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BIOCHEMICAL AND BIOLOGICAL ACTIVITIES OF 2,3-DIHYDRO-6-[3-(2-HYDROXYMETHYL)PHENYL-2-PROPENYL]-5-BENZOFURANOL (L-651,896), A NOVEL TOPICAL ANTI-INFLAMMATORY AGENT

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Abstract—The biochemical and biological profile of a topical anti-inflammatory agent, 2,3-dihydro-6-[3-(2-hydroxymethyl)phenyl-2-propenyl]-5-benzofuranol (L-651,896), is described. L-651,896 inhibited the 5-lipoxygenase of rat basophilic leukemia cells with an IC_{50} of 0.1 μM and leukotriene synthesis by human PMN and mouse macrophages with IC_{50} values of 0.4 and 0.1 μM respectively. L-651,896 also inhibited prostaglandin E_2 synthesis by mouse peritoneal macrophages (IC_{50} = 1.1 μM). This compound inhibited ram seminal vesicle cyclooxygenase activity at considerably higher concentrations, and this effect was directly related to substrate concentration. When applied topically to the mouse ear, L-651,896 lowered elevated levels of leukotrienes associated with arachidonic acid-induced skin inflammation and delayed hypersensitivity induced by oxazolone. However, while L-651,896 inhibited the increased vascular permeability induced by arachidonic acid, it had no effect on the edema associated with the immune-based response to oxazolone in the same tissue. Thus, it is possible that leukotrienes may play a role in some but not all inflammatory responses.

In addition to the well established proinflammatory activity of prostaglandins (PG)** [1], leukotrienes (LT) have also been shown to be potent effectors of the classical signs of inflammation, modulating vascular permeability in a variety of vascular beds [2–6]. Leukotriene B_4, a potent chemotaxin for phagocytic cells [7], has been found in elevated concentrations in the involved skin of psoriatic patients [8], the lesions of which are characterized by focal accumulations of polymorphonuclear leukocytes (PMN). Since cells participating in inflammatory responses, particularly PMN, macrophages and certain types of mast cells, are rich sources of various leukotrienes, it is reasonable to postulate that inhibition of both cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism could provide a more effective way of combating the pain and inflammation associated with a variety of acute and chronic inflammatory diseases. Initial pharmacological and clinical studies with agents claimed to have 5-lipoxygenase or weak dual lipoxygenase/cyclooxygenase inhibitory activity such as BW755C [9], sulfasalazine [10] and benoxaprofen [11] have not provided unequivocal biochemical and functional evidence to address this issue.

We now report on the anti-inflammatory activity of 2,3-dihydro-6-[3-(2-hydroxymethyl)phenyl-2-propenyl]-5-benzofuranol (L-651,896, Fig. 1), a novel inhibitor of leukotriene and prostaglandin biosynthesis in a number of *in vitro* and *in vivo* systems.

MATERIALS AND METHODS

Reagents

L-651,896 (Fig. 1) was prepared by C-alkylation of 5-hydroxydihydrobenzofuran with *o*-bromocinnamyl bromide (97% NaH in benzene, 75°, 6 hr, 66% yield). The resulting 6-(*o*-bromocinnamyl)-5-hydroxydihydrobenzofuran (m.p. 110–112°) was converted to the *o*-cyano derivative (2 Eq. CuCN in *N*-methylpyrrolidinone, 175°, 4.5 hr, 68% yield, m.p. 137–139°) which was then reduced sequentially with diisobutyl-aluminum hydride (2.3 Eq. in benzene, 5° for 1 hr and then room temperature for 1 hr) and sodium borohydride (absolute ethanol, room temperature). The final product recrystallized from isopropyl alcohol had a melting point of 118–119°.

Tissue culture reagents including M-199 medium, Eagle's minimum essential medium (MEM), fetal

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** Abbreviations: PG, prostaglandin; LT, leukotriene; HIPS, heat-inactivated porcine serum; AA, arachidonic acid; PMA, phorbol myristate acetate; RIA, radioimmunoassay; MEM, Minimal Essential Medium; PMN, polymorphonuclear leukocytes; 5-HETE, 5-hydroxyeicosatetraenoic acid; and HEPES, 4(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

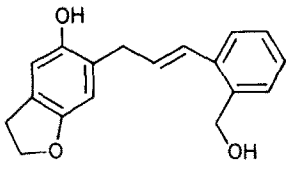

Fig. 1. Structure of L-651,896 (2,3-dihydro-6-[3-(2-hydroxymethyl)phenyl-2-propenyl]-5-benzofluranol.

calf serum and porcine serum were purchased from the Grand Island Biological Co., Grand Island, NY. The porcine serum was inactivated by heating at 56° for 30 min (HIPS). A23187 was purchased from Calbiochem, San Diego, CA. Zymosan was purchased from ICN Nutritional Biochemicals, Cleveland, OH, and phorbol myristate acetate from the Sigma Chemical Co., St. Louis, MO. Arachidonic acid was from NuChek Prep., Inc., Elysian, MN. [5,6,8,9,11,12,14,15-^{3}H(N)]Arachidonic acid (80 Ci/mmol), [1-^{14}C]arachidonic acid (52 mCi/mmol) and ^{125}I-labeled albumin (bovine serum) (BSA), 1–5 mCi/mg, were obtained from the New England Nuclear Corp., Boston, MA. Leukotrienes B_4 and C_4 were provided by J. Rokach, Merck-Frosst, Montreal, Canada. PGE_2 was purchased from the Ono Pharmaceutical Co., Osaka, Japan. Leukotriene antisera were from our laboratories [12, 13], and PGE_2 antisera were purchased from Miles Research Products, Elkhart, IN. Whatman TLC plates (LKDF) were obtained from Pierce, Rockford, IL, and 4-ethoxy-methylene-phenyloxazol-5-one(oxazolone) was from the Sigma Chemical Co., St. Louis, MO.

Animals

Female CFW-1 mice were purchased from Charles River Laboratories, Wilmington, MA. Male Sprague–Dawley rats were from Taconic Farms, Germantown, NY. The animals were maintained on standard pellet diet and water *ad lib.*

Mouse peritoneal macrophages

Resident mouse peritoneal macrophages were collected by peritoneal lavage of female CFW-1 mice and placed into cell culture as previously described [14]. The cells were incubated overnight in tissue culture medium M-199 containing 1% HIPS. After washing the cells with this medium, L-651,896 was added by diluting concentrated DMSO solutions 1 to 1000 into the medium and the cells were incubated for 30 min in 1 ml M-199/HIPS. Zymosan, 50 μg/ml, was then added, and the incubations were continued for an additional 2 hr. The amounts of immunoreactive LTC_4 and PGE_2 in the culture medium were determined by RIA.

In other studies, macrophages were incubated overnight in M-199/HIPS with [^{3}H]arachidonic acid ([^{3}H]AA) to radiolabel cellular phospholipids [14]. The radiolabeled cells were washed with tissue culture medium and subsequently preincubated with test compound and then stimulated with zymosan or PMA. Aliquots of the culture medium (40 μl) were directly spotted on Whatman LK5DF silica gel TLC plates. The chromatograms were developed with ethyl acetate–methanol–acetic acid (95:5:1). The PGE_2 zone was removed from the plate, and the radioactivity was determined by scintillation counting.

Rat peritoneal polymorphonuclear leukocytes (PMN)

Elicited PMN were prepared from peritoneal exudates as follows: 8 ml of 12% sodium caseinate was injected intraperitoneally into male rats. After 18–20 hr the rats were killed with CO_2 and the peritoneal cavities were lavaged with Eagle's MEM (pH 7.7) without $NaHCO_3$ but containing Earle's salts, L-glutamine, and 30 mM HEPES. The PMN were isolated by centrifugation, washed with MEM, filtered through lens paper to remove clumps, and adjusted to a concentration of 1×10^7 cells/ml. Aliquots (0.5 μl) of concentrated dimethyl sulfoxide (DMSO) solutions of L-651,896 were added to 5×10^6 PMN in 0.5 ml of MEM and incubated at 37°. After 2 min A23187 was added to 10 μM, and the incubations were continued for an additional 4 min. The reactions were terminated by the addition of 0.5 ml methanol, the protein was removed by centrifugation, and the amounts of LTB_4 in the aqueous-methanol extract were determined by radioimmunoassay [13].

Human peripheral blood polymorphonuclear leukocytes

Human PMN were separated from heparinized (10 units/ml) venous blood of healthy volunteers by centrifugation on Ficoll–Hypaque (1.077 to 1.08 g/ml). Contaminating erythrocytes were lysed with ammonium chloride.

Aliquots (0.25 μl) of concentrated DMSO solutions of L-651,896 were preincubated at 37° with 0.8 to 1.0×10^6 PMN in 0.25 ml MEM. A23187 (10 μM) was added, and the incubations were continued for an additional 5 min. The reactions were terminated by the addition of 0.25 ml methanol, the denatured protein was removed by centrifugation, and the amounts of LTB_4 in the aqueous methanol extract were determined by radioimmunoassay [13]. The immunoreactive LTB_4 was found to cochromatograph on reverse phase HPLC with authentic LTB_4.

5-Lipoxygenase assay

RBL-1 from the American Type Culture Collection were grown in suspension cultures. Cells were harvested by centrifugation at 2000 *g* for 7 min, washed, and centrifuged. The washed cells were suspended at 1×10^8 cells/ml in cold phosphate-buffered saline and sonicated at 0° for 60 sec in a Heat System Sonifier at power setting 4. The sonicate was centrifuged at 15,000 *g* for 15 min. The lipid layer was aspirated and discarded, and the supernatant fraction was centrifuged at 100,000 *g* at 4° for 1 hr. The lipid layer was again removed and the supernatant fluid, which contains the 5-lipoxygenase enzyme activity, was removed and used for assay purposes.

5-Lipoxygenase activity in 100,000 *g* supernatant fluids was determined by measuring the conversion of arachidonic acid to 5-HETE radiometrically. A mixture of 0.5 μl of L-651,896 and 25 μl of enzyme preparation was incubated for 5 min and then treated

with 0.5 μl of either [^{14}C]- or [^{3}H]-arachidonic acid at 50 μM containing 20,000 to 150,000 cpm. The reaction proceeded for 6 min at room temperature and was stopped by the addition of methanol containing 5 μg unlabeled arachidonic acid and 5 μg 5-HETE. The entire mixture was spotted on a silica gel TLC plate and developed with ether–hexane–acetic acid (50:50:2). The distribution of radioactivity was determined on a Bioscan Imaging Detector System.

12-Lipoxygenase assay

(Ethylenedinitrilo)tetraacetic acid was added to human platelet rich plasma (PRP) to a final concentration of 1 mM. The platelets were collected by centrifugation at 2000 *g* for 20 min and suspended in 25 mM Tris–HCl, pH 7.7, at a volume equal to one-fourth of the original PRP. The suspension was lysed with three successive freeze–thaw cycles. The lysate was centrifuged at 100,000 *g* for 1 hr, and the supernatant fluid was used as the source of 12-lipoxygenase.

L-651,896 was added in 0.5-μl aliquots of methanol to an incubation mixture containing 90 μl of the enzyme preparation (approximately 300 μg protein), 3 mM reduced glutathione, and 9 μl of 25 mM Tris–HCl buffer, pH 7.4. After 2 min of incubation at 37° 1.8 nmol [^{14}C]-AA was added in 0.5 μl methanol and the incubation was continued for an additional 10 min. The reactions were terminated by addition of 200 μl of methanol. After centrifugation to remove denatured protein, the 50-μl aliquots of the supernatant fluid were spotted on silica gel TLC plates. The chromatograms were developed in hexane–ethylether–acetic acid (60:40:1). The distribution of radioactivity was determined by scanning, utilizing a Bioscan Imaging Detector System.

Cyclooxygenase assay

Microsomes containing cyclooxygenase activity were prepared from ram seminal vesicles as previously described [15, 16]. For assay of cyclooxygenase activity, reactions were performed at 30° in an oxygen monitor chamber containing 3.0 ml of 100 mM potassium phosphate buffer at pH 7.0 and 2.6 mg of microsomal prostaglandin cyclooxygenase. Arachidonic acid at final concentrations ranging from 2 to 100 μM was added to initiate the reactions. Initial rates of oxygene uptake were determined from the maximum reaction velocities that occurred within the first 15 sec. Each data point represents results from experiments in which the quantities of AA indicated (2, 5, 20 or 100 μM) were added to incubations containing three different levels of L-651,896 at each substrate concentration.

All experiments were run in duplicate.

Arachidonic acid-induced mouse ear inflammation

Arachidonic acid (500 μg) and compound were co-applied in 25 μl of acetone–pyridine–water (97:2:1) to the ears of two groups of five mice. One group was killed after 15 min, and LTC_4/LTD_4 and PGE_2 levels in the ears were determined by radioimmunoassay [17]. The other group was killed after 45 min, and accumulation of ^{125}I-labeled albumin in the ear tissues was determined [17].

Oxazolone-induced mouse ear inflammation

Mice were sensitized topically by applying 100 μl of a solution of 4% oxazolone in acetone solution to shaved abdominal skin. Negative controls were treated with acetone only. After 7 days, the mice were challenged by application of 20 μl of a 1% solution of oxazolone to the ear in acetone–corn oil (3:1). L-651,896 was coapplied in the oxazolone solution. After 24 hr, a 6-mm tissue biopsy of the ear was placed into 3 ml of methanol in a tared vial. The wet weights of the tissues were determined as an index of inflammation.

One milliliter of 0.1 M sodium acetate buffer, pH 4.2, was added to the methanol, and the ear tissues were homogenized (Polytron, Brinkmann Instruments, Rexdale, Ontario) into this solution. The homogenate was centrifuged for 10 min at 1500 *g* to remove denatured protein and the supernatant fluid was removed. The pellet was reextracted with 1 ml methanol. After centrifugation this supernatant fluid was combined with the original aqueous-methanol extract.

The aqueous-methanol extract was diluted with water to 15% methanol and applied to a prewashed reverse phase C-18 Sep Pak cartridge (Waters). The cartridges were washed with 10 ml of 15% methanol and 20 ml of water. The eicosanoids were eluted with 5 ml methanol. The methanol extracts were evaporated under nitrogen and further purified on reverse phase HPLC (Supelco, 5 μm C18 column maintained at 34°) and eluted with methanol–water–acetic acid (73:27:0.05) with 0.5 mM oxalic acid, pH 5.6, at a flow rate of 1 ml/min. Radioimmunoassays (RIA) for LTB_4 and LTC_4 were performed on the HPLC fractions with retention times corresponding to those of authentic leukotrienes. PGE_2 was also determined by RIA of the SepPak eluate fluid. The recoveries for LTB_4, LTC_4 and PGE_2 were 56, 31 and 90% respectively.

RESULTS

Effects of L-651,896 on cell-free lipoxygenases and cyclooxygenase

RBL-1 5-lipoxygenase and human platelet 12-lipoxygenase. L-651,896 inhibited the 5-lipoxygenase activity of 100,000 *g* supernatant fractions from sonicated RBL-1 leukemia cells with an IC_{50} of 0.1 μM (Fig. 2). L-651,896 also inhibited human platelet cytosolic 12-lipoxygenase with an IC_{50} of 5.9 μM (Fig. 3).

Rat seminal vesicle microsomal cyclooxygenase. The inhibition of cyclooxygenase by L-651,896 was dependent upon the concentration of substrate arachidonic acid. This inhibition of microsomal cyclooxygenase was measured as O_2 uptake catalyzed by the ram seminal microsomes at substrate concentrations between 2 and 100 μM arachidonic acid [16]. Percent inhibitions were established at 2, 5, 20 and 100 μM arachidonic acid, using the initial velocities from the oxygen uptake traces. As the substrate concentration decreased from 100 to 2 μM, there was a marked increase in the potency of L-651,896 (Table 1).

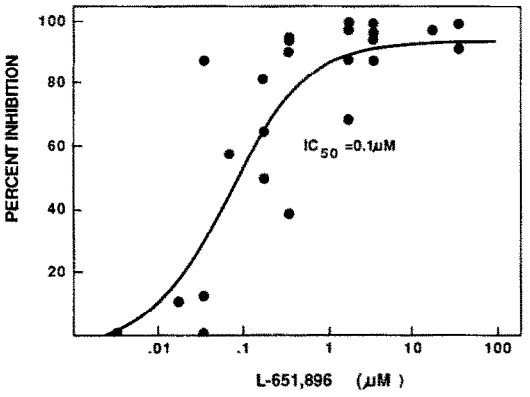

Fig. 2. Effect of L-651,896 on RBL-1 5-lipoxygenase activity. The RBL-1 cells were cultured as described in Materials and Methods. 5-Lipoxygenase activity was determined in the supernatant fluid of the sonicated cells as described in the text. The data were collected from six separate experiments, and the plot was computer drawn using a four-parameter curve fit. The IC_{50} was also computer derived.

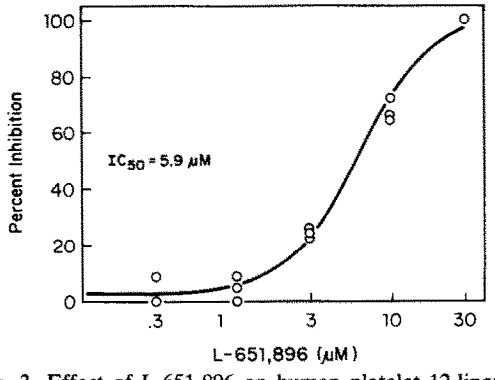

Fig. 3. Effect of L-651,896 on human platelet 12-lipoxygenase. Human platelets were collected and lysed, and the 100,000 *g* supernatant fluid was collected as described in Materials and Methods. The effect of L-651,896 on 12-HETE formation from arachidonic acid by this preparation was measured. The data were collected from four separate experiments, and the plot was computer drawn using a four-parameter curve fit. The IC_{50} was also derived.

Table 1. Inhibition of ram seminal vesicle microsomal cyclooxygenase by L-651,896 at different substrate concentrations

Arachidonic acid (μM)	L-651,896 (μM)	% Inhibition
100	500	88
	400	63
	300	26
20	200	84
	100	43
	50	8
5	50	60
	33	46
	17	34
2	25	63
	17	44
	8	39

Assay conditions are described in Materials and Methods.

Effects of L-651,896 on leukotriene and prostaglandin synthesis by intact cells

Mouse peritoneal macrophages. In mouse peritoneal macrophages zymosan stimulates the synthesis and release of LTC_4 and PGE_2 [14]. This was inhibited in a concentration-dependent manner by L-651,896 (Fig. 4) with IC_{50} values of 0.1 and 1.1 μM respectively. Thus, L-651,896 was 11-fold more potent as an inhibitor of leukotriene than of prostaglandin synthesis. Since L-651,896 inhibited 5-lipoxygenase in RBL-sonicates (Fig. 2) and ram seminal vesicle microsome preparations (Table 1), we conclude that lowering of LTC_4 and PGE_2 levels in these macrophage cultures is due to enzyme inhibition.

We have shown previously that PMA stimulates the synthesis of prostaglandins but not that of leukotrienes [14]. In PMA-treated cells, L-651,896 inhibited PGE_2 synthesis in a concentration-dependent manner yielding an IC_{50} of 3 μM when analyzed at 2 hr (Fig. 5). Furthermore, the inhibition of PMA-

Table 2. Reversible inhibition by L-651,896 of PMA-stimulated PGE_2 synthesis by mouse peritoneal macrophages

	PGE_2 synthesis			
	Agents present		Agents removed	
	cpm*	% Inhibition	cpm	% Inhibition
No addition	2,480 ± 451		1,715 ± 211	
PMA, 5×10^{-7} M	68,420 ± 580		65,060 ± 4,140	
L-651,896 (35 μM) + PMA	5,340 ± 440	96	64,280 ± 1,220	1
Aspirin (56 μM) + PMA	15,220 ± 1,000	81	25,760 ± 700	62

Two groups of cells were preincubated for 15 min with L-651,896 or aspirin. One set of cells was then washed five times with 1 ml M-199 containing 1% delipidated bovine serum albumin and then with two additional 2-ml aliquots of M-199. One milliliter of M-199 was then added to each culture, and 10^{-7} M PMA was added. At various times aliquots were directly chromatographed on TLC, and the amount of [^{3}H]PGE_2 was determined as described in Materials and Methods.

* The average of duplicate observations ± the range except for the "No addition" control. The "No addition" control was the mean ± SD, N = 4.

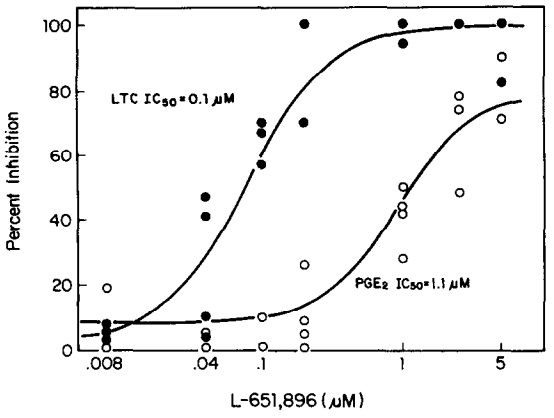

Fig. 4. Effect of L-651,896 on zymosan-stimulated PGE_2 (○) and LTC_4 (●) synthesis by resident mouse peritoneal macrophages. Resident mouse peritoneal macrophages (approximately 2×10^6 cells) were incubated for 30 min with L-651,896 in 1 ml M-199 containing HIPS. Zymosan (50 μg) was added, and the incubations were continued for an additional 2 hr. The media were collected and the amounts of immunoreactive LTC_4 and PGE_2 were determined by RIA. The data represent the mean percent inhibitions from four separate experiments. The control level of LTC_4 was 2 ng/culture and zymosan increased the level to 22.8 ± 6.1 ng/culture (mean ± SE). The control levels of the PGE_2 was 0.4 ng/culture and zymosan increased the level to 18.5 ± 4.8 ng/culture (mean ± SE).

stimulated PGE_2 synthesis by L-651,896 was found to be completely reversible, whereas inhibition by aspirin was only partially reversible (Table 2).

Rat and human PMN. LTB_4 synthesis by elicited peritoneal rat and human peripheral blood PMN incubated with the ionophore A23187 was also inhibited by L-651,896 with IC_{50} values of 0.2 and 0.4 μM respectively (Fig. 6).

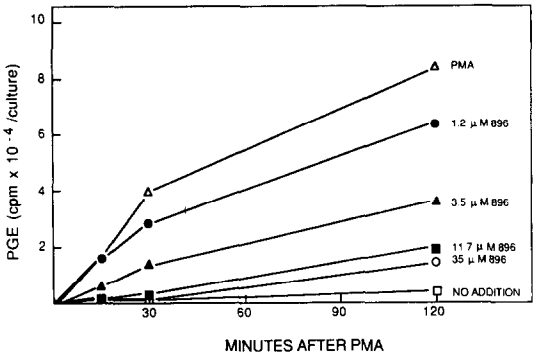

Fig. 5. Effect of L-651,896 on PMA-stimulated PGE_2 production by macrophages. Cultures of mouse peritoneal macrophages were preincubated for approximately 20 hr with [^{3}H]arachidonic acid as described in Materials and Methods. After washing the cells, L-651,896 was incubated with the radiolabeled cells for 15 min. Phorbol myristate acetate (PMA), 1×10^{-7} M, was then added, and the incubations were continued for 15–120 min. Aliquots of the culture medium were removed at various times and chromatographed and the radioactivity associated with PGE_2 was determined as described in Materials and Methods.

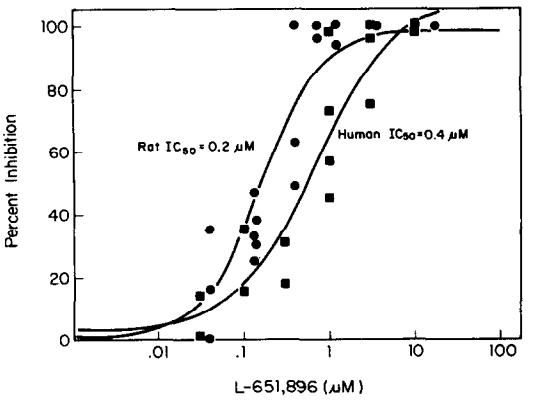

Fig. 6. Effect of L-651,896 on LTB_4 production by rat and human polymorphonuclear leukocytes. Elicited rat peritoneal polymorphonuclear leukocytes (PMN) were prepared from peritoneal exudates as described in Materials and Methods. L-651-896 was incubated with 5×10^6 PMNs in 0.5 ml MEM containing 10 mM HEPES (MEM) at 37°. After 2 min, A23187 (10 μM) was added and the incubations were continued for an additional 4 min. The reactions were terminated by the addition of 0.5 methanol, and the amount of LTB_4 was determined by RIA as described in Materials and Methods and Ref. 18. The levels of LTB_4 production by control and A23187-stimulated cells were 0.9 ± 0.3 and 43.7 ± 4.9 ng/10^6 cells respectively (●). The data are from five separate experiments. Human PMNs were prepared from heparinized (10 units/ml) venous blood of healthy volunteers as described in Materials and Methods. L-651,896 was incubated for 2 min at 37° with 0.8 to 1×10^6 PMNs in 0.25 ml MEM. A23187 (10 μM) was added and the incubations were continued for an additional 5 min. The amount of LTB_4 synthesized (■) was determined by RIA as described in Materials and Methods. The levels of LTB_4 production by control and A23187-stimulated cells were 0.05 ± 0.05 and 29.2 ± 3.0 ng/l × 10^6 cells (mean ± SE). The data are from four separate experiments.

Effects of L-651,896 in in vivo *models of skin inflammation*

Arachidonic acid-induced ear inflammation in the mouse. We have shown recently that topical administration of arachidonic acid to mouse ears results in elevated tissue levels of leukotrienes, principally LTC_4, LTD_4 and prostaglandin E_2. These changes are closely followed by increases in vascular permeability and the development of edema [17]. L-651,896 was co-applied with arachidonic acid in order to test its effects on elevated levels of eicosanoids and the increased vascular permeability. It was directly compared with indomethacin in this model. The application of arachidonic acid caused an increase in PGE_2 from 0.3 to 16.5 ng/site in 15 min and an increase in LTC_4/LTD_4 from 0.3 to 3.4 ng/site. The ED_{50} for L-651,896 to lower LTC_4/LTD_4 levels was 29 nmol/site, to lower PGE_2 levels was 309 nmol/site, and to block increased vascular permeability was 18 nmol/site (Table 3). For indomethacin, the ED_{50} to lower LTC_4/LTD_4 levels was 936 nmol/site, to lower PGE_2 levels was less than 0.8 nmol/site, and to inhibit permeability was 45 nmol/site (Table 3). The potency of L-651,896 to block increased vascular permeability (ED_{50} = 18 nmol) was similar to its potency to lower LTC_4/LTD_4 levels (ED_{50} = 29 nmol). In this context, L-651,896 was three times

Table 3. Comparison of L-651,896 and indomethacin as inhibitors of arachidonic acid-induced increases in LTC_4/LTD_4, PGE_2 levels and vascular permeability in the mouse ear

Compound	Dose (nmol/site)	PGE_2 (% Inhibition)	LTC_4/LTD_4 (% Inhibition)	Permeability (% Inhibition)
L-651,896	11.7	24	28	37
	35.4		52	71
	88.7	17	84	64
	177	+10	84	80
	355	66	92	
	709	65	84	
	1773	64	96	
	2660	84	100	
	3546	90	100	
ED_{50}, nmol/site		309	29	18
Indomethacin	0.8	65	+16	
	2.8	72	+84	18
	9.2	99	+20	0
	28	100	+24	39
	70			67
	140	100	+48	68
	279	100	+8	
	698	100	36	
	1397	100	84	
ED_{50}, nmol/site		<0.8	934	45

Arachidonic acid (500 μg) and compound were co-applied to the ears of two groups of mice (N = 5). One group was killed after 15 min, and LTC_4/LTD_4 and PGE_2 levels in the ears were determined by radioimmunoassay [18]. The other group was killed after 45 min, and the accumulation of ^{125}I-labeled albumin in the ear tissues was determined [17].

more potent than indomethacin in blocking arachidonic acid-induced permeability, although L-651,896 was approximately 400-fold less potent than indomethacin (309 vs 0.8 nmol/site) in lowering PGE_2 levels in this model.

Oxazolone-induced delayed hypersensitivity in mouse ears. The topical application of oxazalone to the ears of mice sensitized to this agent 1 week earlier resulted in pronounced edema and increased levels of leukotrienes and prostaglandins within the tissue.

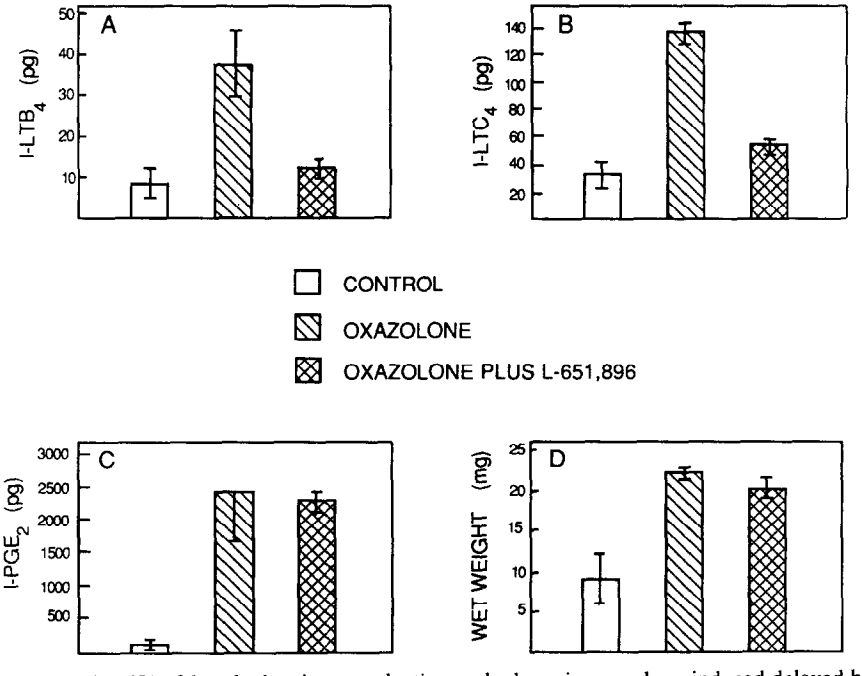

Fig. 7. Effect of L-651,896 on leukotriene production and edema in oxazolone-induced delayed hypersensitivity. L-651,896, (400 μg) was coapplied in the oxazolone solution to ears of mice as described in Materials and Methods. After 24 hr the mice were killed, and the ear tissue biopsies were weighed. The eicosanoid content of the tissues was determined by radioimmunoassay. The data are the mean ± SEM of three separate experiments. (A) LTB_4; (B) LTC_4; (C) PGE_2 and (D) wet weight.

The co-applciation of L-651,896 significantly reduced tissue concentrations of LTB_4 and LTC_4 (Fig. 7, A and B). Under these conditions the increased levels of PGE_2 were not lowered by L-651,896 (Fig. 7C). Furthermore, the oxazalone-induced swelling was not affected (Fig. 7D). Thus, in this model of delayed hypersensitivity, the lowering of the levels of 5-lipoxygenase products was not sufficient to inhibit the edematous response.

DISCUSSION

Although exogenously administered leukotrienes have been shown to mimic the cardinal features of the acute inflammatory response, the role of endogenous leukotrienes in these processes is much less clear [19]. The detection of leukotrienes in inflammatory lesions in the skin of humans [20, 21] and animal models [17] is not sufficient evidence to conclude that they are endogenous mediators of inflammation. Such a role would be more likely if the selective inhibition of their synthesis were shown to be accompanied by anti-inflammatory activity. Since L-651,896 has been shown to be an inhibitor of leukotriene synthesis *in vitro* and when applied topically *in vivo*, it has been used to explore the role of leukotrienes in the increased vascular permeability and edema associated with two different types of cutaneous inflammation.

In the model of oxazolone-induced hypersensitivity, L-651,896 lowered elevated levels of LTB_4 and LTC_4 measured 24 hr after challenge. Such activity was not accompanied by reduction in wet weight increase associated with the edematous response seen at this time. Thus, the lowering of leukotrienes levels does not inhibit edema formation. Kunkel *et al.* [22] have shown lowering of levels of these mediators by NDGA, nafazatron and BW755C in a model of immune based granuloma formation induced by *Schistosoma mansonii* eggs. In these studies granuloma formation was inhibited, but vascular permeability and edema formation were not measured.

In the model of arachidonic acid-induced inflammation, the doses of L-651,896 that inhibited increased vascular permeability approximated those found to lower leukotriene levels. Thus, further consideration should be given to the possibility that leukotrienes have a role in the mediation of vascular permeability increases in this system. However, it is still possible that another mechanism, not involving the inhibition of eicosanoid synthesis mediates the effect of L-651,896. The most obvious possibility is free radical scavenging activity which according to Burton *et al.* [23] is a potent property of this class of compounds. Comparative studies of L-651,896 and 5-lipoxygenase inhibitors lacking radical scavenging activity would help to resolve this possibility.

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