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Effects of a 5-lipoxygenase inhibitior (L-651,392) on primary and late pulmonary responses to ascaris antigen in the squirrel monkey

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Abstract

Allergic squirrel monkeys when exposed to an aerosol of *Ascaris suum* either develop a reproducible, immediate bronchoconstriction or an immediate bronchoconstriction followed by a reproducible late response. Pretreatment of ascaris-challenged squirrel monkeys with a potent, selective, orally active 5-lipoxygenase inhibitor, L-651,392 (4-bromo-2,7-dimethoxy-3,4-phenothizin-3-one), at a dose of 5 mg/kg p.o. resulted in near complete inhibition of the increases in pulmonary resistance (R_L) and decreases in dynamic compliance (Cdyn) normally observed following exposure to the antigen. A lower dose (1 mg/kg p.o.) of L-651-392 produced only a significant inhibition of the decreases in Cdyn. In monkeys known to develop dual responses to antigen, L-651,392 (5 mg/kg p.o.) significantly attenuated the immediate response and markedly inhibited the late response. These results suggest an important role for leukotrienes in primary and late phase allergen-induced bronchoconstriction.

Introduction

Certain conscious allergic squirrel monkeys when challenged with an aerosol of *Ascaris suum* undergo an acute bronchoconstriction which resolves spontaneously and is reproducible for individual animals. The response can be measured as an increase in pulmonary resistance (R_L) and a decrease in dynamic compliance (Cdyn) [1]. In a certain proportion of these animals a reproducible, late bronchoconstriction is observed commencing 2 to 6 h and peaking between 4 and 10 h after antigen challenge [2]. The late response consists of a small increase in R_L and a larger decrease in Cdyn.

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Leukotrienes are thought to be important mediators of immediate hypersensitivity reactions such as human bronchial asthma because they induce bronchoconstriction, may contribute to airway hyperresponsiveness, increase mucous secretion, increase vascular permeability and slow mucocilliary transport [3]. In the present work we report on the effects of a selective, orally active 5-lipoxygenase inhibitor, 4-bromo-2,7-dimethoxy-3H-phenothiazin-3-one (L-651,392) [4], on primary and late responses to inhaled antigen in the conscious squirrel monkey.

Materials and Methods

Materials

The following materials were used: *Ascaris Suum* antigen (Greer Laboratories), L-651,392 (4-

bromo-2,7-dimethoxy-3H-phenothiazin-3-one), synthesized and prepared at Merck Frosst, methocel 400 cps (Dow), Tween 80 (Atlas).

Ascaris-Induced Bonchoconstriction in Conscious Squirrel Monkeys

Male squirrel monkeys (*Saimiri Sciureus*), weight range 0.6–1.0 kg, were obtained from primate import, Fort Washington, N.Y., trained to sit in chairs and breath through face masks. Pulmonary mechanics were monitored as previously described [1, 2]. Briefly the monkeys were challenged with an aerosol of *Ascaris* antigen (40000 or 80000 protein nitrogen units/ml) on a regular basis at intervals no shorter than 2 weeks. Air flow was measured through a pneumotachograph attached to the face mask and connected to a differential pressure transducer. Pleural pressure was measured with a fluid filled catheter inserted into the thoracic cavity under local anesthetic and attached to a fluid filled differential pressure transducer. Both transducers were connected to a Buxco pulmonary mechanics computer. Pulmonary flow resistance (R_L) was expressed as the ratio of transpulmonary pressure to respiratory airflow at points of equal volume ($cmH_2O/ml/s$) and dynamic lung compliance (Cdyn) as the ratio of tidal volume to transpulmonary pressure at points of zero flow (ml/cmH_2O). Experiments were carried out under the guidelines of the Canadian Council of Animal Care.

Effects of L-651,392 on Ascaris-Induced Bronchoconstriction

L-651,392 was administered p.o. as a suspension in 0.4% methocel and 0.5% Tween 80 (dose volume 1 ml/kg) 2 h prior to the administration of antigen. The initial studies at a dose of 5 mg/kg were carried out in a group of 5 monkeys (# 1, 2, 5, 17 and 18) known to develop an early phase response following antigen challenge. Three of these monkeys (# 2, 5 and 17) and two additional monkeys (# 32 and 34) were used for testing the lower dose (1 mg/kg). Mean values for R_L and Cdyn were calculated at 5 min intervals prior to and for 1 h after antigen challenge. Results after antigen challenge were expressed as a % change relative to the baseline values. For control purposes the mean responses to 3–4 separate challenges in the absence of drug were used. In order to test for the effects of the drug, the values obtained over the first 1 h after challenge were averaged and the averaged values for each monkey with each treatment on either pulmonary parameter were tested for normality (Wilk-Shapiro test) and analyzed by the paired *t*-test.
In order to test the effects of the inhibitor on the late response a group of 2 monkeys were treated once and a third monkey was treated twice on two separate occasions and the primary response analyzed as above using a dose of L-651,392 of 5 mg/kg p.o. The time at which the late phase response was maximal varied between experiments. In order to group the data together the peak of the late response on each individual experiment was designated as time 0. Results expressed as percentage changes relative to the baseline values were then calculated at 15 min intervals before and after the peak response. These calculations were carried out separately for R_L and Cdyn because in 50% of the experiments the peak increases in R_L occurred prior to the peak decreases in Cdyn. In order to carry out statistical analysis, for each experiment the values of R_L for a 30 min period before and after the peak of the late phase and for Cdyn for a period of 60 min before and after the peak were meaned. The mean values were then tested for normality (Wilk-Shapiro test) and analyzed by the paired *t*-test. The control data on each monkey represents the mean of 3 separate control experiments.

Results

Administration of L-651,392 (1 or 5 mg/kg p.o.) had no significant effect on the baseline pulmonary parameters prior to the administration of *Ascaris*. Figures 1 and 2 show the effects of an aerosol of *Ascaris* antigen on changes in R_L and Cdyn for 60 min after challenge in the groups of monkeys with and without 1 or 5 mg/kg p.o. L-651,392. In the first group of monkeys the mean increase in R_L over a 60 min period was $52.7 \pm 8.5\%$ and the mean decrease in Cdyn $-34.5 \pm 5.3\%$ (results expressed as means $\pm$S.E.M., $n=5$). Following pretreatment of this first group of animals with L-651,392 (5 mg/kg p.o.) the mean increase in R_L was $2.9 \pm 18.3\%$ ($p<0.01$ compared to control) and the mean change in

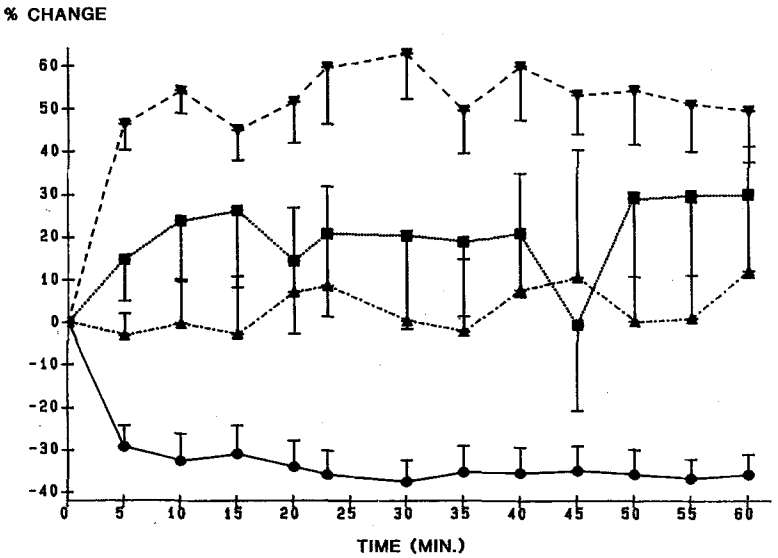

Figure 1
The effects of L-651,392 (5 mg/kg p.o.) on immediate bronchoconstriction induced by an aerosol of ascaris in conscious squirrel monkeys shown as increases in R_L for control (▼) or treated animals (▲) and decreases in Cdyn for control (●) and treated animals (■). Results are shown ± S.E.M., $n = 5$. Control data on each monkey represents the mean from 3–4 separate experiments.

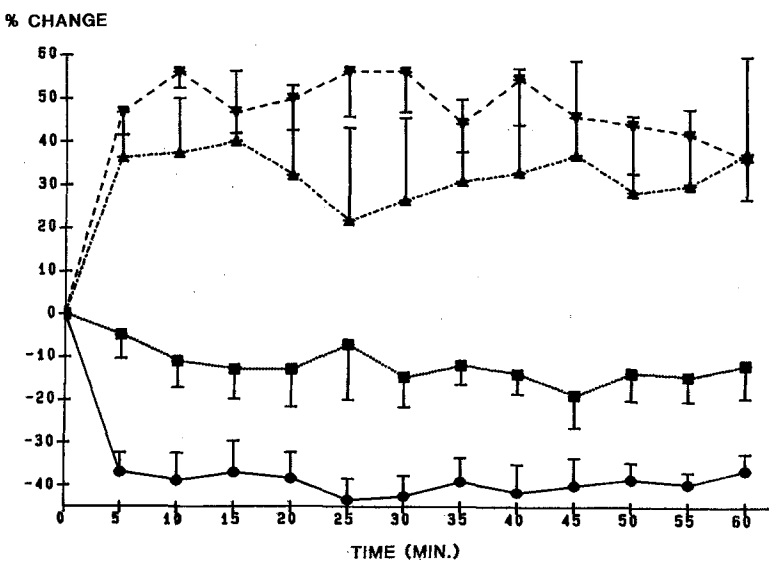

Figure 2
The effects of L-651,392 (1 mg/kg p.o.) on immediate bronchoconstricition induced by an aerosol of ascaris in conscious squirrel monkeys shown as increases in R_L for control (▼) or treated animals (▲) and decreases in Cdyn for control (●) and treated animals (■). Results are shown ± S.E.M., $n = 5$. Control data on each monkey represents the mean from 3–4 separate experiments.

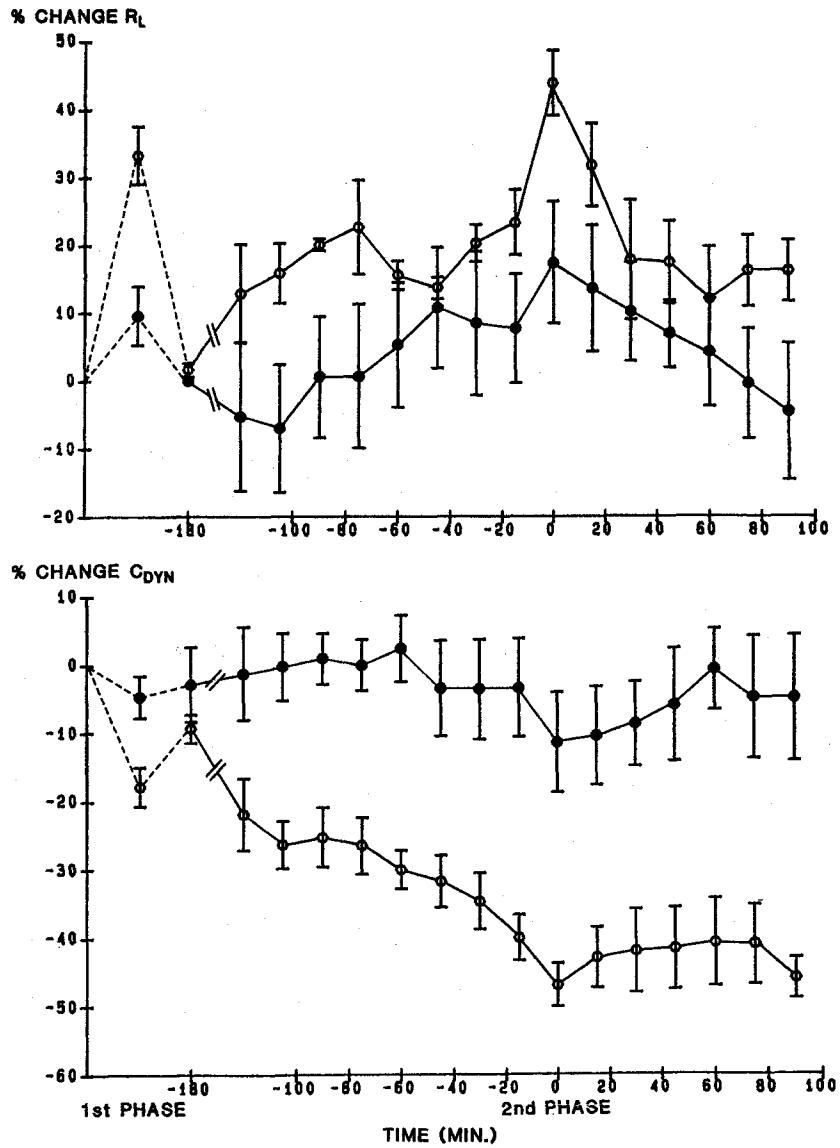

Figure 3
The effects on L-651,392 (5 mg/kg p.o.) on early and late bronchoconstriction induced by an aerosol of ascaris in conscious squirrel monkeys (Fig. 3A, increases in R_L; Fig. 3B, decreases in Cdyn). The early response is shown as the mean value over the first 60 min and the late response was calculated from the peak value (set at=0) in each experiment as defined in the methods. Control data (○) and data from animals treated with the inhibitor (●) are shown ±S.E.M., $n=4$. Control data for each experiment represents the mean of 3 separate experiments.

Cdyn 20.4±15.5% ($p<0.02$ compared to control). In the second group of animals the mean increase in R_L over the 60 min period was 48.0±6.6% and the mean decrease in Cdyn −39.3 ±4.9% (means ±S.E.M., $n=5$). Pretreatment of this second group with L-651,392 (1 mg/ kg p.o.) resulted in a significant inhibition ($p<0.02$) of the decrease in Cdyn to −12.5±6.6% but not of the increase in R_L ($p=0.07$) which was 33.8±16.6%. The selective inhibition of the de-

creases in Cdyn by the lower dose of the compound may reflect a selective distribution of the inhibitor within the lower airways.

In order to test the effects of L-651,392 on the late response, monkeys were treated p.o. using the dose that produced good inhibition of the primary response (5 mg/kg, p.o.). As was observed in the monkeys with single responses, significant inhibition of the immediate response to antigen was also observed in these experiments. The mean increase in R_L over the 60 min time period for the control group was $33.3 \pm 4.3\%$ and the mean decrease in Cdyn was $-17.9 \pm 2.9\%$ (results expressed as means $\pm$ S.E.M., $n=4$). Following pretreatment with inhibitor the mean increase in R_L was $9.6 \pm 4.4\%$ ($p<0.01$ compared to control) and the mean decrease in Cdyn $-4.7 \pm 3.1\%$ ($p<0.001$ compared to control). After partial or total recovery a second bronchoconstriction was observed in control animals commencing between 2 and 6 h peaking 4–10 h after the antigen aerosol. The mean increase in R_L was $27.4 \pm 4.7\%$ and mean decrease in Cdyn $-38.9 \pm 4.3\%$ (means $\pm$ S.E.M., $n=4$) (Fig. 3). The late phase was significantly inhibited by pretreatment with L-651,392 (Fig. 3), the mean increase in R_L being $11.4 \pm 8.7\%$ ($p<0.05$) compared to control) and the mean decrease in Cdyn $-5.1 \pm 6.3\%$ ($p<0.02$ compound to control; results expressed as means $\pm$ S.E.M., $n=4$).

Administration of L-651,392 produced no significant changes in baseline pulmonary parameters prior to administration of *Ascaris* to any of the groups of animals. This can be illustrated in the group of monkeys with dual responses by the mean values for Cdyn and R_L for the 15 min period immediately prior to challenge. Without pretreatment the Cdyn was 1.94 ± 0.23 ml/cmH_2O and the R_L 0.055 ± 0.002 cmH_2O/ml/sec. Following pretreatment with L-651,392 (5 mg/kg) the Cdyn was 1.89 ± 0.26 ml/cmH_2O and the R_L 0.060 ± 0.004 cmH_2O/ml/sec. All results are shown as means $\pm$ S.E.M. ($n=4$).

Discussion

L-651,392 is a potent inhibitor of leukotriene biosynthesis as determined by inhibition of leukotriene B_4 or leukotriene C_4 synthesis in rat peritoneal polymorphonuclear leukocytes, murine mastocytoma cells, murine peritoneal macrophages and human polymorphonuclear leukocytes. Inhibition of crude 5-lipoxygenase enzyme preparations from rat basophil leukemia cells or highly purified porcine 5-lipoxygenase has been observed in the presence of either NADH or NADPH indicating that a reductive product of L-651,392 is the active species. Selectivity for the 5-lipoxygenase enzyme was indicated by the failure to inhibit thromboxane B_2 synthesis in murine mastocytoma cells, prostaglandin E_2 synthesis by murine peritoneal macrophages, a cyclooxygenase preparation from ram seminal vesicle microsomes, 12-lipoxygenase from human platelets or porcine leukocytes, 15-lipoxygenase from soybean and cytochrome P_{450} from rat liver microsomes [4]. Unpublished results indicate that the compound has no effect on „mediator release" in general as exemplified by failure to inhibit histamine release from antigen-challenged smooth muscle preparations and enzyme release from challenged polymorphonuclear leukocytes. Oral activity has been demonstrated in hyperreactive rats where inhibition of antigen-induced dyspnea was observed [5].

The immediate response observed following administration of an aerosol of *Ascaris* to conscious squirrel monkeys in similar to that observed with an aerosol of leukotriene D_4 [6] and is thought in primates to be related to the release of mast cell derived mediators such as histamine and leukotrienes [7, 8]. Evidence in support of a role of leukotrienes in this response has been obtained in the present work using a specific 5-lipoxygenase inhibitor as well with studies with a leukotriene D_4 receptor antagonist [9]. In addition inhibition of the immediate response by a thromboxane A2/contractile prostaglandin antagonist (L-640,035) in squirrel monkeys has suggested the existence of interactions between cyclooxygenase and lipoxygenase products in antigen-induced bronchoconstriction in the squirrel monkey [1]. The exact implications of the inhibition of the primary response by L-640,035 is not entirely clear as the response is not inhibited by a cyclooxygenase inhibitor, indomethacin (McFarlane, unpublished results).

Late reactions have been considered to be clinically important in human asthma because of their severity and their responsiveness to corticosteroids [10, 11]. The late reaction in the squirrel monkey has been considered to be similar to man

because of the time course, the site of the response (primarily the small airways) and the lack of response to β agonists [2]. The pathogenesis of the late response is unclear but recent evidence has suggested a role for mast cell-derived mediators in man because of the presence of leukotriene B_4, neutrophil chemotactic activity and thromboxane B_2 in plasma during such reactions [12–14]. In addition leukotrienes could arise from emigrating inflammatory cells, such as eosinophils, which appear in response to chemotactic factors released during the immediate response. It is also of interest that administration of an aerosol of leukotriene D_4 to allergic sheep [15] and to two squirrel monkeys [6] known to develop late phase responses to *Ascaris* resulted in the development of late responses to the aerosol of the leukotriene.

The present studies with the selective 5-lipoxygenase inhibitor, L-651,392, are supportive for a role for leukotrienes in both primary and late phase responses induced by antigen in the squirrel monkey. Other evidence for a role for leukotrienes in allergen-induced bronchoconstriction has been obtained in allergic sheep where a leukotriene D_4 receptor antagonist has been shown to significantly reduce the early response and block the late response to inhaled *Ascaris* [16]. These results indicate that in man attenuation of primary responses and inhibition of late responses to allergen should be observed following pretreatment with either 5-lipoxygenase inhibitors or leukotriene D_4 receptor antagonists.

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