Evaluation of LY163443, 1-\{2-hydroxy-3-propyl-4-\{4-(1H-tetrazol-5-ylmethyl)phenoxy\}methyl\}phenyl\ethanone, as a pharmacologic antagonist of leukotrienes D\textsubscript{4} and E\textsubscript{4}

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Summary. LY163443, 1-\{2-hydroxy-3-propyl-4-\{4-(1H-tetrazol-5-ylmethyl)phenoxy\}methyl\}phenyl\ethanone, antagonized LTD\textsubscript{4}-induced contractions of guinea pig ileum, trachea, and lung parenchyma. Tracheal contractions to LTE\textsubscript{4} were also inhibited by LY163443. The compound had minimal effect against ileal responses to LTC\textsubscript{4} and parenchymal contractions to LT\textsubscript{B}\textsubscript{4}. Furthermore, LY163443 had little effect against contractions of isolated smooth muscles to histamine, bradykinin, PGF\textsubscript{2\alpha}, carbachol, serotonin or U46619. LY163443, given by oral administration to guinea pigs, blocked LTD\textsubscript{4}-induced increases in total pulmonary impedance (TPI). Similar responses elicited by histamine or U46619 were unaffected. Increases in TPI in response to i.v. administration of LTC\textsubscript{4} were antagonized by LY163443 given by the same route. Ovalbumin challenge also increased TPI in guinea pigs previously sensitized against this antigen. In such animals, pretreated with pyrilamine, propranolol, and indomethacin, oral administration of LY163443 blocked the increase in TPI caused by ovalbumin. Additionally, LTD\textsubscript{4} given intradermally to guinea pigs caused a vascular leakage which was suppressed by prior oral administration of LY163443. Finally, LY163443 relaxed isolated guinea pig trachea previously contracted with LTD\textsubscript{4}, histamine, or carbachol. Relaxation of tissues contracted by these latter two agonists suggested some inherent airway smooth muscle relaxant properties of the molecule. This was further demonstrated by showing some bronchodilator activity in an in vivo setting. Thus, this pharmacologic profile indicates that LY163443, or a member of the same chemical family, warrants consideration as a possible therapeutic agent in the treatment of asthma and in diseases characterized by an overproduction of LTD\textsubscript{4} and LTE\textsubscript{4}. Key words: LY163443 — LTD\textsubscript{4}/LTE\textsubscript{4} antagonist — Leukotriene receptors — Antigen-induced bronchospasm — Isolated smooth muscles

Introduction

Current dogma portrays the sulfidopeptide leukotrienes, a family of C\textsubscript{20} fatty acids derived from arachidonic acid, as responsible for the pathologic sequelae of asthma and perhaps numerous other diseases (Goetzl et al. 1984). Since their discovery as slow reacting substance of anaphylaxis (Samuelsson 1983), many investigators have sought to develop drugs capable of antagonizing their actions. To this end, a diverse group of compounds have been reported to inhibit the actions of LTD\textsubscript{4} and LTE\textsubscript{4} (Krell et al. 1984). In contrast, LTC\textsubscript{4} antagonists have been more difficult to develop.

Draven et al. (1980) and Krell et al. (1981) noted variations between leukotriene receptors using FPL 55712, the first recognized leukotriene antagonist (Augstein et al. 1973). This agent blocked in vitro smooth muscle contractions elicited by LTD\textsubscript{4} but had minimal effects on responses to LT\textsubscript{C}\textsubscript{4}. These experiments raised the hypothesis of separate pharmacologic receptors for LT\textsubscript{C}\textsubscript{4} and LT\textsubscript{D}\textsubscript{4}. Lee et al. (1984) have suggested that differences between LT\textsubscript{D}\textsubscript{4} and LTE\textsubscript{4} receptors are possible. In addition to being specific for the various leukotrienes, evidence has been presented to show that leukotriene receptors are heterogeneous between tissues (Fleisch et al. 1982).

Recently, we described the chemistry and pharmacology of LY171883, a tetrazole substituted acetophenone, which is an orally effective antagonist of LTD\textsubscript{4} and LTE\textsubscript{4} (Fleisch et al. 1985). As with FPL 55712, it does not antagonize in vitro responses to LT\textsubscript{C}\textsubscript{4}. The acetophenone moiety of these molecules seems to direct the antagonist toward the LTD\textsubscript{4} and LTE\textsubscript{4} receptor sites and away from the tissue macromolecule that responds to LT\textsubscript{C}\textsubscript{4}.

The present communication deals with the pharmacologic analysis of LY163443, an aryloxyacetophenone (Fig. 1), that has a similar pharmacologic profile as, and is more potent than, LY171883. These compounds and their congeners represent a new generation of drugs that might prove useful in treating diseases associated with excessive production of leukotrienes.

Methods and materials

Male Hartley guinea pigs (Murphy Breeding Laboratories, Plainfield, IN, USA) weighing 200—400 g were used in these studies.

For in vitro studies, LY163443 was initially dissolved in 0.5 M sodium bicarbonate to a concentration of 10^{-3} M. Further dilutions were made in Krebs' bicarbonate solution. A 15 mg/ml solution in sodium bicarbonate (0.5 M) was made for the in vivo experiments. Subsequent dilutions were carried out in sodium bicarbonate.

Guinea pig ileum, trachea, and lung parenchyma. Guinea pigs were killed by decapitation. A section of terminal ileum was
removed, the lumen cleaned, and the tissue cut into smaller segments of approximately 2 - 3 cm. Each ileum was tied to the bottom of a tissue holder leaving the lumen open. The ilea were then transferred to tissue baths, attached to transducers by means of thread, and equilibrated for approximately 1 h under a maintained passive force of 0.5 g.

Tracheas were excised, cleaned, and cut into ring segments. The tissues were then placed on supports constructed from two 1-inch, 30-gauge disposable stainless steel hypodermic needles (Hooker et al. 1977), and transferred to organ baths. Approximately 1 h was allowed for equilibration under a maintained passive force of 2 g.

Strips of parenchyma were removed from the outer edge of the lung and the ends secured by cotton thread. The tissues were then placed in organ baths under a passive force of 0.5 g. The procedure essentially followed the descriptions by Lulich et al. (1976) and Drazen and Schneider (1978).

All isolated tissues were suspended in 10 ml organ baths containing Krebs' bicarbonate solution of the following composition in millimoles/liter: KCl, 4.6; KH₂PO₄, 1.2; MgSO₄, 7H₂O, 1.2; NaCl, 118.2; NaHCO₃, 24.8; and dextrose, 10.0. The concentration of Ca²⁺, in the form of CaCl₂·2H₂O, varied with the tissue used: ileum, 1.2 mM; lung parenchyma, 1.8 mM; and trachea, 2.5 mM. In those experiments with trachea, indomethacin, 3 x 10⁻⁶ M, was incorporated into the buffer to prevent the production of prostaglandins which might moderate the contractile response. Temperature was maintained at 37°C and the bathing solutions aerated with 95% O₂ and 5% CO₂. Isometric measurements were made with a Grass FT03C force-displacement transducer and recorded on a Grass Model 79D polygraph as changes in grams of force.

**Cutaneous vascular permeability.** Cutaneous vascular permeability was assessed using the method of Katayama et al. (1978) as modified by Rinkema et al. (1984). The backs of guinea pigs were shaved with an electric clipper 16 - 24 h prior to the experiment. On the day of the experiment, animals were anesthetized with 45 to 50 mg/kg pentobarbital sodium given i.p. The left jugular vein was cannulated with a polyethylene catheter (PE 50) for administration of drugs by the i.v. route. Blood pressure was measured with a Statham Gould Inc. (Oxnard, CA, USA) pressure transducer (P23ID) connected to a polyethylene catheter placed in the right carotid artery. A third cannula was inserted into the trachea and the animal ventilated with room air by means of a Harvard (Harvard Apparatus, So. Natick, MA, USA) rodent respirator set to deliver a tidal volume of 1 ml/100 g of body weight at a speed of 50 breaths/min. Succinylcholine, 5 mg/kg, was given i.v. to suppress spontaneous respiration. This dose of succinylcholine lasted for the duration of the experiment. The total pulmonary resistance to insufflation, or total pulmonary impedance (TPI), was measured with a Statham pressure transducer (P23ID) connected to a T-tube on the tracheal cannula. This procedure is a modification of the Konzett-Rossler (1940) technique. Output signals from the pressure transducers were recorded on a Grass (Quincy, MA, USA) polygraph (Model 79D). Body temperature was maintained within normal limits by means of a Deltaphase Isothermal Pad (Braintree Scientific Inc., Braintree, MA, USA).

Dose-response curves to histamine, LTD₄, LTC₄, or U46619 were determined by giving randomized doses of these agonists i.v. Five minutes were allowed between administration of the agonists for recovery. During this period, the lungs were hyperinflated to aid in return to baseline.

A separate group of guinea pigs were passively sensitized against ovalbumin by i.p. administration of 0.2 ml antisem 2 days preceding the experiment. Animals sensitized in such a manner undergo a generalized anaphylaxis after i.v. administration of antigen. We used this model to determine ifLY163443 could reduce responses to leukotrienes released by an immunological reaction. The hyperimmune serum had been previously prepared by actively sensitizing male Hartley guinea pigs with 2 mg of ovalbumin in 50% complete Freund’s adjuvant given i.p. on days 1 and 5. On day 21, the animals were bled and the serum collected and stored at -20°C.

In most experiments, LY163443 was orally administered to guinea pigs, with the aid of a feeding tube connected to a syringe, 2 h prior to agonist or antigen challenge. Deviations from this protocol are noted in the Results section and on appropriate figures. Responses were quantitated as percent maximum possible increase in TPI. The maximal response was determined by clamping off the trachea distal to the cannula and recording pressure generated in the system by the respirator. This maneuver was carried out at the end of the experiment.