PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **182**, 296–300 (1986)

Inhibition of Leukotriene Actions by the Calcium Channel Blocker, Nisoldipine[1] (42342)

ALLAN M. LEFER AND ATSUO YANAGISAWA

Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Abstract. Nisoldipine, a calcium channel blocker having a highly potent effect on vascular smooth muscle relative to cardiac muscle, was tested to determine its anti-leukotriene properties. Nisoldipine, at concentrations from 1 to 300 ng/ml, significantly attenuated the vasoconstrictor effects of both LTC_4 and LTD_4 in isolated perfused cat coronary arteries and in isolated Langendorff perfused cat hearts. In isolated perfused coronary arteries, nisoldipine exerted a greater percentage inhibition of LTC_4- and LTD_4-induced constriction than of the constriction induced by the thromboxane analog, carbocyclic thromboxane A_2 (CTA_2). In isolated cat lung fragments, higher concentrations of nisoldipine were required to inhibit leukotriene formation (i.e., 10–200 μM). These concentrations of nisoldipine markedly inhibited the formation of the chemotactic leukotriene (LTB_4) as well as the peptide leukotrienes (LTC_4 and LTD_4) stimulated by A-23187. Both types of leukotrienes were inhibited to a comparable degree. Thus, nisoldipine has significant anti-leukotriene actions. At normally employed concentrations, nisoldipine inhibits leukotriene actions on vascular smooth muscle, and at higher concentrations, it inhibits leukotriene formation.

© 1986 Society for Experimental Biology and Medicine.

Leukotrienes (LTs) are biologically potent products of the lipoxygenase pathway of arachidonic acid metabolism. There are two different branches of leukotriene A_4 metabolism converting this essentially inactive parent leukotriene into biologicaly active LTs. One branch forms LTB_4, a nonpeptide leukotriene which is a potent chemoattractant and chemotactic agent (1). The other branch of LTA_4 metabolism forms LTC_4 which can be converted to LTD_4 and then to LTE_4. These three peptide leukotrienes, known collectively as the slow reacting substance of anaphylaxis (SRS-A), are potent bronchoconstrictors and vasoconstrictors (1, 2). Leukotrienes C_4 and D_4 are very potent coronary vasoconstrictors in both large arteries (3) and arterioles (4). All the biologically active LTs appear to increase capillary permeability. Leukotrienes are formed largely by white blood cells (1), pulmonary tissue (4, 5), and blood vessels (5, 6).

Calcium appears to be essential in both the formation and action of the leukotrienes. In this connection, A-23187, a calcium ionophore, is necessary to generate significant quantities of leukotrienes *in vitro* (6). Furthermore, the peptide leukotrienes appear to act as calcium mobilizers and considerable evidence suggests that LTC_4 and LTD_4 mobilize calcium in exerting their contraction of smooth muscle.

The major purposes of this study were to compare the effects of the potent calcium channel blocker, nisoldipine, on the vascular effects of LTC_4 and LTD_4 and on the ability of lung tissue to generate both peptide and nonpeptide leukotrienes. In this manner, a profile of the calcium requirements of leukotrienes could be evaluated.

Methods. *Preparation of tissue samples.* Cats were anesthetized with pentobarbital sodium (30 mg/kg) and a lobe of the left or right lung was rapidly removed. The tissues were blotted free of blood and minced in Krebs–Henseleit solution. Suspensions of minced tissues were prepared at a concentration of 10 mg/ml and incubated in a Dubnoff shaker bath at 37°C for 60 min. Incubates were given 3 μg/ml A-23187 (6*S*-[6α-(2*S**, 3*S**), 8β(*R**), 9β, 11α]-5-(methylamino)-2-[[3,9,11-trimethyl-8-[1-methyl-2-oxo-2-(1*H*-pyrrol-2-yl)ethyl]-1,7-dioxaspiro[5.5]undec-2-yl]-methyl]-4-benzoxazolecarboxylic acid; Calbiochem, La Jolla, Calif.). Nisoldipine (10 to 200 μM) or its vehicle (i.e., ethanol at 0.01%) or the lipoxygenase inhibitor propyl gallate (Sigma, St. Louis, Mo.; 50 μM) was added to the lung incubate just prior to the 60-min in-

[1] Supported by Research Grant HL-25575 from the National Heart Lung and Blood Institute of the NIH.

0037-9727/86 $1.50

Copyright © 1986 by the Society for Experimental Biology and Medicine.
All rights reserved.

cubation period. Sixty minutes was previously shown to be the time for maximum leukotriene production (5). At the end of the incubation period, the samples were filtered through Whatman No. 3 filter paper at 4°C and the clear filtrates were assayed for peptide leukotriene activity by radioimmunoassay for both LTB_4 and for the peptide leukotrienes LTC_4, LTD_4, and LTE_4.

Radioimmunoassay. Aliquots (25 to 100 μl) of filtrate were used for the radioimmunoassay of all leukotrienes. The peptide leukotriene antibody cross-reacts almost equally with LTC_4, LTD_4, and LTE_4, but not with prostaglandins, hydroperoxides, endoperoxides, or thromboxanes (5). $[^3H]LTD_4$ obtained from New England Nuclear Corporation (Boston, Mass.) and authentic LTD_4 (Merck–Frosst Laboratories, Dorval, Quebec, Canada) were used as a standard for the assay. The limit of detection was 0.05 pmole LTD_4 per assay. LTB_4 was assayed in the same samples using the radioimmunoassay of leukotriene B_4 (Amersham). The antiserum cross-reacted 100% with LTB_4, but only slightly with other lipoxygenase products: LTC_4 and LTD_4, 0.03%; diHETES, 1.7%; 5-HETES, 0.03%; 12-HETE, 2.0%; arachidonic acid and prostaglandins, <0.03%. All values are expressed as picomoles per milliliter of incubate.

Isolated perfused cat coronary arteries. Adult male cats weighing between 2.5 and 4.5 kg were anesthetized with sodium pentobarbital intravenously (30 mg/kg). The hearts were quickly excised and placed in ice-cold, oxygenated Krebs–Henseleit (K–H) solution. Both the left anterior descending artery and the right coronary artery were isolated and cannulated with a 20-gauge steel catheter adapter. The cannula was tied in place with 3-O silk and surrounding tissue was carefully dissected away from each artery according to previously described methods (7). The coronary arteries were then perfused with oxygenated (95% O_2 + 5% CO_2) K–H buffer warmed to 37°C for 1 hr before drug testing began as employed by Smith *et al.* (14). Each artery was placed in a 10-ml chamber and the K–H solution was circulated by a Harvard peristaltic pump. Perfusion pressure was continuously monitored by a Statham P23Db pressure transducer and recorded on a Grass Model 7 oscillograph. In a few experiments, entire cat hearts were isolated and perfused retrogradely via a constant flow of 40–50 ml/min through the aorta and the coronary circulation (i.e., Langendorff procedure) according to the method of Roth *et al.* (3). LTD_4 (50 ng/ml) and nisoldipine (50 ng/ml) were infused separately over a period of 3–5 min and then together for 3–5 min. Coronary perfusion pressure was continuously recorded.

Stock solutions of LTC_4 and LTD_4 were prepared in distilled water, and were stored at −78°C until just prior to use when the stock solutions were diluted in K–H solution before addition to the perfusate of the coronary artery preparation. The vehicle at the volumes employed (i.e., 5 to 25 μl) failed to exert any significant vasoactive effect. Nisoldipine (Miles Laboratories, West Haven, Conn.) dissolved in ethanol was added to coronary arteries to achieve a final concentration of 0.3 to 300 ng/ml. The coronary constrictor effects of LTC_4 and LTD_4 were tested in the presence and absence of nisoldipine.

Statistics. All values are expressed as means ± SEM. Student's *t* test was used to compare statistical significance between groups and was confirmed by analysis of variance (ANOVA).

Results. When added to isolated perfused cat coronary arteries, nisoldipine exerts a significant vasodilator effect (i.e., a decrease in perfusion pressure during constant flow perfusion). At 100 ng/ml, nisoldipine decreased perfusion pressure by 19 ± 3 mm Hg ($P < 0.01$ from control values). This can be seen in Fig. 1. The nisoldipine vasodilator effect stabilized, and when LTD_4 is added to the perfusate, a marked blockade of the usual va-

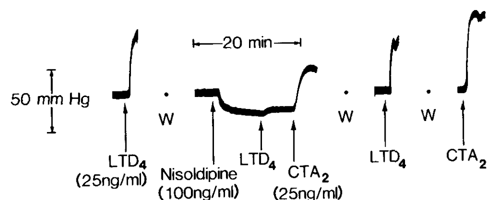

FIG. 1. Representative recordings of perfusion pressure in a cat coronary artery under constant flow perfusion. LTD_4-induced constrictions were almost completely inhibited by nisoldipine (100 ng/ml), whereas carbocyclic thromboxane A_2 (CTA_2) constrictions were only partially inhibited. W, washout of bath; calibrations of 50 mm Hg and 20 min are shown.

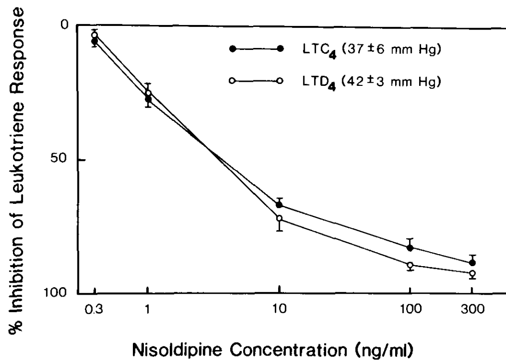

FIG. 2. Concentration–response curves showing percentage inhibition of LTC_4 (closed circles)- and LTD_4 (open cirlces)-induced constriction of cat coronary arteries by nisoldipine (0.3 to 300 ng/ml). All values are means ± SEM for six arteries. Values in parentheses next to LTC_4 and LTD_4 are mean responses ± SEM to the initial leukotriene administration in mm Hg at concentrations of 25 ng/ml.

soconstrictor effect occurred (Fig. 1). However, when the thromboxane agonist, CTA_2 was added to the perfusate in the presence of nisoldipine, a significant vasoconstrictor effect occurred. When the nisoldipine was washed out, the LTD_4-induced constriction was fully restored (Fig. 1). When CTA_2 was added to the bath in the absence of the calcium channel blocker, its constrictor effect was more pronounced. Thus nisoldipine exerted about a 35% inhibition of CTA_2-induced constriction under these conditions in contrast to about a 90% inhibition of the LTD_4 effect.

Figure 2 illustrates the dose-dependent nature of the antagonism of peptide leukotriene coronary constriction by nisoldipine. It is apparent that there is a dose-related blockade ranging from 5 to 6% at 0.3 ng/ml nisoldipine to a blockade of 93 to 95% at 300 ng/ml of nisoldipine. Moreover, nisoldipine appeared to be equieffective in blocking LTC_4 and LTD_4. At 1 and 10 ng/ml, nisoldipine exerted either no dilation or only a modest dilation of the coronary arteries, so that the leukotriene blockade is not dependent upon a significant vasodilator effect of the nisoldipine. In five perfused cat hearts, nisoldipine at 100 ng/ml blocked the coronary constrictor effect of 25 ng/ml of LTD_4 by 94 ± 5%. Thus, nisoldipine also blocks the coronary constrictor effects of leukotrienes in the intact coronary circulation.

In addition to antagonizing the coronary constrictor effects of LTC_4 and LTD_4, nisoldipine also exerted inhibitory effects on leukotriene synthesis in cat pulmonary fragments stimulated by A-23187. Table I summarizes these results. A-23187, at 3 μg/ml, significantly stimulated the production not only of the peptide leukotrienes (LTC_4 and LTD_4), but also of the nonpeptide chemotactic leukotriene (LTB_4). A-23187 appeared to produce more LTB_4, but this may reflect the binding properties of the different antibodies rather than a true difference in leukotriene synthesis. At 10 *μM*, nisoldipine inhibited leukotriene production by about 45% ($P < 0.05$). However, nisoldipine at 100 *μM* markedly attenuated the production of both types of leukotrienes. At 200 *μM*, nisoldipine almost totally inhibited leukotriene production by the lung fragments. Thus, nisoldipine exerts significant inhibition of leukotriene formation in isolated cat lung fragments stimulated by the calcium ionophore, A-23187.

Discussion. Our results clearly show that the calcium channel blocker nisoldipine exerts significant anti-leukotriene effects. The nature of these inhibitory effects is both on the for-

TABLE I. INFLUENCE OF NISOLDIPINE ON LEUKOTRIENE GENERATION IN MINCED CAT LUNG

	LTB_4		$LTC_4 + LTD_4$	
Condition	0 min	60 min	0 min	60 min
A-23187 alone	3 ± 0.3	61 ± 1.8	2 ± 0.2	18 ± 0.1
A-23187 + Nisold (10 *μM*)	2 ± 0.5	35 ± 3.9	3 ± 0.4	10 ± 0.5
A-23187 + Nisold (100 *μM*)	3 ± 0.4	16 ± 2.8	2 ± 0.3	4 ± 0.1
A-23187 + Nisold (200 *μM*)	3 ± 0.5	6 ± 0.9	2 ± 0.3	3 ± 0.5
A-23187 + Propyl gallate (50 *μM*)	3 ± 1.0	7 ± 1.4	3 ± 0.2	3 ± 0.3

Note. All values are means ± SEM for four experiments each performed in duplicate and expressed as pmole/ml.

mation of leukotrienes and on the vasoconstrictor action of the peptide leukotrienes. Thus, nisoldipine effectively inhibits formation of both major branches of leukotriene biosynthesis. The most likely explanation for this effect is via inhibition of calcium influx into leukotriene synthesizing cells effectively inhibiting either phospholipids necessary to release arachidonic acid from cell membranes or inhibition of 5-lipoxygenase, the major enzyme responsibe for leukotriene formation via arachidonic acid metabolism (8). Since a calcium ionophore, A-23187, is the most effective stimulator for leukotriene formation, it is not surprising that a calcium channel blocker inhibits leukotriene formation (9). The degree of inhibition of leukotriene synthesis by nisoldipine may indeed be more significant against other more physiologic stimulators of leukotriene formation (e.g., hypoxia, immunologic stimuli) than A-23187.

There is prior precedent that calcium channel blockers inhibit eicosanoid synthesis. Calcium channel blockers (i.e., verapamil and nisoldipine) have been shown to inhibit thromboxane A_2 formation in rabbits *in vivo* (10) as well as *in vitro* in platelet rich plasma (11). Levine (12) previously showed that verapamil and nifedipine inhibited leukotriene as well as PGE_2 synthesis in rat basophil leukemia cells. However, Weichman *et al.* (13) recently showed that verapamil and nifedipine failed to inhibit LT release in monkey lung fragments in response to immunologic challenge with immunoglobulin E (IgE). The lipoxygenase inhibitor nordihydroguaretic acid did inhibit LT formation in this system. These results suggest that immunologic challenge may either release preformed leukotriene or activate leukotriene synthesis by a mechanism not involving 5-lipoxygenase, and that this alternate mechanism is not calcium dependent.

The concentrations of nisoldipine necessary to achieve marked inhibition of leukotriene synthesis are high. The blood pressure lowering effects of 100 to 200 μM concentrations would be very severe. It is unlikely therefore that the calcium channel blockers could be used *in vivo* to be effective lipoxygenase inhibitors despite the potential utility of this effect. Moreover, this effect of nisoldipine may be a nonspecific effect of this agent not related to its calcium channel blockade at these high concentrations.

In contrast, nisoldipine exerted marked antagonism of the coronary constrictor effects of LTC_4 and LTD_4 at reasonable concentrations of the calcium channel blocker. Previously, other calcium channel blockers have been shown to block the vasoconstrictor effects of eicosanoids. Smith *et al.* (14) reported that nifedipine and verapamil totally antagonized the coronary constrictor effect of carbocyclic thromboxane A_2 (CTA_2) in the cat coronary artery. This was confirmed by Towart and Perzborn (15) using nimodipine in a similar system. With regard to leukotrienes, Fiedler *et al.* (16) showed that nifedipine significantly reversed the coronary constrictor effects of LTD_4 in intact anesthetized dogs. Lefer *et al.* (17) also reported that nicardipine totally antagonized the coronary constrictor effects of both LTC_4 and LTD_4 in the perfused cat coronary artery, although nicardipine failed to antagonize the bronchoconstrictor effect of either LTC_4 or LTD_4 in isolated guinea pig tracheal strips. Weichman *et al.* (13, 18) have clearly shown that tracheal smooth muscle is not dependent upon calcium for its leukotriene-induced contraction. However, recently Ezra *et al.* (19) claim that verapamil, nifedipine, and diltiazem do not antagonize the coronary constrictor effects of LTD_4 in the pig, although verapamil did blunt the electrocardiographic changes observed after LTD_4 injection suggestive of an anti-ischemic effect. Whether this is a species difference of the porcine coronary circulation or is due to a methodological difference is not clear from the available evidence. Nevertheless, significant evidence is available showing that calcium channel blockers inhibit the coronary constrictor effects of leukotrienes.

The reason for the lesser degree of antagonism of CTA_2-induced constriction by nisoldipine is not clear from these experiments, since nifedipine and verapamil at the same concentration (i.e., 100 ng/ml) almost totally antagonized the CTA_2-induced coronary constriction (14). It may therefore be a difference between nisoldipine and other calcium channel blockers. In this regard, nisoldipine possesses a very high selectivity for vascular smooth muscle relative to cardiac muscle (20). Concentrations on the order of 100 to 1000 times higher than effective vascular inhibitory concentrations are necessary to inhibit cardiac

function in contrast to verapamil, diltiazem, or nifedipine (19). These results are also of considerable significance since coronary arteries have been shown to produce significant amounts of leukotrienes (21).

We gratefully acknowledge the expert technical assistance of Judith Komlosh and George Papanicolaou during the course of these studies. We also thank Dr. Alexander Scriabine of the Miles Institute, Inc., West Haven, Connecticut, for the supply of nisoldipine and Dr. Joshua Rokach of Merck–Frosst Laboratories, Dorval, Quebec, Canada, for the supply of LTC_4 and LTD_4 used in this study.

1. Samuelsson B, Hammarstrom S, Murphy RC, Borgeat P. Leukotrienes and slow reacting substance of anaphylaxis (SRS-A). Allergy **35**:375–381, 1980.
2. Lefer AM. Eicosanoids as mediators of ischemia and shock. Fed Proc **44**:275–280, 1985.
3. Roth DM, Reibel DK, Lefer AM. Altered coronary vascular responsiveness to leukotrienes in alloxan-diabetic rats. Circ Res **54**:388–398, 1984.
4. Aharony D, Dobson P, Bernstein PR, Kusner EJ, Krell RD, Smith JB. Determination of SRS-A release from guinea pig lungs by a radioimmunoassay. Biochem Biophys Res Commun **117**:574–579, 1983.
5. Lefer AM, Roth DM, Lefer DJ, Smith JB. Potentiation of leukotriene formation in pulmonary and vascular tissue. Naunyn-Schmiedeberg's Arch Pharmacol **326**: 186–189, 1984.
6. Piper PJ, Letts LG, Dalton SA. Generation of a leukotriene-like substance from porcine vascular and other tissues. Prostaglandins **25**:591–599, 1984.
7. Ogletree MJ, Smith JB, Lefer AM. Actions of prostaglandins on isolated perfused cat coronary arteries. Amer J Physiol **235**:H400–H406, 1978.
8. Bach MK. Prospects for the inhibition of leukotriene synthesis. Biochem Pharmacol **33**:515–521, 1984.
9. Parker CW. The lipoxygenases in leukocytes and other mammalian cells. In: Chakrin LW, Bailey DM, eds. The Leukotrienes: Chemistry and Biology. Orlando, Fla., Academic Press, pp125–162, 1984.
10. Okamatsu S, Peck RC, Lefer AM. Effects of calcium channel blockers on arachidonate-induced sudden death in rabbits. Proc Soc Exp Biol Med **166**:551–555, 1981.
11. Lefer AM, Hock CE. Mechanisms of the anti-shock effects of nimodipine. In: Betz E, Deck K, Hoffmeister F, eds. Nimodipine: Pharmacology and Chemical Properties. Stuttgart, Schattauer, pp229–238, 1985.
12. Levine L. Inhibition of the A-23187-stimulated leukotriene and prostaglandin biosynthesis of rat basophil leukemia (RBL-1) cells by non-steroidal anti-inflammatory drugs, anti-oxidants, and calcium channel blockers. Biochem Pharmacol **32**:3023–3026, 1983.
13. Weichman BM, Muccitelli RM, Tucker SS, DeVan JF. Effect of calcium antagonists on the biosynthesis and contractile effects of peptidoleukotrienes in rhesus monkey lung. J Pharmacol Exp Therap **233**:345–351, 1985.
14. Smith EF, Lefer AM, Nicolaou KC. Mechanisms of coronary vasoconstriction induced by carbocyclic thromboxane A_2. Amer J Physiol **240**:H493–H497, 1981.
15. Towart R, Perzborn E. Nimodipine inhibits carbocyclic thromboxane-induced contractions of cerebral arteries. Eur J Pharmacol **69**:213–215, 1981.
16. Fiedler VB, Mardin M, Abram TS. Nifedipine on cardiovascular leukotriene D_4 action in the anaesthetized dog. Eur J Pharmacol **104**:159–164, 1984.
17. Lefer AM, Leprán I, Roth DM, Smith JB. Specificity of anti-leukotriene actions of nicardipine. Pharmacol Res Commun **16**:1141–1150, 1984.
18. Weichman BM, Muccitelli RM, Tucker SS, Wasserman MA. Effect of calcium antagonists on leukotriene D_4-induced contractions of the guinea-pig trachea and lung parenchyma. J Pharmacol Exp Ther **225**:310–315, 1983.
19. Ezra D, Boyd LM, Goldstein RE, Feuerstein G. Effects of calcium entry blockers on coronary constriction and myocardial ischemia due to leukotriene D_4. J Pharmacol Exp Ther **233**:229–234, 1985.
20. Kazda S, Garthoff B, Ramsch K-D, Schluter G. Nisoldipine: New drug annual. In: Scriabine A, ed. Cardiovascular Drugs. New York, Raven Press, pp243–258, 1983.
21. Piper PJ, Letts LG, Dalton SA. Generation of a leukotriene-like substance from porcine vascular and other tissues. Prostaglandins **25**:591–599, 1983.

Received December 11, 1985. P.S.E.B.M. 1986, Vol. 182.
Accepted March 14, 1986.