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EFFECTS OF CYCLOOXYGENASE AND LIPOXYGENASE INHIBITORS ON INFLAMMATION ASSOCIATED WITH OXAZOLONE-INDUCED DELAYED HYPERSENSITIVITY*

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Abstract—Oxazolone-induced delayed hypersensitivity in mice produced swelling with concomitant increased tissue levels of leukotrienes and prostaglandins. Pharmacological agents were coapplied topically with oxazolone at the time of challenge in an attempt to modulate the immune-based inflammation. Dexamethasone inhibited both swelling and increases in eicosanoid levels. Indomethacin reduced prostaglandin levels but failed to inhibit swelling or reduce leukotriene levels. L-651,896 (2,3-dihydro-6-[3-(2-hydroxymethyl)phenyl-2-propenyl]-5-benzofuranol), a 5-lipoxygenase inhibitor, reduced leukotriene levels but did not reduce swelling or prostaglandin levels. A combination of indomethacin and L-651,896 reduced eicosanoid levels but did not reduce swelling. These data suggested that the reduction in tissue levels of 5-lipoxygenase or cyclooxygenase oxygenation products of arachidonic acid either singularly or together did not result in the concomitant reduction of the inflammation associated with oxazolone-induced delayed hypersensitivity.

The arachidonic acid (AA) metabolites, prostaglandins (PG) and leukotrienes (LT), have been implicated in a wide variety of inflammatory conditions. Delayed hypersensitivity (DH) is an immune-based inflammation, characterized by immune cell infiltration and associated fibrin deposition, in which causative roles for LTs and/or PGs have been postulated and subsequently investigated. Elevated levels of AA, PGE_2 and LTB_4-like material have been detected in the skin in human contact dermatitis induced by both nickel and chromate allergens [1]. Increased levels of LTB_4 and LTC_4 have also been found in peritoneal exudate fluids from mice sensitized with dinitrofluorobenzene and challenged intraperitoneally with spleen cells conjugated with dinitrophenol.‡ We have reported increased levels of LTs in other models of inflammation [2, 3]. A recent review article details the occurrence and putative roles of various LTs in both animal models of inflammation and human inflammatory disease [4]. Thus, these findings of increased LT levels have suggested that these eicosanoids may play a role in inflammation. A possible causative role for PG in DH has also been studied. Using a variety of DH models, different investigators have obtained conflicting results using the cyclooxygenase inhibitor, indomethacin. Lipsmeyer [5] and Schrier [6] showed augmentation of responses, whereas Ball and Ney [7] and Tarayre *et al.* [8] found inhibition of responses.

We recently reported the pharmacological profile of a new 5-lipoxygenase inhibitor, L-651,896 (2,3-dihydro-6-[3-(2-hydroxymethyl)phenyl-2-propenyl]-5-benzofuranol) [9]. In the present study, we examined the effects of L-651,896, as well as indomethacin and dexamethasone administered either alone or in combination for their ability to affect inflammation and modulate tissue concentration of PGs and LTs associated with oxazolone-induced DH. These studies suggested that neither PGs nor LTs mediated the inflammatory response.

METHODS

Chemicals. Oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one) was purchased from the Aldrich Chemical Co., Milwaukee, WI, U.S.A. PGE_2 was purchased from the Ono Pharmaceutical Co. Ltd., Osaka, Japan; PGE_2 antisera was from Miles Research Products, Elkhart IN, U.S.A. [^{3}H]PGE_2 and [^{3}H]LTC_4 were from New England Nuclear, Boston MA, U.S.A. [^{3}H]LTB_4 was from Amersham, UK. LTB_4 and LTC_4 were obtained from Merck Frosst, Montreal, Canada. Dexamethasone, indomethacin, L-651,896, LTB_4 antisera and LTC_4 antisera were prepared at the Merck Sharp & Dohme Research Laboratories, Rahway, NJ, U.S.A. [10, 11].

Animals. Female CF-1 outbred mice (4–6 weeks of age) were purchased from the Charles River Breeding Laboratories, Kingston, NY, and maintained on a standard pellet diet and water *ad lib.* The

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‡ B. D. Jaffee, E. A. Jones, J. J. Keenan, S. R. Lundy and N. R. Ackerman, Third International Conference of the Inflammation Research Association, White Haven, PA (1986).

mice were housed for at least 4 weeks prior to use. Ten mice were used per group.

Sensitization and challenge. Mice were sensitized topically on the shaved abdomen with 100 μl of a 4% solution of oxazolone in acetone. Control mice received only acetone. Seven days after sensitization, the mice were challenged on one ear with 20 μl of a 1% solution of oxazolone in acetone/corn oil (3:1). The test compounds were solubilized in the challenge solution and co-applied with the oxazolone. Twenty-four hours after challenge, a 6 mm disc was excised from the ear and placed in a preweighed vial containing 3 ml methanol. The wet weights of the ears were determined as an index of inflammation.

Extractions and analysis. The excised tissues were homogenized (Kinematic Polytron) in 3 ml methanol after the addition of 1 ml sodium acetate buffer (0.1 M, pH 4.2). The homogenizer probe was washed with 4 ml of water which was then combined with the original homogenate. The combined fluid was centrifuged at 1000 *g* for 10 min. The supernatant fluid was adjusted to 15% methanol, loaded onto a prewashed C18 Sep-Pak (Waters, Milford MA) which was then washed with 10 ml of 15% methanol and 20 ml of water. The eicosanoids were eluted from the Sep-Pak with 5 ml of methanol. Radioimmunoassay (RIA) for PGE_2 was performed on this eluent as previously described [12]. Measurements of LTB_4 and LTC_4 by RIA were performed after further fractionation by reversed phase high performance liquid chromatography (HPLC) (5 μm Supelcosil C18 column; 65% methanol, 35% H_2O, 0.05% glacial acetic acid, 0.5 mM oxalic aicd, pH 5.6, 1 ml/min, 34°).

The efficiency of extraction was determined by addition of tritiated standards to the tissue and measuring the amount of radioactivity recovered. The recovery of radiolabeled LTB_4, LTC_4 and PGE_2 from processed tissue was 56, 31 and 90% respectively (two experiments). The data were adjusted accordingly.

RESULTS

After the challenge solution of oxazolone was applied, inflammation, LTB_4, LTC_4 and PGE_2 were measured at various times between 0 and 72 hr. A typical time course is presented in Fig. 1. The inflammation and levels of LTB_4 and LTC_4 were maximal by 24 hr and virtually back to control values by 72 hr. The PGE_2 levels were maximal between 24 and 48 hr and also returned to control levels by 72 hr. The 24-hr time point was thus chosen to measure the effects of pharmacological agents on these responses.

The identities of LTB_4 and LTC_4 were confirmed by comparison with authentic standards on HPLC and by specific immunoreactivity of HPLC fractions (Figs. 2 and 3). L-651,896, 400 μg, failed to inhibit inflammation and PGE_2 formation but did lower oxazolone-induced increased tissue levels of LTB_4 (Fig. 2) and LTC_4 (Fig. 3) by 87 and 78% respectively (Table 1). In addition, L-651,896 lowered the oxazolone-induced increased amounts of LTD_4 and LTE_4 as well as the sulfone or sulfoxide metabolites of LTC_4 (Fig. 3). L-651,896 caused a dose-dependent (100, 200, 400 μg/ear) reduction in LTB_4 formation (50, 61, 89%) and LTC_4 formation (53, 87, 90%).

The C18 Sep-Pak purified extracts contained components that cross-reacted with both the LTB_4 and LTC_4 antisera (Figs. 2 and 3). These components were more lipophilic than eicosanoids as they were retained by the C18 reverse phase column eluted

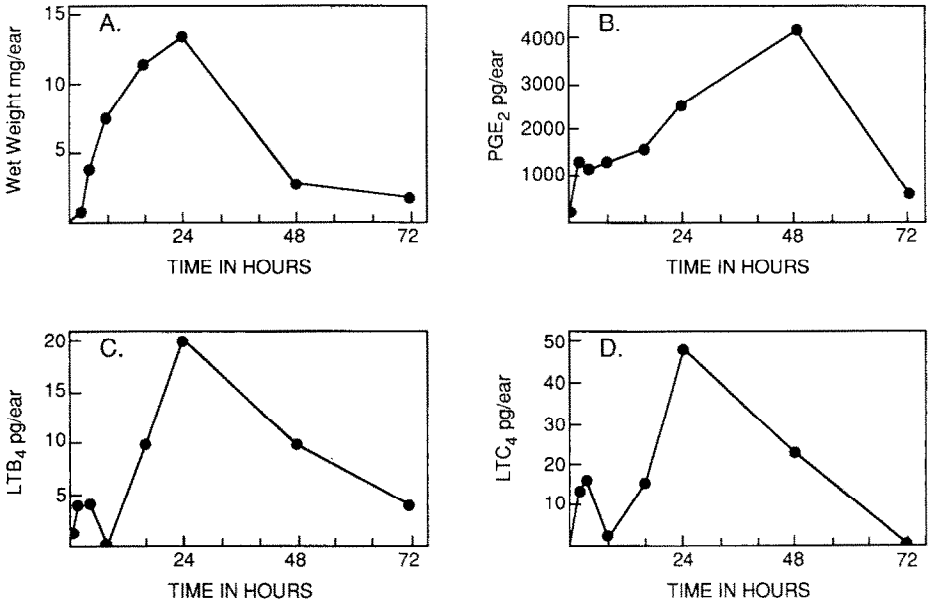

Fig. 1. Time courses of oxazoline-induced inflammation and eicosanoid levels in ears of mice sensitized to oxazolone. Oxazolone was topically applied to ears of sensitized mice as described in Methods. At various times ten mice were killed and the changes in wet weight/ear, PGE_2, LTB_4 and LTC_4 in the tissue were determined as described in Methods. (A) wet weight/ear; (B) PGE_2; (C) LTB_4; and (D) LTC_4.

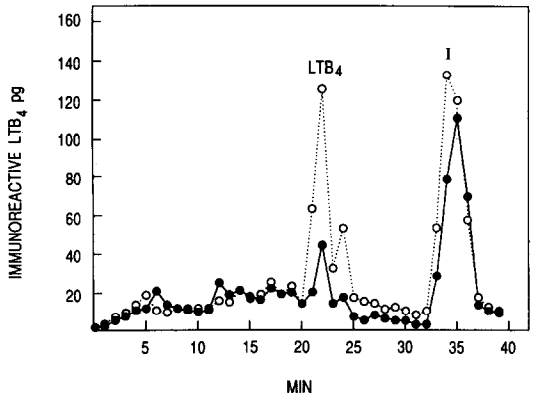

Fig. 2. LTB_4—immunoreactivity resolved by HPLC. Mouse ear extracts from twenty combined ear biopsies were chromatographed by HPLC as described in Methods. The fractions were collected and assayed by RIA with LTB_4 antisera. After 25 min the column was washed with 100% methanol, and lipophilic components (Peak I), cross-reactive with the LTB_4 antisera, were eluted. The data are expressed as pg equivalents of LTB_4. Key: (○· · ·○) extract of oxazolone-treated ears; and (●——●) extract of oxazolone/L-651,896-treated ears.

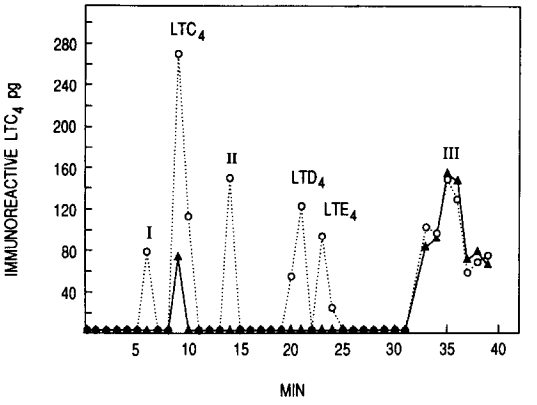

Fig. 3. Peptidoleukotriene immunoreactivity resolved by HPLC. Mouse ear extracts from twenty combined ear biopsies were chromatographed by HPLC as described in Methods. The fractions were collected and assayed by RIA with LTC_4 antisera. After 25 min the column was washed with 100% methanol. Peak I: LTC_4 sulfone, or LTC_4 sulfoxide; Peak II: not identified; and Peak III: a mixture of lipophilic components cross-reactive with the LTC_4 antisera. The data are expressed as pg equivalents of LTC_4. Key: (○· · ·○) extract of oxazolone-treated ears; and (▲—▲) extract of oxazolone/L-651,896-treated ears.

with the aqueous-methanol solvent but were eluted with 100% methanol. The identify of these materials was not investigated, and the tissue content of these materials was not reduced by L-651,896. Thus, all tissue extracts were purified by HPLC and the amounts of LTB_4 and LTC_4 in the appropriate fractions quantified by RIA.

Dexamethasone, when topically applied at a concentration of 10 μg/ear, inhibited oxazolone-induced inflammation as assessed by wet weight (Table 1). In addition to preventing the increase in inflammation, dexamethasone lowered the oxazolone-induced elevated amounts of PGE_2, LTB_4 and LTC_4 (Table 1). Indomethacin, 50 μg/ear, inhibited PGE_2 formation by 90%; however, inflammation and LT levels were not reduced. As neither the inhibition of prostaglandins nor leukotriene synthesis affected the inflammation, indomethacin and L-651,896 were then applied together. The levels of PGE_2, LTC_4 and LTB_4 were lowered significantly; however, the oxazolone-induced inflammation was not affected (Table 1).

DISCUSSION

Increased levels of PG and LT have been found associated with inflamed tissues and exudates [2–4]. In addition, the exogenous administration of these eicosanoids has been shown to mimic some cardinal symptoms of acute inflammatory responses [13]. However, these two facts are not sufficient evidence to conclude that either PGs or LTs are causal mediators of DH. However, a positive correlation between the endogenous tissue concentration of a specific LT and/or PG and the extent of inflammation would provide a more convincing role that a specific eicosanoid plays a role as an inflammatory mediator in DH.

Steroids have been the mainstay in the therapy of severe allergic diseases; however, due to undesirable

Table 1. Effects of agents on eicosanoid levels and inflammation in oxazolone-induced delayed hypersensitivity

Treatment	Dose (μg/ear)	% Inhibition			
		Inflammation	PGE_2	LTB_4	LTC_4
Dexamethasone	10	89 ± 9*	86 ± 8*	88 ± 6*	96 ± 3*
Indomethacin	50	21 ± 10	90 ± 13*	30 ± 6	20 ± 20
L-651,896	400	15 ± 7	5 ± 8	87 ± 12*	78 ± 10*
Indomethacin+ L-651,896	50 400	14 ± 2	98 ± 2*	72 ± 9*	69 ± 16*

The test agents were coapplied with the oxazolone challenge solution as described in Mehods. After 24 hr the amount of inflammation and the concentrations of eicosanoids in the tissues were determined. Ten mice were used in each experimental group. The data are the mean ± SD of three separate experiments.

* $P < 0.05$.

side effects, other chemotherapeutic agents are needed. Steroids have been shown to inhibit the cellular formation of both PGs and LTs [14, 15]. This inhibition is thought to be mediated by the steroid-induction of lipomodulin, which inhibits phospholipase A_2 hydrolysis of cellular phospholipids to yield substrate arachidonic acid [16]. In these studies, dexamethasone coapplied topically with oxazolone lowered the oxazolone-induced increased tissue levels of both leukotrienes and prostaglandins. Concomitant with the reduction of eicosanoids levels was also the reduction of inflammation as assessed by wet weight.

We have reported recently that L-651,896 is a potent and relatively specific inhibitor of 5-lipoxygenase [9]. In the present studies of oxazolone-induced hypersensitivity, L-651,896 lowered elevated levels of both LTC_4 and LTB_4. However, this reduction of LT content of inflamed tissue did not result in a diminution of the inflammation. In a similar manner, the coadministration of indomethacin with oxazolone selectively lowered the increased tissue levels of PGE_2. However, the inflammatory responses as evaluated by increases in weight were not changed significantly. Finally, the topical coapplication of both indomethacin and L-651,896 together significantly lowered the tissue content of both LT and PG. However, in contrast to the dexamethasone-mediated lowering of PG, LT and inflammation, the combination of indomethacin and L-651,896 did not modulate the oxazolone-induced inflammatory response. These data suggested that reduction of neither 5-lipoxygenase nor cyclooxygenase products either alone or together caused a concomitant reduction of inflammation associated with oxazolone-induced delayed hypersensitivity.

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