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# INHIBITION BY REV-5901 OF LEUKOTRIENE RELEASE FROM GUINEA-PIG AND HUMAN LUNG TISSUE *IN VITRO**

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**Abstract**—The 5-lipoxygenase inhibitor REV-5901 [α-pentyl-3-(2-quinolinylmethoxy)benzene-methanol] was evaluated for effects on mediator release *in vitro* from fragmented guinea-pig and human lung. In guinea-pig lung, REV-5901 inhibited the antigen-induced release of immunoreactive leukotriene $D_4$ ($iLTD_4$) with an $IC_{50}$ of 9.6 ± 2.9 μM and immunoreactive leukotriene $B_4$ ($iLTB_4$) with an $IC_{50}$ of 13.5 ± 2.2 μM. REV-5901 also inhibited the calcium ionophore-induced release of immunoreactive leukotrienes from human lung *in vitro* with $IC_{50}$ values of 11.7 ± 2.2 μM versus peptide leukotrienes and 10.0 ± 1.1 μM versus $iLTB_4$. The inhibition of release of $iLTD_4$ and $iLTB_4$ with similar $IC_{50}$ values suggests that REV-5901 acts by inhibiting 5-lipoxygenase in this system. At concentrations as high as 50 μM, REV-5901 did not inhibit the release of thromboxane $B_2$ ($TxB_2$), 6-keto-prostaglandin-$F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$), or histamine from either lung. The lack of effect on $TxB_2$ and 6-keto-$PGF_{1\alpha}$ indicates that REV-5901 did not inhibit phospholipase $A_2$, cyclooxygenase, or thromboxane synthetase. Inhibition of leukotriene release by REV-5901 could not be reversed by washing. Among various 5-lipoxygenase inhibitors, the order of potency for inhibition of $iLTD_4$ release from guinea-pig lung was AA-861 > REV-5901 > phenidone > nafazatrom > NDGA > BW755C. These findings suggest that REV-5901 is a selective and relatively potent inhibitor of leukotriene release from lung tissue *in vitro*.

Leukotrienes (LT) are products of the 5-lipoxygenase pathway of arachidonic acid metabolism and are potent mediators of immediate hypersensitivity and inflammatory-type responses [1]. The sulfidopeptide leukotrienes are potent bronchoconstrictors in many species including humans [1–5]. In addition, leukotrienes may induce airway hyperresponsiveness, a primary characteristic of asthma [4–6]. $LTB_4$ stimulates chemotaxis and chemokinesis of leukocytes [7] and has been implicated in a variety of inflammatory disorders [8, 9]. Hence, compounds that inhibit the 5-lipoxygenase pathway could prove to be effective agents for the treatment of asthma and other disorders [10].

A number of 5-lipoxygenase inhibitors have been described, including nordihydroguaiaretic acid (NDGA) [11–14] 1-[2-(β-naphthyloxy)ethyl]-3-methyl-2-pyrazolin-5-one (Nafazatrom®) [15], 3-amino-1-[*m*-(trifluoromethyl)phenyl]-2-pyrazoline (BW755C) [11, 14, 16, 17], *N*-(3-phenoxycinnamyl)-acetohydroxamic acid (BWA4C) [18], *N*-[3-(5,6,7,8-tetrahydro-2-naphthyl)prop-2-enyl]-acetohydroxamic acid (BWA797C) [18], 6,9-deepoxy-6,9-phenylimino-$\Delta^{6,8}$-prostaglandin $I_1$ (U60257) [19], 1-phenyl-3-pyrazolidone (phenidone) [20], and 2,3,5-trimethyl-6-(12-hydroxy-5,10-dodeca diynyl)-1,4-benzoquinone (AA-861) [21]. Many of these compounds are antioxidants, and several have been reported to inhibit other enzymes in addition to 5-lipoxygenase [11–21].

REV-5901 [α-pentyl-3-(2-quinolinylmethoxy)-benzene-methanol] is an active site directed reversible inhibitor of guinea-pig neutrophil 5-lipoxygenase [22, 23]. It appears to be selective for 5-lipoxygenase, having little or no activity against platelet 12-lipoxygenase, soybean 15-lipoxygenase, or cyclooxygenase [22, 23]. REV-5901 has also been reported to be a weak competitive antagonist of peptide leukotrienes [24].

This report describes the effects of REV-5901 on the release of leukotrienes, cyclooxygenase products and histamine from guinea-pig lung *in vitro* and expands the studies involving the effects of REV-5901 on mediator release from human lung *in vitro*, recently reported by Tennant and coworkers [25].

## METHODS

*Animals*. Male Hartley guinea pigs were sensitized 21–28 days before use by intraperitoneal injection of 5 mg ovalbumin (OA) in saline solution on day 1 and 10 mg OA in saline solution on day 3 [26].

*Preparation and antigen challenge of guinea-pig lung*. Animals were killed by decapitation, and the lungs were removed. The large airways and blood vessels were dissected away and the lung parenchyma was cut with a McIlwain tissue chopper into segments of approximately 1 × 1 × 2 mm. The tissue was washed three times with Tyrode solution and divided into aliquots weighing approximately 600 mg. Each sample of lung tissue was resuspended in 3.8 ml of Tyrode solution containing cysteine ($10^{-2}$ M) with or without experimental compound. Separate experiments confirmed that cysteine did not interfere with

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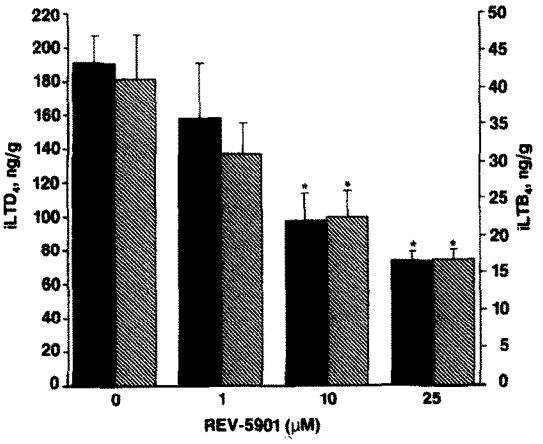


Fig. 1. Effect of REV-5901 on antigen-induced release of $iLTD_4$ and $iLTB_4$ from guinea-pig lung *in vitro*. Data are means ± SE for net $iLTD_4$ and net $iLTB_4$ release. N = 3 experiments, triplicate observations in each. Key: (■) $iLTD_4$; (▨) $iLTB_4$. (*) $P < 0.05$ compared to control.

the inhibitory effect of REV-5901 as judged by inhibition of $iLTB_4$ release (data not shown). Experimental compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted to final concentration with Tyrode solution. Solvent did not exceed 0.3% (v/v). Samples were preincubated with compounds at 37° for 10 min. Incubations with antigen (in triplicate) were started by adding OA (10 μg/ml final concentration) and continued for 45 min, except when indicated otherwise. At the end of the incubation the extracellular fluid was decanted through cheesecloth.

The extracellular fluid was divided and prepared for assay of mediators as follows: (a) 0.9 ml was added to 0.1 ml of 4 N $HClO_4$ and stored at −5° for histamine assay; (b) 0.2 ml was stored at −70° for 6-keto-prostaglandin-$F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) and thromboxane $B_2$ ($TxB_2$) radioimmunoassay (RIA); (c) the remainder of each sample was adjusted to pH 8 with 0.1 N NaOH, bubbled with argon gas, and stored at −70° for leukotriene determination by radioimmunoassay. Tyrode (4 ml) was added to the remaining lung tissue in each tube. These samples were placed in a boiling water bath for 10 min to extract the residual histamine. After decanting through cheesecloth, the samples were stored at −5° for histamine assay. Release values for all mediators except histamine are reported as ng mediator per g (wet weight) of lung tissue. Histamine release is expressed as a percentage of total tissue histamine. REV-5901, up to 50 μM, and DMSO, up to 0.3% (v/v), had no effect on any of the assay procedures used.

*Preparation and A23187 challenge of human lung.* Macroscopically normal human lung tissue was obtained from specimens resected from patients with carcinoma of the lung. Large airways and blood vessels were dissected away. The tissue was cut into segments with a tissue chopper and resuspended in modified Krebs buffer containing $10^{-2}$ M cysteine, with or without test compound. The tissue was challenged in a manner similar to that described above, except that calcium ionophore A23187 (5 μg/ml) rather than antigen was used to induce mediator release. The extracellular fluid was treated as described above and stored frozen until assayed.

*$iLTD_4$ assay.* Peptide leukotrienes were determined by radioimmunoassay. The procedure was similar to that described earlier [27], except that antiserum with a higher titer was used. This antiserum was prepared by injecting rabbits with a conjugate of $LTD_4$ and keyhole limpet hemocyanin. The conjugate was prepared using 1,5-difluoro-2,4-dinitrobenzene as coupling agent as described by Young *et al.* [28]. The conjugate contained 12 mol $LTD_4$ per mol hemocyanin. The antiserum (1:1000 titer) was sensitive to $LTD_4$, with 50% inhibition obtained at 7.5 nM $LTD_4$. The percent cross-reactivity of the antiserum was as follows ($LTD_4$ = 100): $LTC_4$, 177; $LTE_4$, 45; $LTD_3$, 21; $LTB_4$, 0.04; 5(*R,S*)-hydroxyeicosatetraenoic acid [5(*R,S*)-HETE], 0.08; 15-HETE, <0.04; arachidonic acid, <0.27; $PGF_{2\alpha}$, $PGD_2$, $PGE_2$, $TxB_2$, cysteine and glutathione, all <0.03. Cysteine ($10^{-2}$ M), which we have demonstrated to be an inhibitor of the dipeptidase that converts $LTD_4$ to $LTE_4$, in guinea-pig and human lung [29], was included in the incubation medium in all experiments to block the formation of $LTE_4$.

An assessment of the amount of $LTC_4$ versus $LTD_4$ released by antigen from sensitized guinea-pig lung was performed as follows. Control extracellular fluid samples (antigen challenge, no drug treatment) from approximately fifteen experiments were thawed, pooled, and passed through a Gelman filter (0.45 μm). After acidification to pH 5.5, the filtrate was loaded onto pre-equilibrated Sep Pak $C_{18}$ columns. After washing the columns, peptide leukotrienes were eluted with 100% methanol, pooled, and concentrated by evaporation to approximately 1 ml. This material was analyzed by HPLC using a $C_{18}$ ODS reverse phase column and elution with methanol:water:acetic acid (68:32:1, pH 5.8, 1.5 ml/min). Fractions were collected at 1-min intervals and assayed by RIA. Comparisons to authentic standards of $LTC_4$ and $LTD_4$ were made. Only material corresponding to $LTD_4$ was recovered. Hence, the peptide leukotrienes released from guinea-pig lung are designated in this report as "immunoreactive $LTD_4$" ($iLTD_4$). $LTB_4$ released from guinea-pig lung was identified by HPLC in a similar manner, as reported previously for $LTB_4$ released from rat peritoneal cells [30]. There were not enough samples from human lung to permit determination of the $LTC_4/LTD_4$ ratio. Material released from human lung and determined by RIA is therefore designated immunoreactive peptide leukotriene (iPEPTIDE-LT).

*Assays for $LTB_4$, $TxB_2$ and 6-keto-$PGF_{1\alpha}$.* These mediators were each determined by specific radioimmunoassay using procedures similar to that for $LTD_4$. The percent cross-reactivity for the $LTB_4$ antiserum [31] was as follows ($LTB_4$ = 100): 5*S*,12*S*-diHETE, 5.8; 6-*trans*-$LTB_4$, 2.2; 20-OH $LTB_4$, 0.03; 20-COOH $LTB_4$, <0.01; hydroxyheptadecatrienoic acid (HHT), 0.16; 12*S*-HETE, 0.08; 12 *R,S*-HETE, 0.06; $PGE_2$, $TxB_2$, 6-keto-$PGF_{1\alpha}$, arachidonic acid, and $LTD_4$, all <0.1. The percent cross-reactivity for the $TxB_2$ antiserum was as follows ($TxB_2$ = 100):

Table 1. Effects of 50 μM REV-5901 on antigen-induced mediator release from guinea-pig lung*

| Mediator | Net amount released after: Antigen | Antigen + 50 μM REV-5901 | % Change | P |
|---|---|---|---|---|
| $iLTD_4$ | 64.7 ± 12.6 ng/g† | 7.6 ± 4.2 ng/g | −88.1 | <0.05 |
| $iLTB_4$ | 19.1 ± 3.4 ng/g | 3.0 ± 0.6 ng/g | −84.4 | <0.01 |
| $TxB_2$ | 259 ± 42 ng/g | 219 ± 46 ng/g | −15.6 | >0.05 |
| 6-Keto-$PGF_{1\alpha}$ | 11.2 ± 3.6 ng/g | 9.3 ± 3.2 ng/g | −16.7 | >0.05 |
| Histamine | 19.8 ± 1.2% | 18.2 ± 2.7% | −8.1 | >0.05 |

* At 50 μM, REV-5901 had no significant effect on the spontaneous release of these mediators, values for which in the absence and presence of REV-5901 respectively, were as follows: $LTD_4$, 1.5 ± 0.5 ng/g, 1.9 ± 0.9 ng/g; $LTB_4$, 2.4 ± 0.1 ng/g, 3.8 ± 0.6 ng/g; $TxB_2$, 239 ± 73 ng/g, 260 ± 52 ng/g; 6-keto-$PGF_{1\alpha}$, 36.3 ± 5.3 ng/g, 35.7 ± 5.2 ng/g; and histamine, 1.0 ± 0.3%, 0.8 ± 0.2% (mean ± SE, N = 3–4 experiments, triplicate determinations in each).

† Data are means ± SE; N = 3–4 experiments, triplicate observations in each.

$PGD_2$, 6.5; $PGF_{2\alpha}$, 0.2; $PGE_1$, $PGE_2$, $PGF_{1\alpha}$ and 6-keto$PGF_{1\alpha}$, all <0.2. The percent cross-reactivity for the 6-keto-$PGF_{1\alpha}$ antiserum was as follows (6-keto-$PGF_{1\alpha}$ = 100): 6-keto-$PGE_1$, 1.3; $PGF_{2\alpha}$, 1.13; 13,14-dihydro-6-keto-$PGF_{1\alpha}$, 0.71; $PGF_{1\alpha}$ 0.69; 13,14-dihydro-6,15-diketo-$PGF_{1\alpha}$, 0.39; 6,15-diketo-$PGF_{1\alpha}$, <0.2; arachidonic acid, $TxB_2$, $PGD_2$, $PGE_1$, all <0.05.

*Histamine assay.* Histamine was assayed by an automated fluorometric procedure using a Technicon Autoanalyzer II as described by Siraganian [32].

*Lipoxygenase inhibition.* $(K_i)_{app}$ was determined on isolated guinea-pig 5-lipoxygenase as previously described for reversible inhibitors [33, 34].

*Statistics.* Statistical comparisons were made using Student's *t*-test or, where appropriate, Student's *t*-test for paired observations.

*Materials.* Ovalbumin, 1-phenyl-3-pyrazolidone (phenidone) and nordihydroguiaretic acid (NDGA) were purchased from the Sigma Chemical Co. (St Louis, MO). Calcium ionophore A23187 was purchased from the Calbiochem-Behring Corp. (LaJolla, CA). Orthophthaldialdehyde, cysteine, and dimethyl sulfoxide (DMSO) were obtained from the Aldrich Chemical Co. (Milwaukee, WI). BW755C was a gift of the Wellcome Research Laboratories (Beckenham, U.K.). α-Pentyl-3-(2-quinolinylmethoxy)benzene-methanol (REV-5901), 2,3,5-trimethyl-6-(12-hydroxy-5, 10-dodecadiynyl)-1,4-benzoquinone (AA-861), and 1-[2-(β-naphthyloxy)ethyl - 3 - methyl - 2 - pyrazolin - 5 - one (Nafazatrom®) were synthesized at Imperial Chemical Industries, Pharmaceuticals Division, Alderley Park, U.K., or Riems, France. Leukotriene $D_4$, [14,15-$^3$H(N)]-, 40.0 Ci/mmol; leukotriene $B_4$, [5,6,8,9,11,12,14,15-$^3$H(N)]-, 133.0 Ci/mmol; thromboxane $B_2$, [5,6,8,9,11,12,14,15-$^3$H(N)]-, 139.0 Ci/mmol; and 6-keto-prostaglandin-$F_{1\alpha}$, 6-[5,8,9,11, 12,14,15$^3$H(N)]-, 130.0 Ci/mmol; were purchased from New England Nuclear (Boston, MA).

## RESULTS

*Inhibition of mediator release from guinea-pig lung.* REV-5901 was tested at 1–25 μM using 0.1% DMSO as cosolvent. The limited aqueous solubility of REV-5901 necessitated 0.3% DMSO in order to test the compound at 50 μM. Hence, evaluation of this concentration was done in a separate set of experiments.

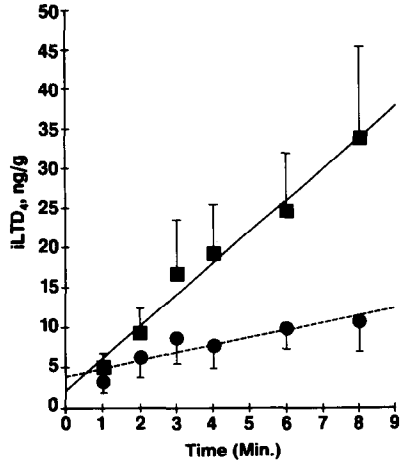


Fig. 2. Time course of $iLTD_4$ release from guinea-pig lung in the presence and absence of 10 μM REV-5901 during the first 8 min after antigen challenge. Key: (■—■) antigen control; and (●—●) antigen plus 10 μM REV-5901. Values are means ± SE, uncorrected for spontaneous release which was 1.9 ± 0.9 ng/g. N = 3 experiments, triplicate observations in each.

Figure 1 illustrates that REV-5901, 1–25 μM, caused concentration-dependent inhibition of antigen-induced $iLTD_4$ release with an $IC_{50}$ of 9.6 ± 2.9 μM. REV-5901 also inhibited antigen-induced $iLTB_4$ release with an $IC_{50}$ of 13.5 ± 2.2 μM (Fig. 1). In contrast, REV-5901, 1–25 μM, had no significant effect on antigen-induced release of $TxB_2$, 6-keto-$PGF_{1\alpha}$ or histamine (data not shown).

In the experiments with 0.3% DMSO, the higher level of cosolvent caused a reduction in the control values for antigen-induced $iLTD_4$ and $iLTB_4$ release (cf. Table 1 vs Fig. 1). We were still able to determine, however, that REV-5901 at 50 μM caused substantial inhibition of antigen-induced $iLTD_4$ and $iLTB_4$

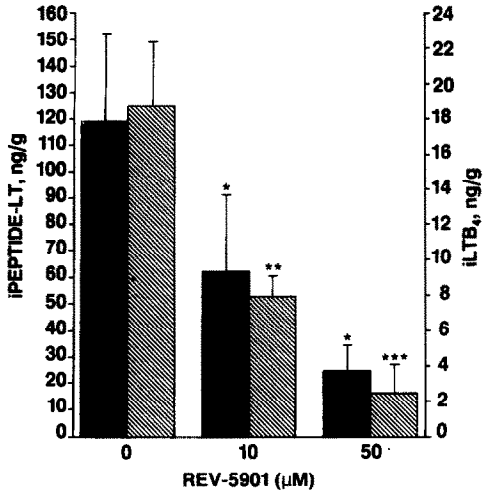


Fig. 3. Effect of REV-5901 on A23187-induced iPEPTIDE-LT release and $iLTB_4$ release from human lung *in vitro*. Values are means ± SE, for net iPEPTIDE-LT release and net $iLTB_4$ release. N = 4–6 experiments, triplicate observations in each. Key: (■) iPEPTIDE-LT; and (▨) $iLTB_4$. (*) $P < 0.05$ compared to control; (**) $P < 0.01$ compared to control; and (***) $P < 0.001$ compared to control.

leukotriene release from guinea-pig lung, we determined the early time course (1–8 min) of antigen-induced $iLTD_4$ release in the presence and absence of 10 μM REV-5901. REV-5901 inhibited release at all time points (Fig. 2). The rate of $iLTD_4$ release in the control samples, as determined by linear regression, was 3.98 ± 0.31 ng/g/min ($r = 0.988$). In the presence of 10 μM REV-5901 the rate was 0.95 ± 0.17 ng/g/min ($r = 0.940$) which constitutes a 76% reduction. Similar levels of inhibition by REV-5901 were also observed at 20 and 40 min after antigen (data not shown).

*Reversibility by washing.* Although REV-5901 is a reversible inhibitor of cell-free 5-lipoxygenase [22, 23], it has been reported to cause irreversible inhibition of leukotriene release from rat polymorphonuclear cells.* Therefore, we evaluated the reversibility of REV-5901 inhibition of $iLTD_4$ release from guinea-pig lung. In preliminary experiments, lung tissue was incubated with 10 μM REV-5901, washed three times with Tyrode solution at room temperature, incubated in Tyrode solution at 37°, and challenged with antigen. Inhibition by REV-5901 was not reversed by this washing procedure (data not shown). Consequently, a more rigorous procedure was used. Specifically, after incubation with 10 μM REV-5901 for 10 min at 37° the lung tissue was washed three times at room temperature with Tyrode solution and incubated for 10 min at 37° in Tyrode solution. The extracellular fluid was decanted and the wash procedure repeated. Control tissue was treated similarly. This wash procedure has been reported to be effective for removing agents such as calcium from guinea-pig lung tissue *in vitro* [35]. In samples where 10 μM REV-5901 was added before the washing procedure, it reduced antigen-induced release of $LTD_4$ from 60.3 ± 13.6 to 30.3 ± 7.5 ng/g ($P < 0.05$, N = 3). In samples where 10 μM REV-5901 was added after the washing procedure, it reduced antigen-induced

Table 2. $IC_{50}$ Values for inhibition of antigen-induced $iLTD_4$ release from guinea-pig lung *in vitro**

| Compound | $IC_{50}$ (μM) | Relative potency† | $(K_i)_{app}$‡ (μM) |
|---|---|---|---|
| AA-861 | 1.5 ± 0.3 | 1 | 0.9 ± 0.3 (3) |
| REV-5901 | 9.6 ± 2.9 | 6 | 7.1 ± 1.3 (7) |
| Phenidone | 29.1 ± 4.3 | 19 | 16.2§ (2) |
| Nafazatrom | 42.1 ± 5.6 | 28 | Not active at 20 |
| NDGA | 72.3 ± 2.6 | 48 | 0.07 ± 0.04 (3) |
| BW755C | 128.8 ± 17.5 | 86 | 6.8§ (2) |

* Data are means ± SE (N = 3–4 experiments, triplicate observations in each).
† Relative potency = $IC_{50}$ test compound/$IC_{50}$ AA-861.
‡ Values are means ± SE (except where noted), with the number of experiments given in parentheses.
§ Average of two experiments.

release (Table 1). Even at this higher concentration, REV-5901 had no significant effect on antigen-induced release of $TxB_2$, 6-keto-$PGF_{1\alpha}$, or histamine (Table 1). Also, REV-5901 (50 μM) did not affect the spontaneous release of any of these mediators.

*Comparison with other inhibitors of 5-lipoxygenase.* Since REV-5901 provided selective inhibition of leukotriene release, it was of interest to compare it with other well-known inhibitors of 5-lipoxygenase in this model. The potencies of these compounds toward inhibiting $iLTD_4$ release from guinea-pig lung varied considerably (Table 2). Only the benzoquinone AA-861 was more potent than REV-5901. Also shown in Table 2 is the $(K_i)_{app}$ for each of these compounds versus isolated guinea-pig polymorphonuclear 5-lipoxygenase.

*Time course of $iLTD_4$ release in the presence and absence of REV-5901.* To gain a better understanding of the mechanism by which REV-5901 inhibited

* Tannenbaum AS, Caruso FS, Sereny N and Carobene G, Safety and tolerance of REV-5901, a novel 5-lipoxygenase inhibitor in man. American College of Clinical Pharmacology, Fourteenth Annual Meeting, Philadelphia, PA, October 1985.

Table 3. Effects of 50 μM REV-5901 on A23187-induced release of $TxB_2$, 6-keto-$PGF_{1\alpha}$, and histamine from human lung*

| Mediator | Net amount released after†: A23187 | A23187 + 50 μM REV-5901 | % Change | P |
|---|---|---|---|---|
| $TxB_2$ | 23.0 ± 5.6 ng/g | 35.0 ± 9.0 ng/g | +52 | >0.05 |
| 6-Keto-$PGF_{1\alpha}$ | 2.7 ± 1.6 ng/g‡ | 2.9 ± 2.8 ng/g | +7 | >0.05 |
| Histamine | 25.7 ± 6.2% | 24.7 ± 5.3% | −4 | >0.05 |

* At 50 μM, REV-5901 had no significant effect on the spontaneous release of these mediators, values for which in the absence and presence of REV-5901, respectively, were as follows: $TxB_2$ 25.9 ± 7.5 ng/g, 30.2 ± 6.7 ng/g; 6-keto-$PGF_{1\alpha}$ 16.2 ± 6.1 ng/g, 16.8 ± 5.6 ng/g; and histamine 6.3 ± 1.6%, 6.5 ± 1.7% (mean ± SE, N = 3–6 experiments, triplicate determinations in each).

† Data are means ± SE, N = 3–6 experiments, triplicate observations in each.

‡ Release of 6-keto-$PGF_{1\alpha}$ in the presence of A23187 was not significantly greater than basal release ($P > 0.05$).

release of $LTD_4$ from 58.4 ± 12.6 to 27.7 ± 8.3 ng/g ($P < 0.05$, N = 3). Inhibition levels for REV-5901, 50.3 ± 1.5% with washing and 55.0 ± 5.9% without washing, were not significantly different ($P > 0.05$), indicating that inhibition was not reversible.

*Inhibition of mediator release from human lung.* Since REV-5901 was a potent and selective inhibitor of leukotriene release from guinea-pig lung, we evaluated its effects on mediator release from human lung as well. The calcium ionophore A23187 caused variable amounts of iPEPTIDE-LT release (range 42 to 255 ng/g, N = 6) in human lung tissue. REV-5901 caused concentration-dependent inhibition of A23187-induced iPEPTIDE-LT release (Fig. 3) which was significant ($P < 0.05$) at 10 μM. The mean $IC_{50}$ value was 11.7 ± 2.2 μM (mean ± SE, N = 6). The effect of REV-5901 on $iLTB_4$ release was also evaluated. Again, REV-5901 produced concentration-dependent inhibition (Fig. 3) which was significant ($P < 0.05$) at 10 μM. The $IC_{50}$ value was 10.0 ± 1.1 μM (mean ± SE, N = 4). REV-5901 (50 μM) had no significant effect ($P > 0.05$) on A23187-induced release of $TxB_2$, 6-keto-$PGF_{1\alpha}$ or histamine (Table 3).

## DISCUSSION

Inhibition of the release of $iLTD_4$ and $iLTB_4$ with similar $IC_{50}$ values suggests that in guinea-pig lung REV-5901 acted by inhibiting 5-lipoxygenase. This is consistent with reports of inhibition by REV-5901 of cell-free 5-lipoxygenase from guinea-pig neutrophils [22] and RBL cells [23]. Since REV-5901 did not inhibit the release of $TxB_2$ and 6-keto-$PGF_{1\alpha}$, it apparently exhibits selectivity for 5-lipoxygenase versus phospholipase $A_2$, cyclooxygenase, and thromboxane synthetase. In this study we have demonstrated greater than 5-fold selectivity for 5-lipoxygenase, based on an $IC_{50}$ of 10 μM for inhibition of $iLTD_4$ release and no significant inhibition of $TxB_2$ or 6-keto-$PGF_{1\alpha}$ release at 50 μM. We observed no significant inhibition of antigen-induced histamine release by REV-5901. This, along with the lack of effect on release of cyclooxygenase products, suggests that REV-5901 did not inhibit leukotriene release by way of a generalized effect on the cell secretory response to antigen challenge.

When compared with a variety of well-known 5-lipoxygenase inhibitors for inhibition of $iLTD_4$ release in this model, REV-5901 was one of the most potent. Only AA-861 [36] exhibited greater potency. This greater potency was also evident in inhibition of cell-free 5-lipoxygenase from guinea-pig neutrophils where the $(K_i)_{app}$ values for AA-861 and REV-5901 were 0.9 and 7.1 μM respectively. Although there appears to be an unusually large difference in the potencies of NDGA and BW755C for inhibition of isolated 5-lipoxygenase versus $iLTD_4$ release, our data agree well with previous reports on these compounds. NDGA is an extremely potent inhibitor of the cell-free enzyme from a variety of cells with $IC_{50}$ values of 0.08 to 0.21 μM [11, 16, 37], but is a 15- to 50-fold less potent inhibitor of LT synthesis in isolated cells [12–14, 38]. Furthermore, Walker *et al.* [39] reported that NDGA is a rather poor inhibitor of SRS-A release ($IC_{50} > 30$ μM) in guinea-pig chopped lung, suggesting that: ". . . the lack of activity may be due to poor access to the lung enzyme. . . ." Similar tissue-dependent variability has been observed for BW755C as well. The $IC_{50}$ values, determined with isolated enzyme, range from 5 to 35 μM [11, 16, 39], and its potency further decreases to 25–50 μM in isolated cells [14, 38, 40]. Similar to NDGA, BW755C is an even weaker inhibitor of LT synthesis in guinea-pig lung with an $IC_{50}$ of 100 μM [17] in agreement with results reported here.

REV-5901 uniformly reduced the rate of $iLTD_4$ release during the early minutes after antigen challenge. This is as expected for a reversible, active site inhibitor of 5-lipoxygenase [33]. Compounds inhibiting $iLTD_4$ release by other mechanisms may have (1) reduced the duration rather than the rate of release, or (2) induced a lag phase such that release would not have begun until a later time point.

REV-5901 is a reversible inhibitor of cell-free 5-lipoxygenase [22, 23]. However, despite a thorough washing procedure, we were not able to remove the inhibitory effect of REV-5901 by washing. This is not necessarily in disagreement with reports that REV-5901 is a reversible inhibitor of cell-free 5-lipoxygenase, however, as it is possible that REV-5901 is

taken up by the cells involved in $iLTD_4$ release and not easily removed by the washing procedure used. Inhibition of leukotriene release from rat polymorphonuclear cells by REV-5901 has also been reported to be irreversible.* Alternatively, there may be physical "trapping" of REV-5901 in the lung tissue. Such trapping has been observed earlier with cromolyn sodium [41].

Our studies on mediator release from human lung indicate that REV-5901 also acts as a selective inhibitor of 5-lipoxygenase in this tissue. REV-5901 exhibited a potency against the calcium ionophore induced release of leukotrienes from human lung similar to that exhibited against the antigen-induced release of leukotrienes from guinea-pig lung. However, at the highest concentration tested (50 $\mu$M), REV-5901 had no inhibitory effect on the release of $TxB_2$, 6-keto-$PGF_{1\alpha}$, or histamine from human lung. This suggests that in this tissue also REV-5901 inhibits leukotriene release by 5-lipoxygenase inhibition rather than by a generalized effect on the mediator release process.

This inhibition of leukotriene release from human lung by REV-5901 is in general agreement with that reported by Tennant and coworkers [25]. They also observed that REV-5901 inhibited leukotriene release but not histamine release from human lung induced by A23187. However, in their studies with antigen-induced mediator release from human lung, REV-5901 inhibited both leukotriene release and histamine release. Our observations with antigen-induced mediator release from guinea-pig lung indicated that REV-5901 inhibited leukotriene release but not histamine release. The reason for this differential effect on antigen-induced histamine release from human lung versus guinea-pig lung is not presently known. The 5-lipoxygenase inhibitor AA-861 also has been shown recently to exhibit species selectivity in its ability to inhibit histamine release [42].

These studies suggest that REV-5901 would be a useful agent for evaluating the effects of selective inhibition of leukotriene synthesis in models of allergic and inflammatory disorders.

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## REFERENCES

1. Piper PJ, Pharmacology of leukotrienes. *Br Med Bull* **39**: 255–259, 1983.
2. Smith LJ, Greenberger PA, Patterson R, Krell RD and Bernstein PR, The effect of inhaled leukotriene $D_4$ in humans. *Am Rev Respir Dis* **131**: 368–372, 1985.
3. Davidson AB, Lee TH, Scanlon PD, Solway J, McFadden ER, Ingram RH, Corey EJ, Austen KF and Drazen JM, Bronchoconstrictor effects of leukotriene $E_4$ in normal and asthmatic subjects. *Am Rev Respir Dis* **135**: 333–337, 1987.
4. Barnes NC, Piper PJ and Costello JF, Actions of inhaled leukotrienes and their interactions with other allergic mediators. *Prostaglandins* **28**: 629–631, 1984.
5. Drazen JM and Austen KF, Leukotrienes and airway responses. *Am Rev Respir Dis* **136**: 985–998, 1987.
6. Patterson R, Harris KE, Bernstein PR and Krell RD, Aerosolized leukotriene $D_4$ converts monkeys that are negative ascaris responders to positive airway responders. *Life Sci* **38**: 1179–1184, 1986.
7. Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME and Smith MJH, Leukotriene $B_4$, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature* **286**: 264–265, 1980.
8. Bray MA, The pharmacology and pathophysiology of leukotriene $B_4$. *Br Med Bull* **39**: 249–254, 1983.
9. Davies P, Bailey PJ and Goldenberg MM, The role of arachidonic acid oxygenation products in pain and inflammation. *Annu Rev Immunol* **2**: 335–357, 1984.
10. Samuelson B, Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. *Science* **220**: 568–575, 1983.
11. Furukawa M, Yoshimoto T, Ochi K and Yamamoto S, Studies on arachidonate 5-lipoxygenase of rat basophilic leukemia cells. *Biochim Biophys Acta* **795**: 458–465, 1984.
12. Humes JL, Sadowski S, Galavage M, Goldenberg M, Subers E, Kuehl FA and Bonney RJ, Pharmacological effects of non-steroidal antiinflammatory agents on prostaglandin and leukotriene synthesis in mouse peritoneal macrophages. *Biochem Pharmacol* **32**: 2319–2322, 1983.
13. Hope WC, Welton AF, Fiedler-Nagy C, Batula-Bernardo C and Coffey JW, *In vitro* inhibition of the biosynthesis of slow reacting substance of anaphylaxis and lipoxygenase activity by quercetin. *Biochem Pharmacol* **32**: 367–371, 1983.
14. Dobson P, Aharony D and Krell RD, Development of a sensitive method for measurement of leukotriene production by isolated cells. *Res Commun Chem Pathol Pharmacol* **42**: 3–23, 1983.
15. Fischer S, Struppler M and Weber PC, *In vivo* and *in vitro* effects of nafazatrom (Bay G 6575), an antithrombotic compound on arachidonic acid metabolism in platelets and vascular tissue. *Biochem Pharmacol* **32**: 2231–2236, 1983.
16. Beetens JR, Loots W, Somers Y, Coene MC and De Clerk F, Ketoconazale inhibits the biosynthesis of leukotrienes *in vitro* and *in vivo*. *Biochem Pharmacol* **35**: 883–891, 1986.
17. Piper PJ and Temple DM, The effects of lipoxygenase inhibitors and diethylcarbamazine on the immunological release of slow reacting substance of anaphylaxis (SRS-A) from guinea-pig chopped lung. *J Pharm Pharmacol* **33**: 384–386, 1981.
18. Higgs GA, Follenfont RL and Garland LG, Selective inhibition of arachidonate 5-lipoxygenase by novel acetohydroxamic acids: effects on acute inflammatory responses. *Br J Pharmacol* **94**: 547–551, 1988.
19. Sun FF and McGuire JC, Inhibition of human neutrophil arachidonate 5-lipoxygenase by 6,9-deepoxy-6,9-(phenylimino)-$\Delta^{6,8}$-prostaglandin $I_1$ (U-60257). *Prostaglandins* **26**: 211–221, 1983.
20. Carlson RP, O'Neill-Davis L, Chang J and Lewis AJ, Modulation of mouse ear edema by cyclooxygenase and lipoxygenase inhibitors and other pharmacologic agents. *Agents Actions* **17**: 197–204, 1985.

* Tannenbaum AS, Caruso FS, Sereny N and Carobene G, Safety and tolerance of REV-5901, a novel 5-lipoxygenase inhibitor in man. American College of Clinical Pharmacology, Fourteenth Annual Meeting, Philadelphia, PA, October 1985.

21. Yoshimoto T, Yokoyama C, Ochi K, Yamamoto S, Maki Y, Ashida Y, Terao S and Shiraishi M, 2,3,5-Trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA-861), a selective inhibitor of the 5-lipoxygenase reaction and the biosynthesis of slow reacting substance of anaphylaxis. *Biochim Biophys Acta* **713**: 470–473, 1982.
22. Aharony D, Stein RL, Redkar-Brown DG, Hubbs SJ, Kusner EJ and Krell RD, The mechanism of leukotriene inhibition of REV-5901. *Fed Proc* **45**: 659, 1986.
23. Coutts SM, Khandwala A, Van Inwegen R, Chakraborty U, Musser J, Bruens J, Jaruvala N, Dally-Meade V, Ingram R, Pruss R, Jones H, Weiss E and Weinryb I, Arylmethyl phenyl ethers: A new class of specific inhibitors of 5-lipoxygenase, In: *Prostaglandins, Leukotrienes and Lipoxins* (Ed. Martyn Bailey J), pp. 627–637. Plenum Press, New York, 1985.
24. Van Inwegen RG, Khandwala A, Gordon R, Sonnino P, Coutts S and Jolly S, REV-5901: An orally effective peptidoleukotriene antagonist, detailed biochemical/pharmacological profile. *J Pharmacol Exp Ther* **241**: 117–124, 1987.
25. Tennant CM, Seale JP and Temple DM, Effects of a 5-lipoxygenase inhibitor, REV-5901, on leukotriene and histamine release from human lung tissue *in vitro*. *J Pharm Pharmacol* **39**: 309–311, 1987.
26. Forsberg K and Sorenby L, The influence of a new corticosteroid, buddesonide, on anaphylactic bronchoconstriction and SRS-A release in the guinea pig. *Agents Actions* **11**: 391–395, 1981.
27. Aharony D, Dobson P, Bernstein PR, Kusner EJ, Krell RD and Smith JB, Determination of SRS-A release from guinea-pig lungs by a radioimmunoassay. *Biochem Biophys Res Commun* **117**: 574–579, 1983.
28. Young RN, Kakushima M and Rokach J, Studies on the preparation of conjugates of leukotriene $C_4$ with proteins for development of an immunoassay for SRS-A. *Prostaglandins* **23**: 603–613, 1982.
29. Aharony D, Dobson PT and Krell RD, *In vitro* metabolism of [$^3$H]-peptide leukotriene in human and ferret lung: A comparison with the guinea pig. *Biochem Biophys Res Commun* **131**: 892–898, 1985.
30. Aharony D, Dobson P and Krell RD, A radiochemical assay for detection of leukotriene $B_4$ production from isolated cells. *J Pharmacol Methods* **11**: 125–135, 1984.
31. Carey F and Forder RA, Radioimmunoassay of $LTB_4$ and 6-*trans*-$LTB_4$: Analytical and pharmacological characterization of immunoreactive $LTB_4$ in ionophore stimulated human blood. *Prostaglandins Leuko Med* **22**: 57–70, 1986.
32. Siraganian R, Refinements in the automated fluorometric histamine analysis system. *J Immunol Methods* **7**: 283–290, 1975.
33. Aharony D and Stein RL, Kinetic mechanisms of inhibition of 5-lipoxygenase. *Fed Proc* **46**: 1971, 1987.
34. Aharony D, Redkar-Brown DG, Hubbs SJ and Stein RL, Kinetic studies on the inactivation of 5-lipoxygenase by 5(*S*)-hydroperoxyeicosatetraenoic acid. *Prostaglandins* **33**: 85–100, 1987.
35. Damiano MA and Barbieri EJ, The release of slow reacting substance of anaphylaxis from guinea-pig lungs: effect of calcium antagonists. *Can J Physiol Pharmacol* **63**: 23–29, 1985.
36. Ashida Y, Saijo T, Kuriki H, Makino H, Terao S and Maki Y, Pharmacologic profile of AA-861, a 5-lipoxygenase inhibitor. *Prostaglandins* **26**: 955–972, 1983.
37. Hogaboom GK, Cook M, Newton JF, Varrichio A, Shorr RGL, Sarau HM and Crooke ST, Purification, characterization and structural properties of a single protein from rat basophilic leukemia (RBL-1) cells possessing 5-lipoxygenase and leukotriene $A_4$ synthetase activities. *Mol Pharmacol* **30**: 510–519, 1986.
38. Chang J, Skorvronek MD, Cherney ML and Lewis AJ, Differential effects of putative lipoxygenase inhibitors on arachidonic acid metabolism in cell-free and intact cell preparations. *Inflammation* **8**: 143–155, 1984.
39. Walker JR, Boot JR, Cox B and Dawson W, Inhibition of the release of slow reacting substance of anaphylaxis by inhibitors of lipoxygenase activity. *J Pharm Pharmacol* **32**: 866–867, 1980.
40. Shoam H and Razin E, BW755C inhibits the 5-lipoxygenase in E-mast cells without affecting degranulation. *Biochim Biophys Acta* **837**: 1–5, 1985.
41. Sung CP, Saunder HL, Krell RD and Chakrin LW, Studies on the mechanism of tachyphylaxis to disodium cromoglycate. *Int Arch Allergy Appl Immunol* **55**: 374–384, 1977.
42. Yamamura H, Taira M, Negi H, Nanbu F, Kohno SW and Ohata K, Effect of AA-861, a selective 5-lipoxygenase inhibitor, on models of allergy in several species. *Jpn J Pharmacol* **47**: 261–271, 1988.