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LCB 2183 inhibits tracheal hyperreactivity and pulmonary inflammation in mouse airways

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Abstract

The pulmonary delayed-type hypersensitivity (DTH) reaction induced by picryl chloride is characterized by enhanced albumin concentration in the airway tissue in the early phase (2 h after challenge with picryl sulphonic acid). During the later phase (48 h after the challenge) enhanced tracheal reactivity to carbachol in vitro and cellular (mononuclear and polymorphonuclear leukocytes) accumulation into the airway tissue in vivo are prominent features. In this present study, the effects of a novel drug, LCB 2183, were examined in the pulmonary DTH reaction. LCB 2183 (25 mg/kg twice daily intragastric gavage) failed to inhibit the enhanced albumin accumulation in the early phase of this response. In contrast, LCB 2183 abolished the tracheal hyperreactivity and the leukocyte accumulation in the airways of picryl chloride-sensitized mice 48 h after the challenge. These results demonstrate that LCB 2183 could be effective in the treatment of airway hyperreactivity and pulmonary inflammation.

Keywords: Delayed-type hypersensitivity; Vascular permeability; Pulmonary inflammation; Airway hyperreactivity, mouse; LCB 2183

1. Introduction

Delayed-type hypersensitivity (DTH; type IV hypersensitivity) reactions have been extensively examined in the skin of man and animal species (Askenase, 1993). This reaction can be divided into at least two sequential components: the DTH-initiating and the DTH-effector phases (Van Loveren and Askenase, 1984). The recruitment of $CD4^+$ T lymphocytes into the extravascular tissue at local sites of antigen challenge is a prominent feature of the effector phase. This recruitment is thought to be dependent on the prior activation of resident mast cells by non-IgE-initiating factors which are produced in lymphoid organs shortly after the initial exposure to the antigen (Kops et al., 1984; Van Loveren et al., 1983). More recently, it has become apparent that DTH reactions can also be elicited in mouse airways using selected low molecular weight haptens (e.g. picryl chloride; Enander et al., 1983; Garssen et al., 1989, 1991). To induce this reaction mice (BALB/c) were initially skin-sensitized with picryl chloride and after 7 days the mice were re-exposed, via intranasal administration, to the same hapten in a water-soluble form (picryl sulphonic acid). The effector phase of the picryl chloride-induced DTH reaction is characterized by an early (≤ 2 h after re-exposure to the hapten) increase in vascular permeability in the airway tissue (Garssen et al., 1989). Furthermore, at later time points after the challenge (24–48 h) tracheal hyperreactivity and leukocyte accumulation (predominantly mononuclear leukocytes but also polymorphonuclear leukocytes) are observed (Garssen et al., 1991). LCB 2183 (Fig. 1) is a pteridinone derivative which is known to inhibit passive cutaneous anaphylaxis in both

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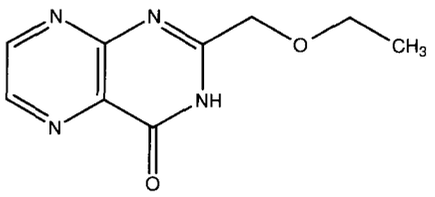

Fig. 1. Chemical structure of LCB 2183.

rats and mice while not influencing the cutaneous responses to intradermal histamine or serotonin (unpublished data). This anti-allergic activity is rendered more interesting by the finding that LCB 2183 is also able to inhibit an oxazolone-induced contact hypersensitivity reaction in mouse skin (Murray et al., 1994).

In this study we examined the ability of LCB 2183 to interfere with airway hyperreactivity and pulmonary inflammation associated with the effector phase of the picryl chloride-induced DTH reaction in the mouse. Both the early phase (2 h) and a later phase (48 h) were investigated.

2. Materials and methods

2.1. Animals

Mice (male BALB/c aged 6–8 weeks) were supplied by the National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands. All experiments were approved by the animal ethics committee at Utrecht University and the National Institute of Public Health and Environmental Protection.

2.2. Induction of pulmonary DTH reaction

Mice were skin-sensitized on day 1 with picryl chloride by applying 0.5% picryl chloride solution (200 μl) or vehicle control (alcohol; 200 μl) to the shaved dorsum and feet of anaesthetized (sodium pentobarbitone; 50 μl; 30 mg/kg i.p.) mice. Seven days later both picryl chloride-sensitized and vehicle-sensitized mice were challenged intranasally, under anaesthesia, with picryl sulphonic acid (50 μl; 0.6% solution). Various parameters were measured during the early phase (2 h after the challenge) and during the later phase (48 h after the challenge).

2.3. Measurement of albumin as an indicator of vascular permeability

Two hours after intranasal challenge mice were ether anaesthetized and perfused by injecting phosphate-buffered saline (PBS) in the right heart ventricle; the abdominal aorta was cut to inhibit buildup of pressure. The lungs turned pale during this procedure. After the perfusion the lungs were removed and homogenized in 10 ml PBS. After freezing and thawing the homogenate was centrifuged at $1000 \times g$ and the albumin concentration in the supernatant was measured using a Cobas-Bio centrifugal analysator.

2.4. Measurement of tracheal reactivity

Mice were killed by an overdose of sodium pentobarbitone (0.3 ml; 60 mg/kg i.p.). Determination of tracheal reactivity was performed as previously described (Buckley and Nijkamp, 1994). The trachea, which was resected in toto, was carefully cleaned of connective tissue using a binocular microscope. A 9-ring piece of trachea, taken from just below the larynx, was transferred to an oxygenated organ bath containing a modified Krebs solution (NaCl, 118 mmol/l; KCl, 4.7 mmol/l; $CaCl_2 \cdot 6H_2O$, 2.5 mmol/l; $MgCl_2 \cdot 6H_2O$, 0.5 mmol/l; $NaHCO_3$, 25.0 mmol/l; $NaHPO_4 \cdot H_2O$, 1.0 mmol/l and glucose 11.1 mmol/l) The trachea was directly slipped onto two supports, one of which was coupled to the organ bath and the other to an isometric transducer. The solution was aerated (95% O_2/5% CO_2) and maintained at 37°C. Isometric measurements were made using a force displacement transducer (Harvard Bioscience, Boston, MA, USA) and a two-channel recorder (Servogar type SE-120; Plato, Diemen, Netherlands) and were expressed as changes in milligram (mg) force. An optimal preload (1 g) was placed on the tissue at the beginning of the experiment. During the equilibration period of 45 min the fluid in the organ bath was exchanged every 15–20 min. At the end of this period cumulative concentration-response curves to carbachol (10^{-8}–10^{-4} M) or responses to a single application of carbachol (3×10^{-7} M) were recorded.

2.5. Histological examination

Forty-eight hours after intranasal hapten challenge the mice received a lethal dose of anaesthesia (50 μl i.p. of a cocktail consisting of 50 mg/ml Ketalar, 2% Rompun and 1 mg/ml atropine). Shortly before they were killed the mice were perfused by injecting a solution of 0.5% bovine serum albumin (BSA) and 5 mM glucose in PBS in the right heart ventricle; the abdominal aorta was cut to inhibit buildup of pressure. After this procedure the lungs had a very pale appearance. Hereafter, the lungs were removed and filled intratracheally with acetic acid formalin fixing solution (0.8% formalin, 4% acetic acid) using a ligature around

Table 1
Evaluation of the number of leukocytes

Accumulation of mononuclear cells	0	1	2	3
Diffusely around bronchioli	–	< 10 cell layers thick	> 10 cell layers thick	entirely surrounding bronchiolus > 10 cell layers thick
Around blood vessels	–	≤ 3 cell layers thick	4–10 cell layers thick	≥ 10 cell layers thick
Interstitial	–	scattered distribution of single cells specimen	dense accumulation covering < 25% of specimen surface	dense accumulation covering > 25% of specimen surface

the trachea. The unfolded lungs were fixed for at least 24 h in the fixative, dehydrated and embedded in Paraplast. Four μm thick sections were stained with haematoxylin and eosin. Evaluation of the number of leukocytes was performed. The parameters were scored according to the scoring method devised by Enander et al. (1983); see Table 1.

2.6. Drug administration

LCB 2183 (25 mg/kg; 100 μl; in sterile distilled water) was given by intragastric gavage at the following time points in relation to challenge time (0 h): −50 h, −42 h, −26 h, −18 h, −2 h, +6 h, +22 h, +30 h. The mice that did not receive LCB 2183 received distilled water at the same time points.

2.7. Data analysis

The non-parametric Wilcoxon test was used to analyze differences in histological scores between the treatment groups. Statistical differences in albumin content of lung homogenates were analyzed using the Student's *t*-test. Data obtained from measuring the tracheal smooth muscle function (tracheal reactivity) was initially analyzed using Bartlett's test for homogeneity of variances. Analysis of variance (ANOVA) using a multifactorial design was used to analyze the data further. P values < 0.05 were considered to be significant.

2.8. Materials

Picryl chloride was obtained from Swannanoa Chemotronix, NL, USA and was recrystallized 3 times from methanol/H_2O before use. Picryl sulphonic acid (PSA; trinitro-benzene sulphonic acid) was obtained from Serva Feinbiochemica, Heidelberg, Germany. Ketalar was obtained from Parke Davis, Spain. Rompun was obtained from Bayer, Leverkusen, Germany. Atropine, sodium pentobarbitone and carbachol were obtained from Onderlinge Farmaceutische Groothandel, Utrecht, Netherlands. LCB 2183 (2-(ethoxymethyl)-4(3*H*)-pteridinone) was provided as a gift by Lipha, Lyon, France.

3. Results

3.1. Effect of LCB 2183 on the increased albumin concentration in the airway tissue

An increased albumin concentration in lung homogenates, as a marker for increased vascular permeability, was observed 2 h after challenge in the picryl chloride-sensitized mice when compared to the vehicle-sensitized mice (Fig. 2). LCB 2183 (25 mg/kg twice daily intragastric gavage) did not affect this enhancement in the picryl chloride-sensitized mice and LCB 2183 had no effect in the vehicle-sensitized mice.

3.2. Effect of LCB 2183 on tracheal hyperreactivity induced by picryl chloride

Mice sensitized with picryl chloride exhibited tracheal hyperreactivity 48 h after challenge with picryl

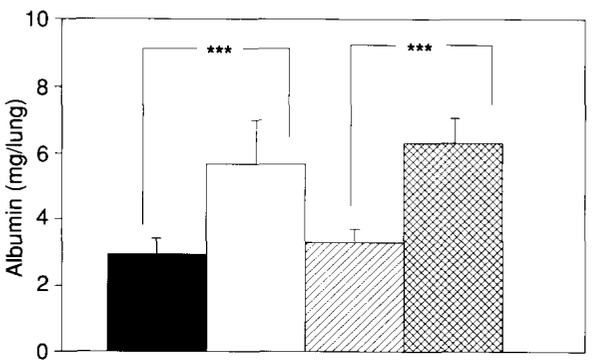

Fig. 2. The effect of LCB 2183 (25 mg/kg twice daily intragastric gavage) on albumin accumulation (mg/kg) in picryl chloride (PCL)-sensitized mice. Responses were measured 2 h after challenge in vehicle-sensitized (black bar), PCL-sensitized (open bar), vehicle-sensitized + LCB 2183 (hatched bar) and PCL-sensitized + LCB 2183 (cross-hatched bar) mice. Results are expressed as mean ± S.E.M. for $n = 8$ mice/group. Significant differences ($P < 0.01$) are indicated by asterisks (***).

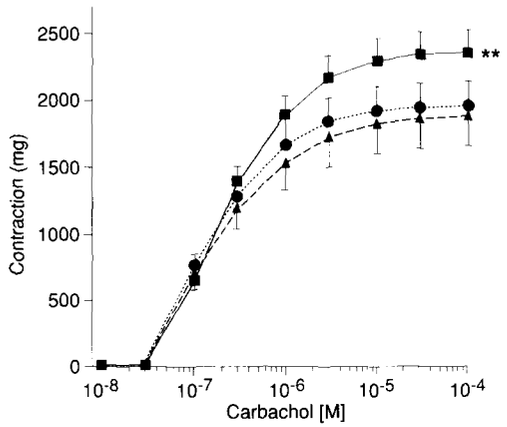

Fig. 3. The effect of LCB 2183 (25 mg/kg twice daily intragastric gavage) on tracheal responsiveness in picryl chloride (PCL)-sensitized mice 48 h after challenge. Concentration-response curves to carbachol were measured in PCL-sensitized (■), PCL-sensitized + LCB 2183 (●) and vehicle-sensitized (control, ▲) mice. Results are expressed as mean ± S.E.M. for $n = 7$–8 mice/group. Significant differences ($P < 0.01$) between the PCL-sensitized and control groups are indicated by asterisks (* *).

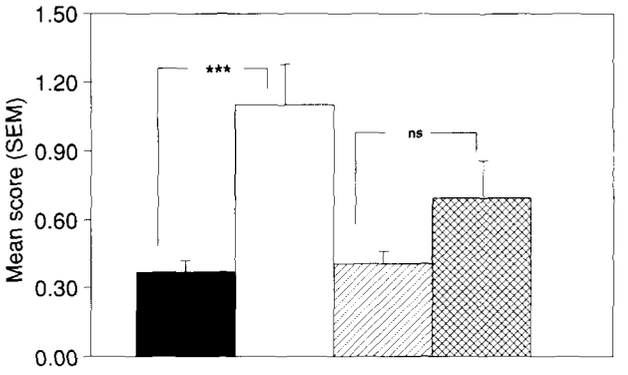

Fig. 4. The effect of LCB 2183 (25 mg/kg twice daily intragastric gavage) on the inflammatory reaction around the bronchioli 48 h after challenge. The inflammation, scored according to the scheme in Table 1, was assessed in vehicle-sensitized (black bar), picryl chloride (PCL)-sensitized (open bar), vehicle-sensitized + LCB 2183 (hatched bar) and PCL-sensitized + LCB 2183 (cross-hatched) mice. Results are expressed as mean score ± S.E.M. for $n = 8$ mice/group. Significant differences ($P < 0.01$) are indicated by asterisks (* * *). ns = not significant.

sulphonic acid (Fig. 3). The E_{max} value (the maximum response) in the picryl chloride-sensitized group was increased by approximately 20% above the vehicle-sensitized group. However, in these experiments there was no change in the EC_{50} (the effective concentration that gives 50% of the response). More importantly, Fig. 3 demonstrates that LCB 2183 (25 mg/kg twice daily intragastric gavage) completely abolished the tracheal hyperreactivity 48 h after the challenge. As a control, naive mice were also treated in vivo with LCB 2183 (25 mg/kg twice daily intragastric gavage) or water according to the regimen previously described. In this experiment the effect of LCB 2183 on the contraction induced by a single application of carbachol (3×10^{-7} M) to the organ bath was examined. There were no significant differences observed between the two groups (results: LCB 2183 group, 1870 ± 212 mg; water group, 2083 ± 121 mg, mean ± S.E.M. for $n = 6$–8 mice).

3.3. Effect of LCB 2183 on leukocyte accumulation

Two days after intranasal challenge with picryl sulphonic acid a significant accumulation of mononuclear leukocytes was seen around bronchioli and pulmonary blood vessels in the picryl chloride-sensitized mice when compared to the vehicle-sensitized mice (Fig. 4 and Fig. 5, Fig. 6a and Fig. 6b). In addition, some accumulation was found in the interstitium. The majority of the accumulating cells were lymphocytes and alveolar macrophages but some polymorphonuclear leukocytes were detected (Fig. 6e). In some animals foamy alveolar macrophages were found outside the inflammatory lesions. In the majority of vehicle-sensitized mice no inflammatory reactions were observed; however, occasionally a slight accumulation of inflammatory cells was seen 48 h after the challenge; this accumulation comprised mainly of polymorphonuclear leukocytes (data not shown). Treatment with LCB 2183 markedly inhibited the pulmonary DTH reaction (Fig. 4 and Fig. 5 and Fig. 6d). Moreover, the inflammatory response around the pulmonary blood vessels in picryl chloride-

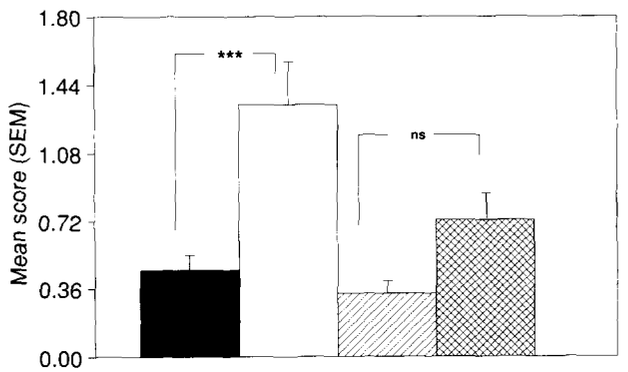

Fig. 5. The effect of LCB 2183 (25 mg/kg twice daily intragastric gavage) on the inflammatory reaction around the blood vessels 48 h after challenge. The inflammation, scored according to the scheme in Table 1, was assessed in vehicle-sensitized (black bar), picryl chloride (PCL)-sensitized (open bar), vehicle-sensitized + LCB 2183 (hatched bar) and PCL-sensitized + LCB 2183 (cross-hatched bar) mice. Results are expressed as mean score ± S.E.M. for $n = 8$ mice/group. Significant differences ($P < 0.01$) are indicated by asterisks (* * *). ns = not significant. In addition the difference between PCL-sensitized (open bar) and PCL-sensitized + LCB 2183 (cross-hatched bar) was significant ($P < 0.05$).

sensitized mice was significantly ($P < 0.05$) inhibited by treatment with LCB 2183 (Fig. 5). In fact after LCB 2183 treatment no significant inflammatory reaction was detected in the picryl chloride-sensitized when compared to the vehicle-sensitized mice (Fig. 4 and Fig. 5, Fig. 6c and Fig. 6d).

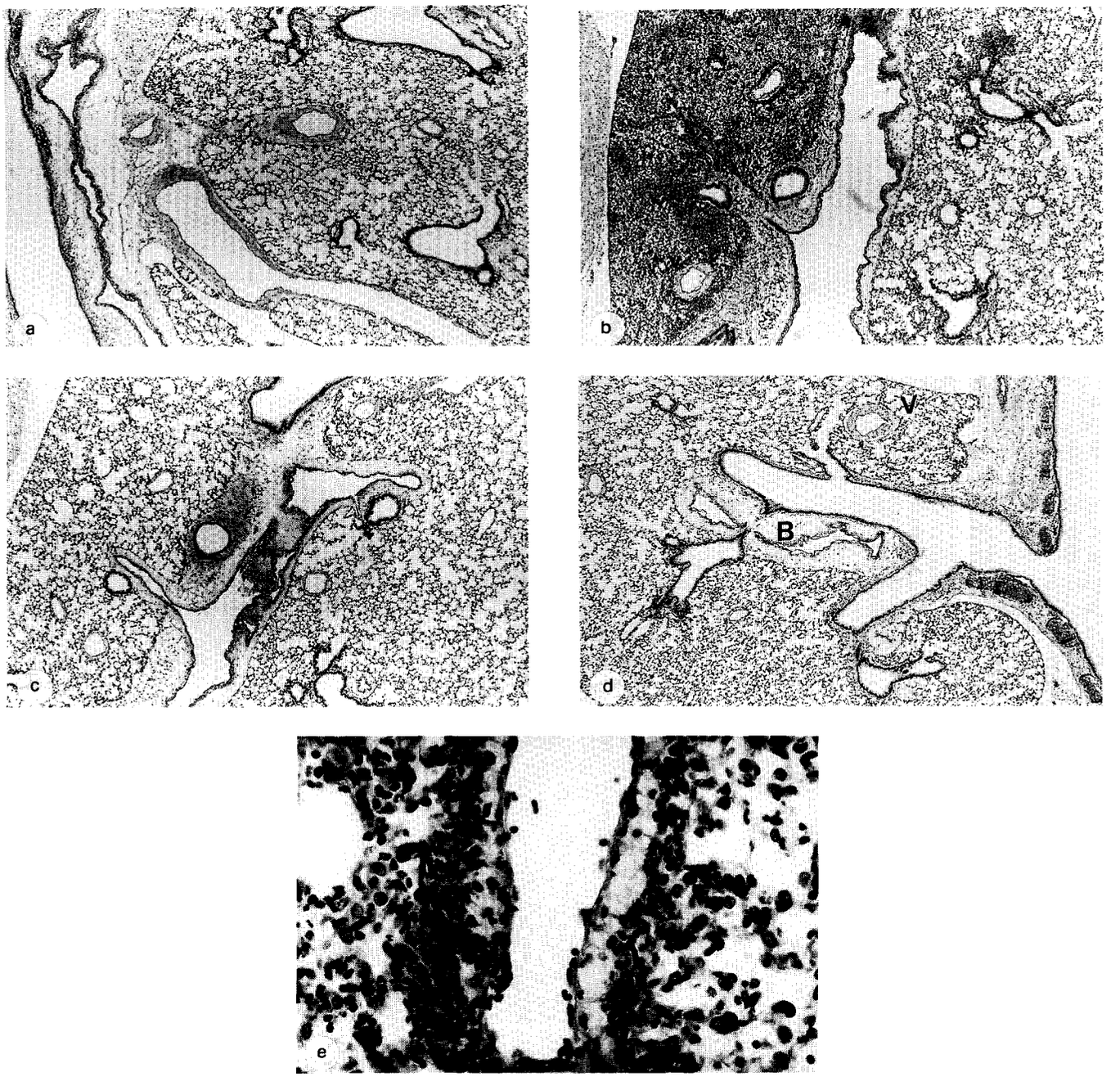

Fig. 6. Inflammatory responses to picryl chloride in lungs of BALB/c mice 48 h after challenge with picryl sulphonic acid. (a) Lung of vehicle-sensitized mouse. No mononuclear infiltrates are found. (b) Lung of picryl chloride-sensitized mouse. Mononuclear leukocytes are observed around blood vessels (V). Some mononuclear accumulation is also observed in the alveolar interstitium. (c) Lung of vehicle-sensitized mouse treated with LCB 2183. No mononuclear leukocyte accumulation is observed. (d) Lung of picryl chloride-sensitized mouse treated with LCB 2183. No mononuclear infiltrates are observed around the blood vessel (V) or the bronchioli (B). (e) High magnification of infiltrating cells around a pulmonary blood vessels in the lung of a picryl chloride-sensitized mouse.

4. Discussion

LCB 2183 markedly suppressed tracheal hyperreactivity and cellular accumulation associated with the late effector phase of the picryl chloride-induced DTH reaction in mouse airways. In contrast, the enhanced albumin accumulation in the airway tissue, 2 h after the antigen challenge, was unaffected by LCB 2183.

Although the mechanisms involved in pulmonary DTH reactions are not fully understood, it is known that T lymphocytes play a crucial role in the orchestration of the initial and later phases of this response (Garssen et al., 1991, 1994). DTH-initiating T cells produce an antigen-binding factor (e.g. picryl chloride factor) that arms mast cells and mediates the early (2 h) response in the skin and in the airways (Askenase et al., 1983, Kops et al., 1984). It is important to state that although picryl chloride factor is analogous to IgE in its ability to arm mast cells these two molecules are biologically and chemically dissimilar (Garssen et al., 1994; Askenase et al., 1983). The early events subsequently allow the recruitment of classical Tdth cells into the extravascular spaces 24–48 h after the challenge (Van Loveren and Askenase, 1984; Garssen et al., 1989). The characterization of these pulmonary inflammatory responses are extensively described by Enander et al. (1983) and Garssen et al. (1989, 1990, 1993). These studies revealed that the inflammatory response is characterized by a significant increase in $CD4^+$ T cells in the lung tissue of picryl chloride-sensitized mice (Garssen et al., 1990). In contrast the percentage of subtypes of bronchoalveolar cells was not altered whereas the total number of cells was increased (Garssen et al., 1993). During the early and late phases tracheal hyperresponsiveness in vitro was observed in picryl chloride-sensitized mice (Garssen et al., 1991). LCB 2183 was effective at inhibiting the later phase but not the early enhanced vascular leakage associated with the pulmonary DTH response. In light of the fact that leukocyte (particular mononuclear leukocyte) infiltration into airway tissue is a prominent feature of the later phase it is possible that LCB 2183 could interfere with leukocyte infiltration or activation.

As described above, the mast cell is thought to occupy a central role in the DTH reaction. It has been hypothesized that mast cell mediators released upon re-exposure to the hapten could be at least partially responsible for the effector phase of the pulmonary DTH reaction. Serotonin is a major vasoactive inflammatory mediator released by murine mast cells. In support of the role for serotonin in DTH reactions, 5-HT_2 receptor antagonists inhibited the mononuclear accumulation around the bronchioli and pulmonary blood vessels in picryl chloride-sensitized mice (Garssen et al., 1989, 1993). These studies raised the possibilty that LCB 2183 is a 5-HT_2 receptor antagonist. However, this is unlikely because (a) LCB 2183 abolished the tracheal hyperreactivity in picryl chloride-sensitized mice 48 h after the challenge whereas 5-HT_2 receptor antagonists failed to inhibit this response (Garssen et al., 1993) and (b) 5-HT_2 receptor antagonists inhibited the early enhanced vascular leakage response (Garssen et al., 1993) whereas LCB 2183 was without effect in this regard.

Although the pharmacological profile of LCB 2183 appears to be very different to 5-HT_2 receptor antagonists in this reaction it remains a possibility that LCB 2183 can influence mast cell activation. The induction of pulmonary DTH reactions in mast cell-deficient mice (W/W^v) has revealed that tracheal hyperreactivity to serotonin in vitro and mononuclear leukocyte accumulation in vivo were mast cell-dependent processes (Garssen et al., 1989,1994). In direct comparison, LCB 2183 also suppressed these airway parameters. Recently, LCB 2183 has been shown to be effective in a cutaneous DTH reaction induced by oxazolone in the mouse (Murray et al., 1994). In this study, LCB 2183 partially inhibited cutaneous ear swelling 2 and 24 h after a topical challenge. Interestingly, the mast cell stabilizing drugs, nedocromil sodium and sodium cromoglycate, were virtually ineffective in this model (Murray et al., 1994). These results argue against the hypothesis that LCB 2183 can influence mast cell activation. However, different mast cell populations are present in the skin and the airways and these differences could be of importance with regard to the influence of LCB 2183 in pulmonary DTH reactions (Irani and Schwartz, 1989). Moreover, nedocromil sodium was effective in inhibiting picryl chloride-induced tracheal hyperreactivity, in passively sensitized mice, 48 h after the challenge with picryl sulphonic acid (Garssen et al., 1994).

In conclusion, LCB 2183 is effective in inhibiting tracheal hyperreactivity and leukocyte accumulation in the airway tissue associated with the later phase of the lymphocyte-dependent pulmonary DTH reaction. The mechanism of action of LCB 2183 is as yet unknown but our data would support an interaction between LCB 2183 and pulmonary mast cells. Whatever the mode of action of LCB 2183, these results highlight the possibility that this novel drug could be an effective therapy in airway disease.

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