Effect of Intravenously Administered Lipoxxygenase Metabolites on Rat Tracheal Mucous Gel Layer Thickness

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Abstract

The effect of intravenous injections of 5-, 12- and 15-hydroxyeicosatetraenoic acids (HETE), leukotrienes D₄ and E₄ (LTD₄, LTE₄) on tracheal mucous gel layer (TMGL) thickness was assessed in rats. When administered in doses ranging from 0.03 pg to 33 ng per rat, the lipoxxygenase metabolites produced significant increases in TMGL thickness. The order of potency of the metabolites was 15-HETE > 12-HETE ≥ 5-HETE > LTD₄ ≥ LTE₄. Imidazole (31.6 mg/kg), intravenously, significantly decreased this response. These findings suggest that the **mono-HETEs**, especially 15-HETE, may be important modulators of airway mucus in the rat.

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Antigen challenge of actively sensitized rats produced an increase in the thickness of the tracheal mucous gel layer (TMGL) which was prevented by treating the rats with a leukotriene (LT) antagonist before antigen challenge [1]. Further work demonstrated that slow-reacting substance injected intravenously stimulated significant increases in rat TMGL thickness which were inhibited by treatment with imidazole [2]. Studies in which mucous glycoprotein secretion from organ cultures of human airways was measured following incubation with purified lipoxxygenase (LO) metabolites demonstrated that monohydroxyeico-satetraenoic acids (mono-HETEs) and sulfidopeptide LTs were potent mucus secretagogues [3,4]. In view of this, studies were conducted to determine the effects of intravenously administered LO metabolites on rat TMGL thickness.

Fasted male Sprague-Dawley rats (150–250 g) were randomly divided into groups of 4 animals each. Each rat received 1.0 ml of an appropriate concentration of one of the LO metabolites prepared in sterile 0.9% saline via the lateral tail vein. Forty-five minutes after injection the thickness of the TMGL was measured as described by Yanni et al. [1]. Briefly, the trachea was removed immediately after the rat was killed. Each trachea was opened along the midline of the dorsal surface and mounted on the convex end of a test tube.

The tracheal tissue was placed in front of a Zeiss slitlamp and pachymeter (Model 30, SLM; Zeiss, New York, N.Y., USA). The slit was focused on the tracheal mucosa to identify the air-mucus interface and the mucus-epithelium interface. The image was then split and repositioned with the air-mucus interface of one image imposed on the mucus-epithelium interface of the second image. The gel thickness was then read directly from the pachymeter scale. Inhibition of the response by imidazole was assessed by administering imidazole intravenously 5 min before

LO metabolite injection. The mean of the imidazole-treated group was compared to that of the appropriate LO-metabolite-injected group by using Student's t test [5]. The mean of each treated group was compared with that of the saline-challenged control group by using Dunnett's t test [6]. Linear regression was used to determine relative potency [7].

5(S)-, 12(S)- and 15(S)-HETEs and LTD₄ and LTE₄ were obtained from Cayman Chemical (Ann Arbor, Mich., USA) and diluted into sterile saline immediately before intravenous administration. Imidazole was purchased from Sigma Chemical (St. Louis, Mo., USA) and prepared as a solution in sterile saline immediately before intravenous administration (vol. 1.0 ml/kg). Ricinoleic acid was the gift of Dr. Gustav Graff, A.H. Robins Company.

All of the LO metabolites administered intrave-

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with imidazole

Table 1. Effect of LO metabolites administered intravenously on rat TMGL thickness (n = 4/group)

^a p < 0.05, Dunnett's t test.

^b Approximate potencies due to lack of parallelism between regression lines.

Table 2. Effect of intravenous imidazole (31.6 mg/kg) on intravenously administered LO-metabolite-induced increases in TMGL thickness (n = 4/group)

Metabolite Dose pg/rat

Increase in TMGL thickness (X ± SD)

176 ± 13^a 203 ± 11^a

153 ± 5^a 157 ± 8^a 200 ± 5^a

without imidazole

p < 0.05, Student's t test.

nously significantly increased TMGL thickness. The monohydroxy acids were more potent than either LTD₄ or LTE₄ following intravenous administration. Ricinoleic acid (50 ng/rat) failed to produce a significant increase in TMGL thickness (table 1). Imidazole 31.6 mg/kg, intravenously, significantly inhibited the responses induced by the LO metabolites (table 2).

The data indicate that intravenous 5-, 12- and 15-HETE, LTD₄ and LTE₄ significantly increase TMGL thickness in a dose-dependent manner (table 1). The failure of ricinoleic acid to stimulate an increase in TMGL thickness indicates that the effect following LO metabolite injection is not a nonspecific effect due to intravenous lipid administration.

Imidazole pretreatment significantly inhibits the LO metabolite's effects on gel layer thickness (table 2), thereby implicating thromboxane in the response. Thromboxane's effects in this model have been demonstrated using a thromboxane A₂ (TXA₂) agonist, carbocyclic thromboxane, and a TXA₂ mimetic, U46619[8].

The potency of 15-HETE following intravenous administration was unexpected and noteworthy. Human airway cultures spontaneously produce 5-, 12- and 15-HETE. An anaphylactic response in these cultures stimulated production of micromolar concentrations of 5- and 15-HETE [3].

Likewise, purified (99%) human tracheal epithelial cells exposed to arachidonic acid have been shown to produce 15-LO products including 15-HETE, but little or no detectable 5-HETE [9]. A study in which bronchoalveolar lavage (BAL) was performed on 5 patients with stable asthma

demonstrated that control lavage fluid contained 414 ± 225 pg/ml of 15-HETE, which rose to $1,404 \pm 592$ pg/ml after antigen challenge [10]. These concentrations of 15-HETE are within the range of concentrations that stimulated increases in TMGL thickness in the present study. Murray et al. [10] further demonstrated that no detectable 5-HETE, LTC₄, LTD₄ or LTE₄ were present in either control or postchallenge BAL fluid. These data along with the potency of 15-HETE presented in this work suggest that LO metabolites, particularly 15-HETE, are involved in mucus secretion.

Acknowledgments

We thank Mrs. Loretta Davis and Ms. Donna Chenault for their help in the preparation of this manuscript.

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