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PRELIMINARY COMMUNICATIONS

INHIBITION OF LEUKOTRIENE BIOSYNTHESIS IN MASTOCYTOMA CELLS BY DIETHYLCARBAMAZINE

W. Rodney Mathews and Robert C. Murphy

Department of Pharmacology,
University of Colorado Medical School,
Denver, CO 80262, U.S.A.

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The recent structure elucidation of slow reacting substance of anaphylaxis (SRS-A) (1) and the recognition of the leukocyte chemotactic properties of the 5,12-dihydroxyeicosatetraenoic acids (2) have suggested an important physiological and pathophysiological role for the leukotriene pathway of arachidonic acid metabolism. A specific inhibitor of the leukotriene pathway would be highly desirable in order to pharmacologically investigate this aspect of the multi-branched arachidonic acid cascade which leads to multiple cyclooxygenase and lipoxygenase products. Some inhibitors have been employed such as eicosatetraynoic acid (3), nordihydroguaiaretic acid (4), and BW733C (5); however, evidence to date suggests that these agents are not specific for the leukotriene branch initiated by the formation of leukotriene A_4 from 5-hydroperoxyeicosatetraenoic acid.

In 1965, Mallen (6) reported the relief of bronchospastic symptoms in patients he was treating for intractable asthma with diethylcarbamazine, an antifilarial agent. Orange et al. (7) found that diethylcarbamazine inhibited the antigen-induced release of SRS-A from rat peritoneal cells as measured by the guinea pig ileum bioassay. Other laboratories have reported similar inhibition of SRS-A production in perfused guinea pig lungs (8) and chopped bovine lungs (9), also using the bioassay. There have been no studies reported on the effect of diethylcarbamazine directly on the biosynthesis of the leukotriene family.

METHODS

Diethylcarbamazine (N,N-diethyl-4-methyl-1-piperazinecarboxamide) was obtained from the Aldrich Chemical Co. (Milwaukee, WI); A23187 from CalBiochem (La Jolla, CA); and prostaglandins $F_{2\alpha}$, 6-keto-$F_{1\alpha}$, E_2, D_2, B_2 and thromboxane B_2 as gifts from Dr. J. Pike, the Upjohn Co. (Kalamazoo, MI). The 5-hydroxyeicosatetraenoic acid was synthesized as previously described (10).

Murine mastocytoma cells, CXBGABMCT-1, were obtained from Litton Bionetics (Bethesda, MD) and carried as an ascites tumor in $CB6F_1$ mice. Cells were harvested from the peritoneal cavity, washed, and suspended in a Ca^{2+}-free incubation buffer (150 mM NaCl, 3.7 mM KCl, 3.0 mM Na_2HPO_4, 3.5 mM KH_2PO_4, 5.6 mM dextrose adjusted to pH 7.0 with 30% NaOH) to 10^7 cells/ml. Aliquots of the cell suspension (20 ml) were incubated with diethylcarbamazine (5-250 μM final concentration) for 15 min; A23187 (2 mg/ml ethanol) was then added to a final concentration of 10 μg/ml and incubation was continued for 15 min.

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Maximum stimulation of the cells was obtained by the addition of Ca^{2+} to a final concentration of 1.9 mM and incubation with gentle shaking maintained for 20 min. The reaction was then stopped by centrifugation at 400 g.

Leukotriene C_4, leukotriene B_4, and the other 5,12-dihydroyeicosatetraenoic acids were quantitatively analyzed by a high pressure liquid chromatographic (HPLC) assay as previously described (11), using prostaglandin B_2 as an internal standard.

Prostaglandin and 5-hydroxyeicosatetraenoic acid (5-HETE) production in mastocytoma cells was estimated using a radioactive arachidonic acid tracer. Cells (30 ml, 10^7 cells/ml) were incubated for 45 min in the presence of 50 μ Ci [^{3}H] -arachidonic acid (90 Ci/mmole; Amersham Corp., Arlington Heights, IL) and 1.9 mM Ca^{2+}. The cells were then centrifuged and resuspended in 30 ml of fresh calcium-free buffer, divided into six 5-ml aliquots and incubated for 15 min in the presence or absence of diethylcarbamazine (5 μM). After addition of A23817 and Ca^{2+} in the sequence described above, the reaction was stopped by addition of 1 vol. of chloroform. Eicosanoid carriers (10 μg each: $PGF_{2\alpha}$, PGE_2, 6-keto-$PGF_{1\alpha}$, TxB_2, PGD_2 and 5-HETE) were added and the arachidonic acid metabolites were extracted by the addition of 1 vol. of 1% formic acid and 5 vol. of chloroform. The extract was esterified with ethereal diazomethane, dissolved in methanol, and subjected to thin-layer chromatography (TLC)(12). The plates were visualized with phosphomolybdic acid and the metabolites were scraped from the TLC plates directly into scintillation vials. The internal standard method was used to determine counting efficiency.

RESULTS

When CXBGABMCT-1 cells were treated with 5-250 μM diethylcarbamazine, a dose-dependent inhibition of leukotriene C_4 and B_4 production (seen in Fig. 1) was observed as compared to drug-free cell incubations. These results were obtained using an HPLC assay specific for each leukotriene (11). A 5 μM dose of diethylcarbamazine produced a 57% inhibition of LTC_4 production and a 41% inhibition of LTB_4 production. The syntheses of the non-enzymatic isomers of LTB_4, viz. Δ^6-trans-LTB_4 and Δ^6-trans-12-epi-LTB_4, were likewise inhibited. The dose-response curves for the inhibition of these leukotrienes were essentially parallel in the dose range used.

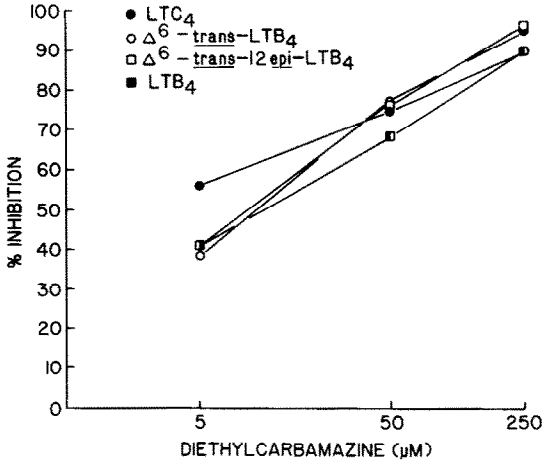

Fig. 1. Dose-related inhibition of leukotriene biosynthesis by diethylcarbamazine in mastocytoma cells stimulated by calcium ionophore.

The effects of diethylcarbamazine on other arachidonic acid metabolites were investigated using radioactively labeled arachidonic acid that was incorporated into the mastocytoma cell arachidonic acid pools by prelabeling. The only significant radioactive cyclooxygenase product was prostaglandin D_2. This result is in agreement with the report indicating that PGD_2 is the major prostaglandin produced by the rat peritoneal mast cell (13). As seen in Table 1, the radioactivity associated with the PGD_2 increased 17% in response to 5 μM diethylcarbamazine as compared to controls.

5-Hydroxyeicosatetraenoic acid was the major mono-hydroxy arachidonic acid metabolite produced by calcium ionophore stimulation of these cells. Since 5-HETE is formed at an important branch point on the leukotriene pathway, its production was also estimated from radioactive arachidonic acid. As shown in Table 1, diethylcarbamazine produced a 27% increase in 5-HETE at a dose (5 μM) that inhibited LTC_4 production by 57%.

TABLE 1. Effect of metabolism of [^{3}H] arachidonic acid stimulated by the calcium ionophore A23187*

Diethylcarbamazine (μM)	PGD_2 †	5-HETE
0	1.04 (3)	1.18 (2)
5	1.25 (2)	1.62 (2)

* CXBGABMCT-1 cells, 5 ml prelabeled with [^{3}H]-arachidonic acid, were treated for 30 min with 5 μM diethylcarbamazine prior to stimulation. The numbers in parentheses equal the number of determinations.

† Amounts are reported as the mean percent of the total dpm applied to the TLC plate.

DISCUSSION

Diethylcarbamazine was shown to inhibit leukotriene biosynthesis in a dose-dependent manner in calcium ionophore stimulated mastocytoma cells. In spite of this inhibition, cyclooxygenase as well as lipoxygenase activity was maintained as indicated by the modest increase in PGD_2 and 5-HETE production. The parallel decrease in both LTB_4 and LTC_4 by diethylcarbamazine suggests that the biosynthesis of their common precursor, LTA_4, has been reduced by the drug treatment. Thus, we suggest that diethylcarbamazine inhibits the enzymatic conversion of 5-hydroperoxyeicosatetraenoic acid (5-HPETE) into leukotriene A_4. The resultant buildup of 5-HPETE could result in the observed increase in 5-HETE and PGD_2 production.

Piper and Temple (14) reported that diethylcarbamazine caused an inhibition of SRS-A production, as measured by bioassay, while production of $PGF_{2\alpha}$ was increased slightly in the antigen stimulated chopped guinea pig lung preparation. These results are in qualitative agreement with this study; however, it was found that a much higher dose was required in the chopped lung preparation in order to obtain the same degree of inhibition seen here. A dose of 250 μM produced only a 20% decrease in SRS-A release while we found

that a 5 μM concentration of drug inhibited leukotriene production approximately 50%. Several differences could possibly explain this discrepancy including possible metabolism and distribution of diethylcarbamazine at the target site, and differences in the assays used to measure SRS-A and leukotrienes. Additionally, the ionophore stimulation procedure is known to differ biochemically from the antigen mediated stimulation.

The inhibition of leukotriene production by diethylcarbamazine provides a useful pharmacological tool to investigate the metabolism of arachidonic acid, where both prostaglandins and leukotrienes are believed to play a physiological role. There is reasonable evidence to suggest that a fair degree of biochemical interplay exists between these two pathways. When used in conjunction with a cyclooxygenase inhibitor such as aspirin or indomethacin to block prostaglandin production, one could separate the direct pharmacologic actions of the prostaglandins from those of the leukotrienes which is a difficult task at the present time.

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REFERENCES

1. R.C. Murphy, S. Hammarstrom and B. Samuelsson, Proc. natn. Acad. Sci. U.S.A. 76, 4275 (1979).

2. A.W. Ford-Hutchinson, M.A. Bray, M.B. Doig, M.E. Shipley and M.J.H. Smith, Nature, Lond. 286, 264 (1980).

3. P. Borgeat, M. Hamberg and B. Samuelsson, J. biol. Chem. 251, 7816 (1977).

4. G.M. Bokoch and P.W. Reed, J. biol. Chem. 256, 4156 (1981).

5. G.A. Higgs, R.J. Flower and J.R. Vane, Biochem. Pharmac. 28, 1959 (1979).

6. M.S. Mallen, Ann. Allergy 23, 534 (1965).

7. R.P. Orange, M.D. Valentine and K.F. Austen, Proc. Soc. exp.Biol. Med. 127, 127 (1968).

8. J.F. Burka and P. Eyre, Int. Archs Allergy appl. Immun. 49, 774 (1975).

9. D.M. Engineer, U. Neiderhauser, P.J. Piper and P. Siros, Br. J. Pharmac. 62, 61 (1978).

10. E.J. Corey and S-I. Hashimoto, Tetrahedron Lett. 22, 299 (1981).

11. W.R. Mathews and R.C. Murphy, Analyt. Biochem. 118, 96 (1981).

12. P. Borgeat and B. Samuelsson, J. biol. Chem. 254, 7865 (1979).

13. L.J. Roberts, R.A. Lewis, J.A. Lawson, B.J. Sweetman, K.F. Austen and J.A. Oates, Fedn Proc. 15, 717 (1978).

14. P.J. Piper and D.M. Temple, J. Pharm. Pharmac. 33, 384 (1981).