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# The effects of a novel sulphidopeptide leukotriene antagonist, BAY x7195, against elicited bronchoconstriction in the anaesthetized guinea-pig

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**1** The novel leukotriene antagonist Bay x7195, has been evaluated against bronchoconstriction induced by leukotriene $D_4$ ($LTD_4$), the thromboxane $A_2$ ($TXA_2$) mimetic U46619, histamine and antigen, in the guinea-pig *in vivo* by use of a modified Konzett-Rössler preparation.

**2** $LTD_4$, given intravenously (i.v.) at 1 or 3 $\mu g\ kg^{-1}$ in the presence of indomethacin and sotalol, caused a 50–70% maximal bronchoconstriction in most animals.

**3** BAY x7195, given i.v., orally (p.o.), by aerosol or dry powder insufflation, in lactose, reduced $LTD_4$-induced bronchoconstriction dose-dependently. The approximate $ID_{50}$ values were 83 $\mu g\ kg^{-1}$, 3 $mg\ kg^{-1}$, 0.0003 % w/v for 20 breaths and 20 $\mu g$ respectively.

**4** The action of BAY x7195 (10 $mg\ kg^{-1}$, p.o.) was long lasting, causing significant inhibition of the $LTD_4$-induced response (88% reduction) 8 h after dosing.

**5** When given intravenously, in the presence of selected antagonists, BAY x7195 caused a dose-related reduction in the antigen-induced response, with an approximate $ID_{50}$ of 2 $mg\ kg^{-1}$.

**6** At 3 $mg\ kg^{-1}$, i.v., a dose which abolished the response to $LTD_4$, BAY x7195 had no effect on U46619- or histamine-induced bronchoconstriction.

**7** BAY x7195 is a potent, selective and long acting antagonist of $LTD_4$-induced bronchoconstriction, in an anaesthetized, ventilated guinea-pig model. It is therefore worthy of clinical evaluation in diseases believed to involve the sulphidopeptide leukotrienes, such as asthma.

**Keywords:** Leukotriene antagonist; $LTD_4$; antigen; bronchoconstriction; airways

## Introduction

For many years the disease of asthma has been defined in terms of its characteristic symptoms. However, there has been considerable speculation about the biological mediators which cause them.

Early investigations into guinea-pig anaphylaxis led to the discovery of 'SRS-A' (slow-reacting substance of anaphylaxis), which was later characterized and found to consist of a family of lipoxygenase derived mediators, the sulphidopeptide leukotrienes (leukotriene $C_4$ ($LTC_4$), $LTD_4$ and $LTE_4$) (Murphy *et al*., 1979; Morris *et al*., 1980). Since their isolation, these mediators have been proposed as candidates for many of the symptoms of asthma.

$LTE_4$ is the biologically stable end product of $LTC_4$ and $LTD_4$ metabolism and its appearance demonstrates the prior formation of these mediators. An increase in urinary $LTE_4$ has been observed following acute antigen challenge in asthmatics (Taylor *et al*., 1989; Westcott *et al*., 1991; Kumlin *et al*., 1992), following late reactions in dual responding asthmatics (Manning *et al*., 1990) and after lysine-aspirin administration in aspirin-sensitive asthmatics (Kumlin *et al*., 1992).

In addition, increased levels of immunoreactive $LTC_4$ have been detected in bronchoalveolar lavage (BAL) fluid from asthmatics (Lam *et al*., 1988; Wardlaw *et al*., 1989) and, in rhinitis, increased levels have been observed in nasal washings after antigen challenge (Shaw *et al*., 1985). The cell type responsible for the synthesis and release of leukotrienes in asthma is not clear, although leukotrienes known to be produced by mast cells, macrophages and eosinophils.

Not only do sulphidopeptide leukotrienes possess potent bronchoconstrictor properties (approx 1000 times more potent than histamine, Griffin *et al*., 1983; Smith *et al*., 1985), but they have also been shown to cause a non-specific increase in bronchial responsiveness (Kern *et al*., 1986), an increase in vascular permeability (Dahlen *et al*., 1981) and to increase mucus secretion (Marom *et al*., 1982). It is clear from this profile of activity that leukotrienes may be significant mediators responsible for many of the characteristics of asthma. For this reason many groups have sought to reduce the effects of these mediators by selectively antagonizing their activity at specific receptors. The use of these antagonists has led to an improvement in lung function in patients with asthma (Cloud *et al*., 1989; Gaddy *et al*., 1992) and reduced the severity of responses to antigen challenge (Fuller *et al*., 1989; Taylor *et al*., 1991; Rasmussen *et al*., 1992) or aspirin administration in sensitive subjects (Christie *et al*., 1991).

BAY x7195 is a novel cysteinyl-leukotriene receptor antagonist (Abram *et al*., 1993) with marked potency against these agonists in guinea-pig isolated tracheal tissue. The negative log molar dissociation constant ($pK_B$) against $LTD_4$ and $LTE_4$ have been found to be 8.4 and 9.1, respectively (Abram *et al*., 1993; latter unpublished). In addition, when the metabolism of $LTC_4$ to $LTD_4$ was inhibited, BAY x7195 also weakly inhibited the $LTC_4$-induced contractions with a $pK_B$ of 6.1 (Abram *et al*., unpublished data).

Gorenne *et al*. (1995) have demonstrated that, in human airways, BAY x7195 exhibits a $pK_B$ of $7.83 \pm 0.16$ against $LTD_4$-induced contractions and reduced IgE-induced contractions with an $IC_{50}$ of $0.31 \pm 0.08$ $\mu M$. These values were similar to those obtained for the leukotriene antagonist ICI 204,219 ($7.72 \pm 0.47$ and $0.16 \pm 0.03$ $\mu M$, respectively).

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In this paper we present the findings of our investigations into the properties of this novel leukotriene receptor antagonist, BAY x7195, against leukotriene $D_4$ and antigen-induced bronchoconstriction in the guinea-pig.

## Methods

### *Lung volume recording*

Male Dunkin-Hartley guinea-pigs (350 – 500 g) (Tuck Ltd) were anaesthetized with sodium pentobarbitone 120 mg $kg^{-1}$ intraperitoneally (i.p.), sufficient to induce anaesthesia and abolish natural respiration. The trachea was cannulated to allow the animal to be attached to a Harvard small animal ventilator, set at 60 breaths $min^{-1}$. Lung overflow volume was measured with the modified Konzett-Rössler model described by Gardiner (1971). An external jugular vein was cannulated for drug administration.

At the end of each experiment the maximal overflow volume (designated 100%) was established by clamping the trachea, thus allowing the full stroke volume to pass into the overflow chamber. An increase in lung overflow volume was taken to indicate bronchoconstriction and the size of each response was expressed as a percentage of the maximal overflow.

In experiments where the test compound, or antigen was administered by aerosol, an ultrasonic nebuliser (DeVilbiss 'Pulmosonic') was introduced into the system, as described by Lees and Payne (1985).

### *Experimental design*

For each antagonist study 32 animals were assigned to four treatment groups in a random fashion. Each animal received vehicle or treatment at a designated time before $LTD_4$ administration. Indomethacin (10 mg $kg^{-1}$) and sotalol (1 mg $kg^{-1}$) were given intravenously (i.v.) 20 min before the $LTD_4$ except in studies where the compound pretreatment time was 20 min, when they were given 22 min before $LTD_4$. A maximum of twelve animals was tested on each day and where studies lasted longer than 1 day, the treatments were evenly divided throughout the experimental days, according to a Latin square design. When treatments were given orally (p.o.), the animals were fasted overnight, before being dosed.

Since leukotrienes are known to cause the release of other mediators, the animals were pretreated with intravenous (i.v.) indomethacin and sotalol. These treatments abolished the contribution of prostanoids and adrenaline ($\beta_2$), respectively. The remaining response was considered to be caused by the direct action of $LTD_4$ on its own receptor(s).

Investigations involving antigen challenge were performed in animals sensitized to ovalbumin (OA). Male Dunkin-Hartley guinea-pigs weighing 250 – 300 g were injected with 100 mg OA, 50 mg i.p. and 50 mg subcutaneously, and left for three weeks for antibodies to develop. On the fourth week the animals weighed 350 – 500 g and were set up as described above. Challenge was with aerosol of OA (0.1% for 20 breaths).

Since antigen challenge releases an array of mediators, the animals were pretreated i.v. with indomethacin (10 mg $kg^{-1}$), sotalol (1 mg $kg^{-1}$), pyrilamine (1 mg $kg^{-1}$), cimetidine (5 mg $kg^{-1}$) and atropine (1 mg $kg^{-1}$) 22 min before antigen challenge. These treatments abolished the contribution of prostanoids, adrenaline ($\beta_2$), histamine ($H_1$ and $H_2$) and the effects of parasympathetic acetylcholine release (muscarinic), respectively. The remaining response was predominantly mediated by leukotrienes.

For each investigation of selectivity 12 animals were assigned to two treatment groups. Animals received either vehicle or BAY x7195, at 3 mg $kg^{-1}$ i.v., 20 min before the administration of a bronchoconstrictor agonist.

Since the responses to both $LTD_4$ and antigen were slow in onset and sustained in duration, with very poor recovery, only a single dose of each of these agents was given to each animal.

### *Protocols*

*Dosing regimens for antagonism studies* Bronchoconstriction was induced by the i.v. administration of 1 or 3 $\mu$g $kg^{-1}$ $LTD_4$. In order to determine the appropriate dose of $LTD_4$, if 1 $\mu$g $kg^{-1}$ failed to produce at least 50% maximal constriction in 'pilot' vehicle-treated animals, 3 $\mu$g $kg^{-1}$ was then given, and subsequently used in the antagonist studies. This dose ranging was performed in 2 or 3 animals and these data were subsequently included in the study.

BAY x7195 was administered i.v., orally, by aerosol for 20 breaths or by dry powder insufflation in lactose (Schiantarelli *et al*., 1982), 20 min, 120 min, 5 min or 5 min, respectively, before $LTD_4$ was given. For investigations of the duration of action after oral dosing BAY x7195 was given at 1, 2, 4, 8 or 24 h before $LTD_4$. In a single study against antigen-induced bronchoconstriction, BAY x7195 was administered i.v. 20 min before the aerosol antigen challenge.

*Selectivity* Investigations of selectivity were made against histamine and the thromboxane mimetic U46619. Histamine was administered over the dose range 1 – 10 $\mu$g $kg^{-1}$, i.v., and U46619 over the dose range 0.1 – 100 $\mu$g $kg^{-1}$, i.v., with maximal recovery allowed between each dose of both agonists. BAY x7195 (3 mg $kg^{-1}$) was given i.v. 20 min before the start of each dose-response curve. No other pretreatment was given in these studies.

### *Statistical analysis of data*

Groups of 8 animals responded very differently to the $LTD_4$ and generally data were not normally distributed. For this reason data are expressed as median $\pm$ semi-interquartile range ((upper quartile - lower quartile)/2). Since only one dose of either $LTD_4$ or antigen was given in each animal, an estimate of the level of inhibition observed in the BAY x7195-treated animals was calculated by comparing the median of the test group with the median of the control group. The $ID_{50}$ (dose which reduced the response by 50%) was calculated by use of linear regression of the values obtained from these calculations.

Statistical comparisons across the groups were made with the Kruskall Wallis test and paired comparisons between each treatment group and the control group with the Mann Whitney U-test. Statistical significance was taken at $P \leqslant 0.05$, with no adjustment made for repeat testing.

For selectivity studies, data were normally distributed. Regression analysis was used to calculate the $ED_{50}$ dose (the dose which caused 50% maximal bronchoconstriction), for each animal. These analyses were performed in 6 animals and combined to give the mean $\pm$ s.e.mean. Differences between the vehicle and treatment groups were assessed by one way ANOVA. Since there were no significant differences between the treatment groups ($P > 0.05$), no further comparisons were made.

### *Materials*

BAY x7195 (4S-4-[4carboxyphenylthio]-7-[4-(4-phenoxybutoxy)-phenyl]-hept-5-(z)-enoic acid) and $LTD_4$ were supplied by Dr T.S. Abram, Department of Chemistry, Bayer plc, (U.K.). Other materials were obtained from the following commercial sources: indomethacin and U46619 (1,5,5-hydroxy-11$\alpha$, 9$\alpha$-(epoxymethano) prosta-5Z, 13E-dienoic acid) from Sigma; histamine acid phosphate and lactose from BDH. Sotalol was a gift from Bristol-Myers Squibb. Sodium pentobarbitone ('Sagatal') was obtained from Willington Medical.

For i.v. and aerosol administration, BAY x7195 was dissolved in 5% w/v $NaHCO_3$. For oral treatments, it was formulated as a suspension in 1% w/v Tylose. For dry powder insufflation, it was mixed with lactose, finely ground in a pestle and mortar and packed into glass capillary tubes to give a final weight of 3.5 mg.

$LTD_4$ was made up in physiological saline and kept on ice, with light excluded, throughout the experiment. Indomethacin was dissolved in 5% w/v $NaHCO_3$ and all i.v. administrations were in a volume of 1 ml $kg^{-1}$ given over 10–15 s.

## Results

In none of the experiments with BAY x7195 was any intrinsic activity observed, following any route of administration.

### *Intravenous administration*

$LTD_4$ (1 $\mu$g $kg^{-1}$, i.v.) caused a marked bronchoconstriction in the anaesthetized ventilated guinea-pig (Figure 1). The administration of BAY x7195, 20 min before $LTD_4$, caused a dose-related reduction in this response (Figure 1). When given at 0.1 mg $kg^{-1}$, BAY x7195 caused a statistically significant reduction in the response (−56%, $P<0.001$). Increasing the BAY x7195 dose to 0.3 mg $kg^{-1}$ yielded a further reduction in the response (−83%) which was also statistically significant. The higher doses of 1 and 3 mg $kg^{-1}$ abolished the response to $LTD_4$ in the majority of animals. From regression analysis of these data, the $ID_{50}$ for BAY x7195 was calculated to be 83 $\mu$g $kg^{-1}$.

### *Oral administration*

Overnight fasting in guinea-pigs reduced airway sensitivity to $LTD_4$, but a response to 1 $\mu$g $kg^{-1}$, i.v., in excess of 50% maximal was observed in the majority of animals assigned to this study. When BAY x7195 was given over the dose range 1–10 mg $kg^{-1}$, 2 h before $LTD_4$, a dose-related reduction in the response was observed (Figure 2). At 3 mg $kg^{-1}$ p.o. BAY x7195 caused a marked and significant reduction in the response (−51%, $P<0.01$) and this was taken to be the approximate $ID_{50}$ dose. When the dose was increased to 10 mg $kg^{-1}$, p.o., an increase in inhibition was observed (−83%, $P<0.01$).

### *Aerosol administration*

Administration of the aerosol vehicle or treatment caused a small increase in overflow volume, a characteristic of this model. Since there was no difference between the size of this effect in the vehicle and that in the treatment groups, the results were expressed as the difference from baseline for each animal tested (Figure 3). BAY x7195, over the aerosol concentration 0.0001% w/v to 0.01% w/v given for 20 breaths, 5 min before $LTD_4$ (3 $\mu$g $kg^{-1}$, i.v.) administration, caused a dose-related reduction in the bronchoconstrictor response. The administration of the higher dose of 0.1% w/v caused no greater reduction in the response. From these data, an approximate $IC_{50}$ was calculated to be 0.0003% w/v for 20 breaths.

### *Dry powder insufflation*

BAY x7195, mixed with lactose and administered by insufflation 5 min before $LTD_4$ (3 $\mu$g $kg^{-1}$, i.v.) administration, caused a dose-related reduction in the ensuing response

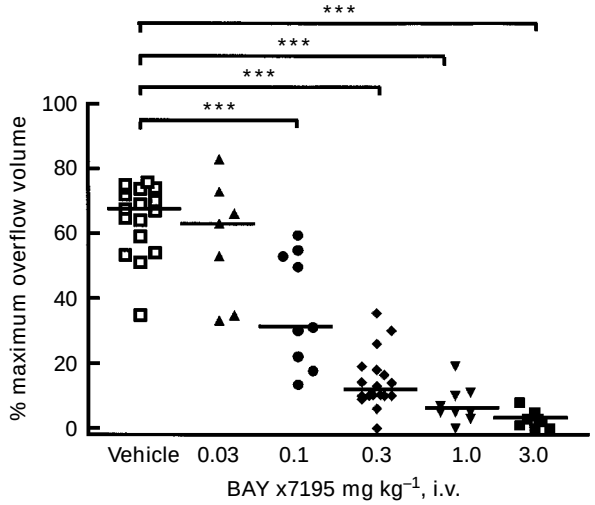


**Figure 1** Effect of BAY x7195 on $LTD_4$-induced bronchoconstriction in anaesthetized, ventilated, guinea-pigs. All animals were pretreated with indomethacin (10 mg $kg^{-1}$, i.v.) and sotalol (1 mg $kg^{-1}$, i.v.) and were given vehicle or BAY x7195 at one dose in the dose range 0.03 to 3 mg $kg^{-1}$, i.v., 20 min before $LTD_4$ administration. Each point represents the response of each animal in each treatment group, with the median shown as a horizontal line. Asterisks denote statistical significance (Mann-Whitney U-test); ***$P\leqslant 0.001$.

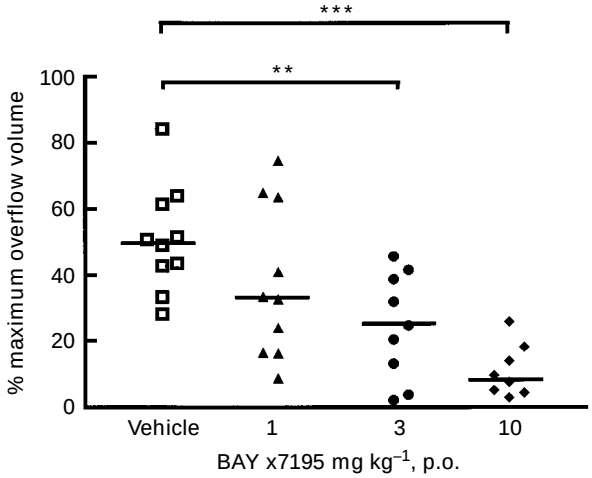


**Figure 2** Effect of BAY x7195 on $LTD_4$-induced bronchoconstriction in anaesthetized, ventilated, guinea-pigs. All animals were pretreated with indomethacin (10 mg $kg^{-1}$, i.v.) and sotalol (1 mg $kg^{-1}$, i.v.) and were given vehicle or BAY x7195 at one dose in the dose range 1.0 to 10 mg $kg^{-1}$, p.o., 120 min before $LTD_4$ administration. Each point represents the response of each animal in each treatment group, with the median shown as a horizontal line. Asterisks denote statistical significance (Mann-Whitney U-test); **$P\leqslant 0.01$, ***$P\leqslant 0.001$.

(Figure 4). From these data the $ID_{50}$ was calculated to be 20 $\mu$g in lactose.

### *Duration of action*

The investigation of duration of action of BAY x7195 (10 mg $kg^{-1}$) revealed a long lasting effect following oral dosing. One hour after dosing a marked and significant reduction in the $LTD_4$ (1 $\mu$g $kg^{-1}$, i.v.)-induced response was observed (−65%, $P \leqslant 0.001$ (Figure 5)). By 2 h after dosing the inhibition had increased to −84% ($P \leqslant 0.01$) and remained high up to 8 h after dosing (−77% at 4 h, −88% at 8 h, $P \leqslant 0.001$). However, by 24 h after dosing there remained only a small, and not statistically significant, reduction in the observed response.

### *Effects against antigen challenge*

Intravenous BAY x7195 was evaluated against antigen challenge (0.1% OA 20 breaths) in previously sensitized guinea-pigs. When given over the dose range 0.1 to 10 mg $kg^{-1}$, i.v., it caused a dose-related reduction in the response, which was statistically significant from 1.0 to 10 mg $kg^{-1}$ (Figure 6). From these data an approximate $ID_{50}$ was calculated to be 2 mg $kg^{-1}$, i.v.

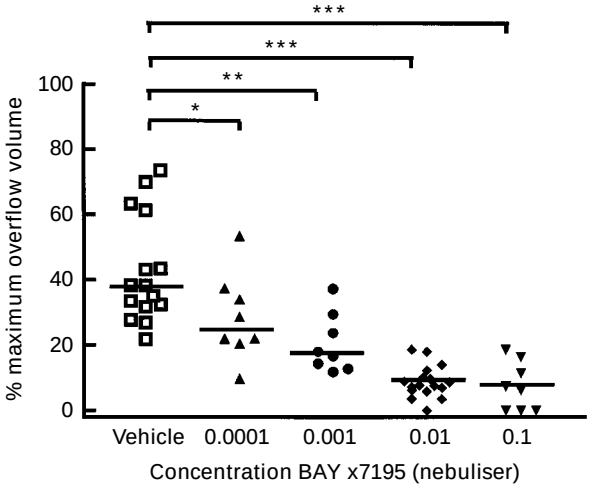


**Figure 3** Effect of BAY x7195 on $LTD_4$-induced bronchoconstriction in anaesthetized, ventilated, guinea-pigs. All animals were pretreated with indomethacin (10 mg $kg^{-1}$, i.v.) and sotalol (1 mg $kg^{-1}$, i.v.) and were given vehicle or BAY x7195 at one dose in the dose range 0.0001% to 0.1% w/v for 20 breaths, 5 min before $LTD_4$ administration. Each point represents the response of each animal in each treatment group, with the median shown as a horizontal line. Asterisks denote statistical significance (Mann-Whitney U-test); $*P \leqslant 0.05$, $**P \leqslant 0.01$, $***P \leqslant 0.001$.

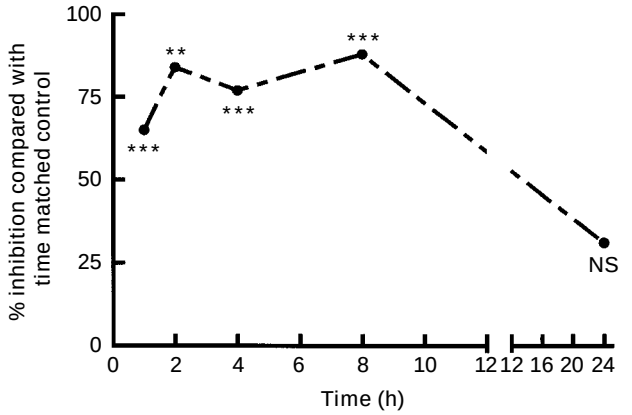


**Figure 5** Duration of action over 24 h, of oral BAY ×7195 (10 mg $kg^{-1}$) against $LTD_4$-induced bronchoconstriction in anaesthetized, ventilated, guinea-pigs. All animals were pretreated with indomethacin (10 mg $kg^{-1}$, i.v.) and sotalol (1 mg $kg^{-1}$, i.v.) and were given vehicle or BAY ×7195 at 1, 2, 4, 8 or 24 h before $LTD_4$ administration. Each point represents the inhibition of the median of the responses from 8 animals compared with the corresponding control median value. Asterisks denote statistical significance (Mann-Whitney U-test); NS, represents not statistically significant ($P > 0.05$), $**P \leqslant 0.01$, $***P \leqslant 0.001$.

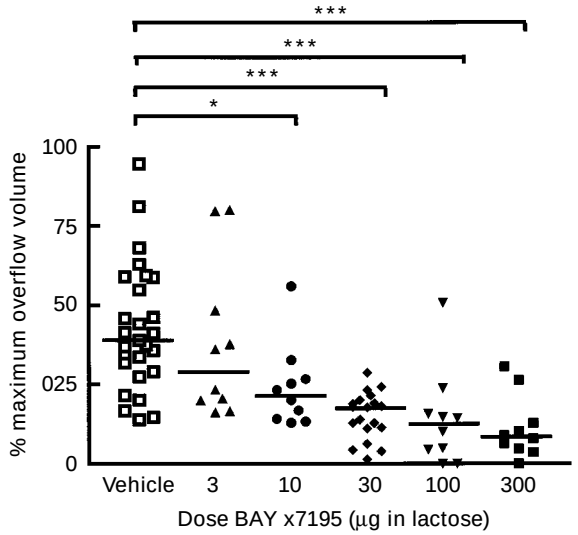


**Figure 4** Effect of BAY x7195 on $LTD_4$-induced bronchoconstriction in anaesthetized, ventilated, guinea-pigs. All animals were pretreated with indomethacin (10 mg $kg^{-1}$, i.v.) and sotalol (1 mg $kg^{-1}$, i.v.) and were given vehicle or BAY x7195 at one dose in the dose range 3 to 300 $\mu$g in lactose, by insufflation, 5 min before $LTD_4$ administration. Each point represents the response of each animal in each treatment group, with the median shown as a horizontal line. Asterisks denote statistical significance (Mann-Whitney U-test); $*P \leqslant 0.05$, $***P \leqslant 0.001$.

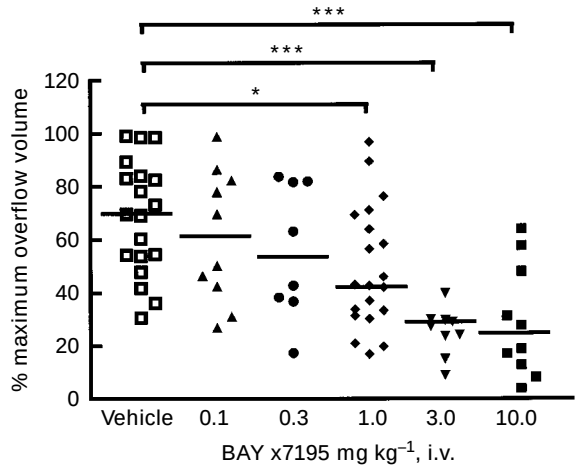


**Figure 6** Effect of BAY x7195 on antigen-induced bronchoconstriction in anaesthetized, ventilated, guinea-pigs. All animals were pretreated i.v. with indomethacin (10 mg $kg^{-1}$), sotalol (1 mg $kg^{-1}$), pyrilamine (1 mg $kg^{-1}$), cimetidine (5 mg $kg^{-1}$) and atropine (1 mg $kg^{-1}$) and were given vehicle or BAY x7195 at one dose in the dose range 0.01 to 10 mg $kg^{-1}$ i.v. 20 min before antigen administration (OA 0.1% w/v for 20 breaths). Each point represents the response of each animal in each treatment group, with the median shown as a horizontal line. Asterisks denote statistical significance (Mann-Whitney U-test); $*P \leqslant 0.05$, $**P \leqslant 0.01$, $***P \leqslant 0.001$.

**Table 1** Effect of BAY x7195 (3 mg kg$^{-1}$, i.v.) on bronchoconstriction induced by non-leukotriene agonists

| *Broncoconstrictor* | $ED_{50}$ ($\mu$g kg$^{-1}$, i.v.) *Vehicle* | *+ Bay x7195* | *Statistical significance* |
|---|---|---|---|
| U46619 (0.1–100 $\mu$g kg$^{-1}$, i.v.) | 1.066±0.933 | 0.945±0.881 | NS |
| Histamine (1.0–10.0 $\mu$g kg$^{-1}$, i.v.) | 6.5±3.39 | 4.8±1.66 | NS |

Selectivity of effect of BAY x7195 on agonist-induced broncoconstriction in anaesthetized, ventilated guinea-pigs. Vehicle or BAY x7195 (3 mg kg$^{-1}$, i.v.) was given 20 min before the start of agonist administration. The thromboxane receptor agonist U46619 was given over the dose range 0.1 to 100 $\mu$g kg$^{-1}$, i.v. Histamine was given over the dose range 1–10 $\mu$g kg$^{-1}$, i.v. Each dose of both agonists was separated from the previous dose by 5 min. From the dose-response data obtained for each animal, an $ED_{50}$ was calculated by regression analysis over the linear part of the curve. The $ED_{50}$ values presented represent the mean and s.e.mean of data from 6 animals. The response to each dose for both vehicle and BAY x7195 treatments were compared by one-way ANOVA. NS: not statistically significant ($P<0.05$).

### *Selectivity*

At 3 mg kg$^{-1}$, i.v., a dose which abolished the response to $LTD_4$, BAY x7195 had no effect on U46619- or histamine-induced bronchoconstriction in this model (Table 1).

## Discussion

In the studies described above, we demonstrated that BAY x7195 is a potent and selective antagonist of $LTD_4$-induced bronchoconstriction in the guinea-pig, with long lasting activity following oral dosing.

Following oral administration, BAY x7195 was some 30–40 fold less potent than following i.v. administration, reflecting the different rates of absorption and distribution of this compound by these two routes. Although this could be interpreted as indicating a reduced availability of the compound following oral administration, the sustained inhibition observed following oral dosing demonstrates that this was not so. From pharmacokinetic studies, data suggest that it may undergo entero-hepatic cycling following initial absorption from the gut. (K.-H. Schlemmer, personal communication).

A marked increase in potency, compared with i.v. administration, was observed when the compound was delivered locally to the lung. Previous in-house studies have suggested that an aerosol generated as described above, delivers 40–50 $\mu$l to the animal over 20 breaths (data not shown). Thus at a concentration of 0.0003% (3 $\mu$g ml$^{-1}$) between 120 and 150 $\mu$g would be administered to the animal. This relatively low dose was potent at reducing the response to $LTD_4$ and the higher dose of 0.01% almost completely abolished the response, leaving only a small residual component of $\leqslant$10% maximal. These data suggest that administration of the compound directly to the airways may increase its availability at the site of action. Administration by dry powder insufflation also caused a marked inhibition of the response, indicating the potency of this compound. However, it is clear that the dose-response curve generated in this way was more shallow than that obtained following aerosol administration, suggesting that the availability of the compound administered by this route is not the same. This could be due to a better distribution in the lung when given by aerosol, rather than by insufflation, or because the compound is more readily absorbed when given in an aqueous solution, rather than as a dry powder.

However, it is clear that administration of BAY x7195, by any route, is efficacious at reducing the bronchoconstrictor response to intravenous $LTD_4$.

When given intravenously before antigen challenge, BAY x7195 was effective in reducing this response. The dose required to reduce the response by 50% was some 20–30 fold higher than that required to reverse the $LTD_4$-induced response. This lower potency against antigen, compared with that against leukotriene administration, has also been found by other investigators (Krell *et al*., 1990). The antigen-induced response is a complex phenomenon and is not mediated solely through leukotriene release. Although the prostanoid, histamine and parasympathetic muscarinic components of the response were abolished in this model, it is possible that other mechanisms occurred which were less susceptible to reversal by BAY x7195. These could include leukotriene-induced changes via another receptor at which BAY x7195 has a lower affinity. Since this model measures changes in total lung volume, these changes may be brought about not only by bronchoconstriction, but also by changes in airway wall thickness, as in localized oedema, or by fluid and secretagogues entering the lung lumen (e.g. mucous secretions). Although leukotrienes have been shown to mediate these changes (Dahlen *et al*., 1981; Marom *et al*., 1982; Piacentini & Kaliner, 1991), it is not clear which, if any, of these mechanisms occurs in this model, or through which receptor type they are mediated.

The evaluation of other peptide-leukotriene antagonists has yielded considerable evidence that these mediators are involved in bronchoconstriction in allergic asthma (Cloud *et al*., 1989; Fuller *et al*., 1989; Taylor *et al*., 1991; Gaddy *et al*., 1992; Rasmussen *et al*., 1992; Taniguchi *et al*., 1993; Nathan *et al*., 1994). They are also considered to be responsible for the bronchoconstriction observed in aspirin-induced asthma (Christie *et al*., 1991; Dahlen *et al*., 1993) and in exercise-induced asthma (Robuschi *et al*., 1992). They may also contribute to the hyper-responsiveness to mediators such as methacholine (Fujimura *et al*., 1993) or histamine (Taki *et al*., 1994). In addition it appears that basal release of these leukotriene mediators may contribute to the underlying deterioration in lung function, in the absence of provocation. The regular administration of leukotriene antagonists gave rise to an improvement in basal lung function and reduced bronchodilator use (Taki *et al*., 1994).

Since BAY x7195 has proven potency in this animal model of lung function, it is worthy of fuller evaluation in a battery of clinical tests where sulphidopeptide leukotrienes have been implicated in the pathology.

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