not modify either the leukotriene D_4 or leukotriene C_4 response curves (Fig. 3). In addition, BAY x7195 (10 μM) did not modify either the acetylcholine- or the histamine-induced contractions in human bronchial preparations (Fig. 4).

3.2. Anti-human IgE-induced contractions

In bronchial preparations treated with the drug combination (atropine, 1 μM, indomethacin, 3 μM and chlorpheniramine, 1 μM) there was a significant relaxation of resting tone (control, 0.00 ± 0.00 g and treated, 0.71 ± 0.11 g; $P < 0.05$). The relaxations induced by BAY x7195 (10 μM), ICI 204,219 (10 μM) and ICI 198,615 (1 μM) in combination with this treatment were 0.61 ± 0.20 g, 0.51 ± 0.13 g and 0.33 ± 0.18 g, respectively. The anti-IgE-induced contractions in human bronchial muscle preparations were $67 \pm 16\%$ and in the presence of the drug combination, $93 \pm 8\%$, expressed as a percentage of the acetylcholine (100 μM)-induced contraction, which was 2.37 ± 0.17 g in 52 preparations from 6 lungs samples. The anti-IgE-induced contractions in human bronchial muscle preparations were inhibited by each of the three leukotriene receptor antagonists in tissues treated with the drug combination (atropine, 1 μM, indomethacin, 3 μM and chlorpheniramine, 1 μM; Fig. 5). These data demonstrate that BAY x7195 and ICI 204,219 were equipotent in this model. The IC_{50} values for these leukotriene

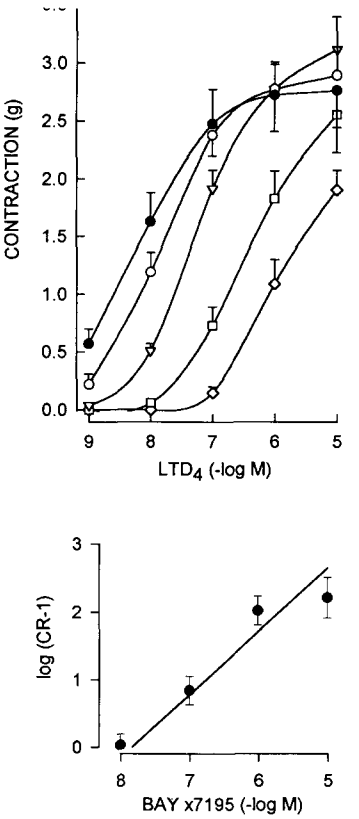

Fig. 2. Top panel. Antagonism of leukotriene D_4 contractions produced in isolated human bronchial muscle preparations in the absence (●) and presence of increasing concentrations of BAY x7195 (○, 10 nM; ▽, 100 nM; □, 1 μM; ◇, 10 μM). All curves after receptor antagonist treatment were significantly shifted to the right (ANOVA). Bottom panel. Schild analysis of the antagonism of leukotriene D_4 in human airways by several concentrations of BAY x7195. Values are means ± S.E.M. from 11 lung samples.

receptor antagonists are presented in Table 2. These leukotriene receptor antagonists did not significantly inhibit the anti-IgE-induced contraction in the absence of the drug combination (atropine, 1 μM, in-

Table 1
A comparison of the effects of cys-LT-1 antagonists in human airways

cys-LT-1 antagonists	Anti-IgE inhibition IC_{50} (μM)	Leukotriene receptor antagonism pA_2 or pK_B
BAY x7195	0.31 ± 0.08	7.83 ± 0.16 [a]
ICI 204,219	0.16 ± 0.03	7.72 ± 0.47
ICI 198,615	0.05 ± 0.02	8.25 ± 0.16

Human airways were pretreated with different cys-LT-1 receptor antagonists and subsequently contracted with either leukotriene D_4 or anti-IgE (dilution, 1:1000). The IC_{50} values against anti-IgE-induced contractions and either the pK_B or pA_2 values against leukotriene D_4 were calculated (see methods). Values are means ± S.E.M. [a] pA_2 value (slope = 1.07 ± 0.15, slope of Schild plot were not significantly different from unity, $n = 11$).

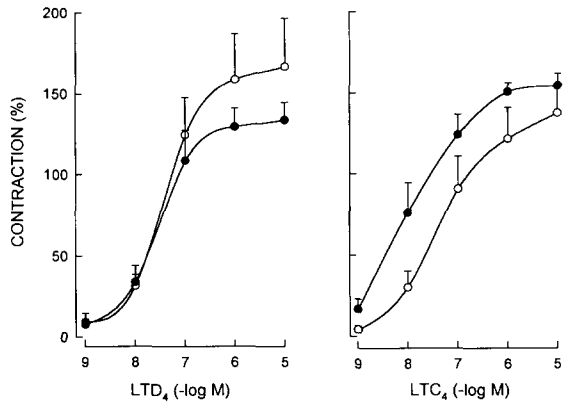

Fig. 3. Effects of BAY x7195 on leukotriene D_4 and leukotriene C_4 contractions in human pulmonary veins. Contractions are expressed as percentages of the response to 5-hydroxytryptamine (5-HT, 10 μM; 1.45 ± 0.21 g in 16 preparations) in control (●) or after incubation with BAY x7195 (10 μM; ○). Values are means ± S.E.M. from four lung samples.

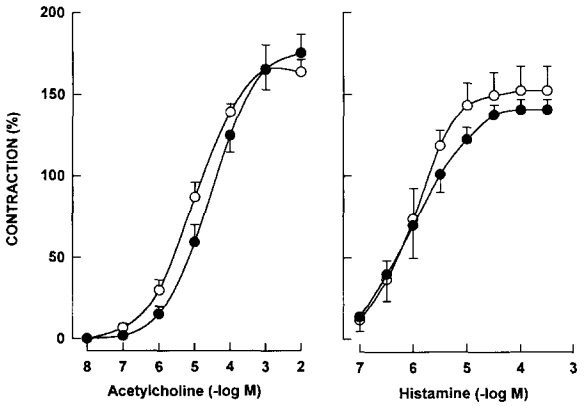

Fig. 4. Effects of BAY x7195 on acetylcholine (left panel) and histamine (right panel) contractions in human bronchial muscle preparations. Contractions are expressed as force in g. Data presented are control (●) and after incubation with BAY x7195 (10 μM; ○). Values are means ± S.E.M. from three lung samples.

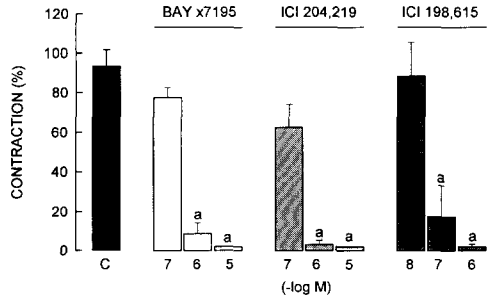

Fig. 5. Antagonism of anti-IgE-induced contractions in human airways by BAY x7195, ICI 204,219 and ICI 198,615. The contractions produced by anti-IgE (dilution, 1:1000) are presented as percentages of the acetylcholine-induced response. C indicates control preparations where the tissues were treated with the drug combination (atropine, 1 μM, indomethacin, 3 μM and chlorpheniramine, 1 μM). Values are means ± S.E.M. from four to six lung samples. [a] Significantly different ($P < 0.05$) from control data (C).

domethacin, 3 μM and chlorpheniramine, 1 μM; data not shown).

4. Discussion

The data presented in this report indicate that BAY x7195 is a potent and selective cysteinyl leukotriene receptor antagonist in isolated human airways. This compound did not block either the acetylcholine-induced contractions in human airways or the leukotriene D_4-induced contractions in isolated human pulmonary veins. These latter results indicate that BAY x7195 acts specifically at the cys-LT-1 receptor present in human airways (Buckner et al., 1986) and not the cys-LT-2 receptors found in human pulmonary veins (Labat et al., 1992).

Antigen challenge in the human lung in vitro causes the release of leukotrienes (Dahlén et al., 1983; Salari et al., 1985; Gorenne et al., 1994). In addition, inhibition of the anti-IgE response in isolated human bronchial muscle preparations by leukotriene receptor antagonists indirectly suggests that these mediators are released and participate in the anti-IgE-induced contractions, data which support previously published results (Björck and Dahlén, 1993; Dahlén et al., 1983; Gorenne et al., 1994; Jones et al., 1988; Undem et al., 1987). Among the inflammatory mediators released during antigen stimulation, the leukotrienes are potent bronchoconstrictors of airway muscle (Dahlén et al., 1980). Many of the studies previously published have suggested that the antigen-induced airway contraction is elicited in tissues pretreated with at least a antihistamine (Björck and Dahlén, 1993). This procedure permits an evaluation of the role of leukotrienes in the contractile response. Other investigators have used atropine and/or indomethacin in combination with the anti-histamine (Jones et al., 1989; Muccitelli et al., 1987; Gorenne et al., 1994). However, the role of prostaglandins in the antigen-induced contraction has not been elucidated, although a suggestion has been made that prostaglandin E_2 may play a role in blocking histamine release, since human airways pretreated with indomethacin release significantly greater quantities of this mediator during stimulation (Undem et al., 1987). Björck and Dahlén (1993) have recently shown data which support a role for both histamine and leukotrienes in anti-IgE-induced contractions in human airways derived from asthmatic patients.

The potency of leukotriene receptor antagonists such as ICI 198,615 and ICI 204,219 was initially established by using isolated airways or various animal models in vivo (Krell et al., 1990). These authors have shown that the former compound was a highly potent selective leukotriene receptor antagonist but lacked consistent bioavailability when administered orally in many species

whereas the latter drug demonstrated an improved profile. Jones and coworkers (1989) performed similar experiments using the leukotriene antagonist, L-660,711 (MK 571), with results which supported those of initial studies involving other compounds (Muccitelli et al., 1987). On the basis of these animal studies a series of compounds are now available which are potent and selective leukotriene receptor antagonists at the cys-LT-1 receptor. The data presented for BAY x7195 (present report) demonstrate a potency similar to that of these previously reported leukotriene receptor antagonists. In isolated human airways, Björck and Dahlén (1993) demonstrated the potency of several cys-LT-1 receptor antagonists in tissues derived from allergic and asthmatic patients. These preclinical evaluations of leukotriene receptor antagonists in human respiratory tissue suggest that such compounds may have potential therapeutic value for the treatment of asthma.

Pharmacological evidence suggesting a role of the leukotrienes in asthma has also been obtained in clinical trials with selective leukotriene receptor antagonists (Barnes et al., 1987; O'Shaughnessy et al., 1993; Britton et al., 1987; Fuller et al., 1989; Christie et al., 1991). However, most of these investigations have involved only acute or short treatment schedules. There is a need for complementary clinical trials with more extended treatments.

The results (present report) show that Bay x7195 is a potent selective leukotriene receptor antagonist in human airways. The compound has a profile of action similar to that observed for ICI 204,219 (Krell et al., 1990). These preclinical data for human respiratory tissue suggest that BAY x7195 may block allergic reactions in lung and therefore warrants evaluation in asthmatic patients.

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