THE INHIBITORY EFFECTS OF BW 755C ON ARACHIDONIC ACID METABOLISM IN HUMAN POLYMORPHONUCLEAR LEUKOCYTES

Olof RÅDMARK, Curt MALMSTEN and Bengt SAMUELSSON
Department of Chemistry, Karolinska Institutet, S-104 01 Stockholm, Sweden

Received 19 November 1979

1. Introduction

Polymorphonuclear leukocytes (PMN) of human peripheral blood have recently been shown to synthesize the lipoxygenase products 5-hydroxy-eicosatetraenoic (5-OH-C20:4), 15-hydroxyeicosatetraenoic acid (15-OH-C20:4) and 5,12-dihydroxy-tetraenoic acid (5,12-diOH-C20:4) when incubated with arachidonic acid and ionophore A23187 [1] (fig.1). The formation of prostaglandins [2] and thromboxanes [3] under similar conditions has been reported. Human PMN have also been reported to produce slow reacting substance of anaphylaxis (SRS-A) when incubated with ionophore A23187 and cystein [4].

SRS activity produced in mouse mastocytomas

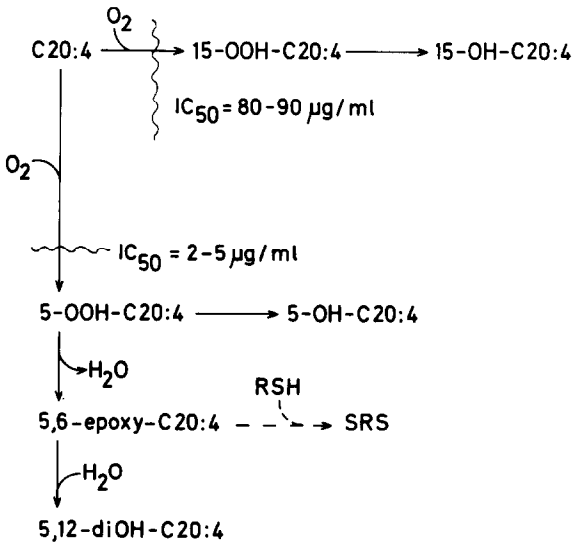

Fig.1. Lipoxygenase pathways of arachidonate in human PMN. Dotted arrow tentative.

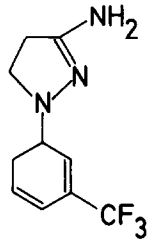

Fig.2. 3-Amino-1-(*m*-(trifluoromethyl)-phenyl)-2-pyrazoline (BW-755C).

was shown to have a structure which indicated that it might be formed from arachidonate via an epoxide intermediate (fig.1) [5,6]. These findings made it of interest to investigate the effect of a combined cyclo-oxygenase and lipoxygenase inhibitor on the formation of SRS-A in human PMN.

The compound used was BW-755C (3-amino-1-(*m*-(trifluoromethyl)-phenyl)-2-pyrazoline) (fig.2) which was reported to be an anti-inflammatory compound that inhibits cyclo-oxygenase as well as lipoxygenase in rat lung [7] and in horse platelets [8].

Thus, we have incubated human PMN with arachidonic acid and ionophore A23187, in the presence of BW-755 at different concentrations. The effects on the biosynthesis of the various metabolites have been investigated, using HPLC and bioassay techniques.

2. Materials and methods

2.1. *Materials*

BW-755C was kindly provided by Dr F. Kuehl, Merck Inst. for Therapeutic Res., MI. Ionophore A23187 was a kind gift from Dr R. L. Hamill, Eli

Lilly Res. Labs., IN and FPL 55712 was a kind gift from Fisons Ltd., Pharmaceut. Div., Loughborough. Arachidonic acid was purchased from Nu-Chek-Prep., MN and indomethacin from Sigma Chem. Co. MO.

2.2. *Cell preparation*

Human PMN were obtained from healthy donors who had not taken any drugs for the last 10 days. The cell suspension was prepared in accordance with [1]. Briefly, the PMN were prepared by centrifugation to remove platelet-rich plasma, dextran sedimentation to separate white cells from red cells, ammonium-chloride lysis to remove traces of erythrocytes and finally gradient centrifugation in Lymphoprep to separate PMN from other white cells. The resultant cell suspension contained mainly polymorphonuclear and eosinopholic granulocytes.

For incubation the cells were suspended in Dulbecco's PBS buffer (pH 7.4) ($3.0–3.5 \times 10^7$ cells/ml).

2.3. *Incubations*

PMN were preincubated for 2 min with or without BW-755C. All samples were also preincubated with indomethacin 5×10^{-5} M to inhibit the cyclo-oxygenase pathway. Arachidonic acid (1.5×10^{-4} M) was added simultaneously with ionophore A23187 (5×10^{-6} M). All substances added were dissolved in ethanol, the volume of which was $\leqslant 1\%$ of the total incubation mixture. Incubation times were 45 s to 10 min for samples subjected to SRS-bioassay, and 4–10 min for those analysed for hydroxy-acids.

2.4. *SRS bioassay*

For SRS assay 1.5 ml aliquots of incubation mixture were filtered (Whatman GF/A) to remove whole cells and 0.5 ml of the filtrate was immediately added to a guinea-pig ileum strip bathed in a 7 ml cuvette with Tyrode's solution containing atropine (10^{-6} M) and pyrilamine maleate (10^{-6} M). Contractions were measured using a Grass force-displacement transducer with a Grass Polygraph 79 C. To measure the inhibitory effect of a given concentration of BW-755C, the average of the contraction amplitudes of two noninhibited incubations, added before and after the sample containing BW-755C, were taken as 100%. The amplitude of the incubation with inhibitor was expressed as a percentage of this mean.

2.5. *Analysis of 5-OH, 15-OH and 5,12-diOH-C20:4*

The incubation was stopped with 1.5 vol. methanol containing 5 μg PGB_2, which was used as an internal standard through the purification. Lipids were extracted with diethyl-ether [1] and separated on a silicic acid column (1 g SiO_2; Mallinkrodt Silicar, CC-4). The column was eluted with 30 ml hexane/diethyl ether, 80/20 (v/v) followed by 30 ml ethylacetate. The ethylacetate eluate was evaporated and dissolved in methanol. Fractions of these samples were injected onto a high-pressure liquid chromatograph (HPLC), equipped with a reversed phase column (Nucleosil C_{18}, 5 μm-spherical, Macherey-Nagel Comp., Düren). The column was eluted with methanol/water/acetic acid, 75/25/0.01 (v/v/v). The ultraviolet detector was set at 232 nm for monohydroxy acids and at 280 nm for dihydroxy acids and PGB_2. The HPLC procedure was performed in accordance with [1] where the identities of the peaks were determined.

Metabolites were quantitated from the ratio of the corresponding HPLC peak areas, to the PGB_2 internal standard.

3. Results

The compound BW-755C inhibited formation of 5-OH-C20:4 and 5,12-diOH-C20:4 in human PMN incubated with arachidonic acid and ionophore A23187 with an IC_{50} of 2–5 μg/ml. Formation of 15-OH-C20:4 was inhibited with an IC_{50} of 80–90 μg/ml. At <50 μg BW-755C/ml the formation of 15-OH-C20:4 was increased, when compared to standard.

Human PMN incubated with ionophore and arachidonic acid also produced SRS activity (cells from most donors produced significant amounts of SRS when incubated with only arachidonic acid, but the quantities of SRS were in all cases markedly increased when ionophore was added simultaneously). Also the SRS formation was inhibited by BW-755C (IC_{50} = 4–20 μg/ml) (fig.3). BW-755C itself did not inhibit contractions of the gut when given simultaneously with SRS to the ileum strip, which indicates that the compound did not exert its effect at the target-organ level. A corresponding amount of ionophore given directly to the gut gave a contraction about one tenth of the one produced by the incubation mixture. It also had a totally different character, and was not inhibited by FPL 55712 [9].

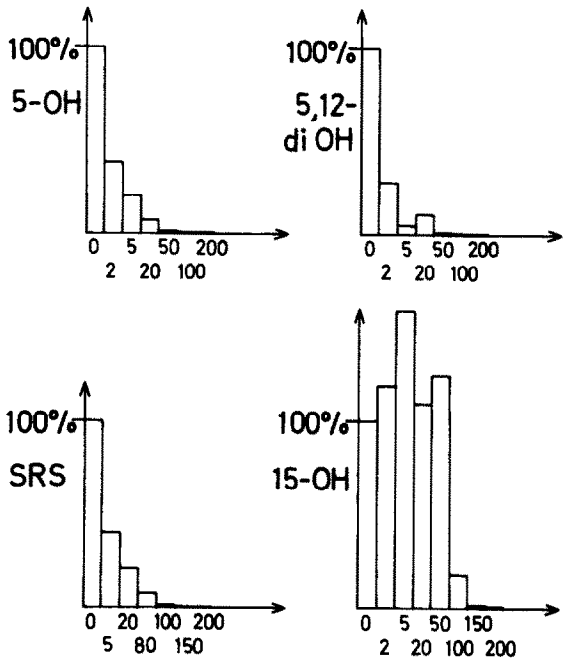

Fig.3. Diagrams showing the effects of BW-755C on the lipoxygenase products in human PMN. Abscissa values are μg BW-755C/ml.

Evidence that the contractions of the guinea pig ileum were caused by SRS was suggested by the insensitivity to histamine and acetylcholine antagonists, by the characteristic time-course of contractions produced, and by the antagonistic effect on the contraction by the SRS-A antagonist FPL 55712 [9]. Prostaglandin and thromboxane formation in the PMN, were inhibited by indomethacin at concentrations that did not affect formation of the hydroxy-compounds investigated.

4. Discussion

Polymorphonuclear leukocytes have a number of functions including generation of SRS. Cyclo-oxygenase and lipoxygenase metabolites of arachidonic acid and other unsaturated fatty acids are thought to have a role in the inflammatory response, as mediators and regulators.

Here we have found that the pyrazoline derivative BW-755C inhibits formation of 5-OH-C20:4, 15-OH-C20:4, 5.12-diOH-C20:4 and SRS in human PMN.

It was shown in [5], that SRS activity obtained from mouse mastocytomas is a metabolite of arachidonic acid. Our finding that SRS formation and oxygenation at C-5 in human PMN, both are inhibited by BW-755C in similar dose–response patterns, indicates that a lipoxygenase product is also involved in the formation of SRS in human cells. Additional studies are required to identify SRS from human leukocytes.

Thus BW-755C might be an important tool for further studies on SRS formation, and the inflammatory response in human leukocytes. The results also indicate that lipoxygenase inhibitors might be of therapeutic value in the treatment of asthma.

Acknowledgements

This study was supported by grants from the Swedish Medical Research Council (project no. 03X-217). We wish to thank Ms Ulla Andersson and Ms Inger Tollman-Hansson for very valuable help and technical assistance.

References

[1] Borgeat, P. and Samuelsson, B. (1979) Proc. Natl. Acad. Sci. USA 76, 2148–2152.
[2] Zurier, R. B. (1976) Adv. Prostaglandin Thromboxane Res. 2, 815–818, Raven, New York.
[3] Goldstein, I. M., Malmsten, C. L., Kindahl, H., Kaplan, H. B., Rådmark, O., Samuelsson, B. and Weissman, G. (1978) J. Exp. Med. 22, 787–792.
[4] Conroy, M. C., Orange, R. P. and Lichtenstein, L. M. (1976) J. Immunol. 116, 1677–1681.
[5] Murphy, R. C., Hammarström, S. and Samuelsson, B. (1979) Proc. Natl. Acad. Sci. USA 76, 4275–4279.
[6] Borgeat, P. and Samuelsson, B. (1979) Proc. Nat. Acad. Sci. USA 76, 3213–3217.
[7] Higgs, G. A., Copp, F. C., Denyer, C. V., Flower, R. J., Tateson, J. E., Vane, J. R. and Walker (1978) Abstr. Proc. VIIth Int. Congr. Pharmacol. no. 843, p. 334, Pergamon Press, Oxford.
[8] Higgs, G. A., Flower, R. J. and Vane, J. R. (1978) Biochem. Pharmacol. 28, 1959–1961.
[9] Augstein, I., Farmer, J. B., Lee, T. B., Sheard, P. and Tattersall, M. L. (1973) Nature New Biol. 245, 215–217.