were used as substrates. These were either self coloured or prepared by colour film coating white tablet cores. The tablets were coated in a 24 inch Accela Cota (Manesty Machines Limited) using a airborne spray system at a spray application rate of 50 ml min^{-1} and inlet air temperature at 60 °C. The film formulation used consisted of a 5% w/v aqueous solution of hydroxypropyl methylcellulose (Pharmacoat 606, Shin-Etsu Chemical Co., Japan) containing glycerol (20% w/w based on polymer) as plasticizer and talc (D. F. Anstead Ltd, Billericay) or precipitated calcium carbonate (J. & E. Sturge Limited, Birmingham) at various concentrations. At the end of each run, the tablets were withdrawn and visually inspected.

With both materials the colour of the main body of the substrate could be clearly seen through the applied film, i.e. the film was essentially transparent. However, within the intagliations the film was opaque and the colour of the substrate was obscured making the intagliations appear white (Fig. 1). The results indicate that, over the main body of the tablet both the talc and the calcium carbonate had orientated equivalent to their state of lowest refractive index, but within the intagliations they were either randomly orientated or orientated equivalent to their state of highest refractive index.

The reasons for orientation can only be a matter for conjecture. The effect is not substrate-dependent since all the substrates used showed the effect. It is possible that orientation will occur under the influence of the residual internal stresses present within the film since these are thought to act parallel to the tablet surface. The mutual rubbing due to tablet-to-tablet contact within the coating drum is another factor to be considered especially since attempts at reproducing the effect on single tablets attached to the inside of the coating drum were unsuccessful.

The results indicate that orientation of both talc and calcium carbonate particles occurs in hydroxypropyl methylcellulose films applied to a variety of tablet substrates. It is possible that other materials will also show this effect but substantiation will be difficult for those materials with very high refractive indices, since in these cases the film will initially be very opaque and differences in opacity due to orientation difficult to detect.

The author wishes to thank Mr S. F. Forse and Mr L. Jessop for assistance in this work.

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J. Pharm. Pharmacol. 1983, 35: 44–45
Communicated August 10, 1982

0022–3573/83/010044–02 $02.50/0

The preferential inhibition of 5-lipoxygenase product formation by benoxaprofen

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Benoxaprofen has been shown to inhibit the formation of 5-lipoxygenase products from rabbit peritoneal cells stimulated with calcium ionophore A23187 (Walker & Dawson 1979). However other workers using isolated soybean lipoxygenase (Kingston 1981) failed to demonstrate any inhibition of lipoxygenase activity using benoxaprofen. We have further studied the effects of the drug on the activities of other lipoxygenases from human platelets, guinea-pig peritoneal cells and the human promyelocytic leukaemic cell line HL60.

Method

Human platelet microsomes were prepared as previously described (Ho et al 1976). The reaction mixture contained 50 mM sodium phosphate buffer pH 7·1, 1 mM EDTA, 0·2 mM indomethacin, 1 mM $CaCl_2$, 0·1% gelatin and 0·4 mg of lyophilized microsome powder in a volume of 0·2 ml. The reaction was initiated by the addition of [1-^{14}C]arachidonic acid (0·5 μCi) (New England Nuclear, Boston, Mass.). After 15 min incubation at 37 °C the reaction was terminated by the addition of 10 μl of 1 M citric acid. Fifty μl of the reaction mixture was applied to a silica gel t.l.c. plate (LQ6D, Quantum Industries, Fairfield, N. Jersey). The plate was developed in the organic phase from ethyl acetate–2,2,4-trimethyl pentane–acetic acid–water (90:50:20:100). The silica gel on the plate was scraped in 1 cm zones, suspended in 5 ml scintillation fluid and the radioactivity determined by liquid scintillation counter. The result was expressed as the ratio of radioactivity recovered from the whole plate.

Guinea-pig peritoneal cells were elicited by an intraperitoneal injection of 2% casein 18 h previously. 1 ml cell suspensions (2·6 × 10^6 cells ml^{-1}) were preincubated in Krebs Ringer Bicarbonate buffer pH 7·4 at 37 °C with different concentrations of benoxaprofen for 5 min. [1-^{14}C]Arachidonic acid (0·1 μCi) and ionophore A23187 (10 μM) were added and the cells incubated for a further 10 min. The incubation was

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Table 1. The effect of benoxaprofen on the lipoxygenase activity of guinea-pig peritoneal cells. These results represent a typical experiment performed in triplicate. These experiments have been repeated at least 4 times with consistent results.

Concn (M)	5-HETE Radioactivity % ± s.e.m.	Redn %	P	5,12-DiHETE Radioactivity % ± s.e.m.	Redn %	P
Control	16·8 ± 0·9			4·9 ± 0·5		
3×10^{-5}	6·5 ± 0·2	61	<0·001	1·7 ± 0·2	65	<0·01
10^{-4}	1·9 ± 0·12	89	<0·001	0·2 ± 0·1	95	<0·001
2×10^{-4}	0·7 ± 0·1	96	<0·001	0·2 ± 0·1	96	<0·001

terminated by acidification to pH 3 with 0·2 M citric acid, diluted and extracted twice with 5 ml ethyl acetate. The ethyl acetate phase was concentrated under a stream of nitrogen and applied to a silica gel t.l.c. plate, and developed at 4 °C in a toluene–dioxane–acetic acid system (66:34:1·5). The t.l.c. plates were scanned by a Berthold radiochromatographic scanner, and the amount of radioactivity within each peak was automatically quantified and expressed as a percentage of the total radioactivity on the plate.

The HL60 cell line was differentiated with dimethyl sulphoxide for 7 days and incubated using the conditions described by Bonser et al (1981) 1 ml cell suspensions (5×10^6 cells ml^{-1}) were preincubated at 37 °C with different concentrations of drug for 5 min. [1-^{14}C]Arachidonic acid (0·1 μCi) and A23187 (10 μM) were added and the cells incubated for a further 5 min. The reaction products were extracted and quantified in a manner similar to those described for the guinea-pig peritoneal cell system.

Results

Whole human platelets incubated with arachidonic acid produce 12-hydroxyeicosatetraenoic acid (12-HETE) (Nugteren 1975). Similarly the microsomal preparation incubated under the conditions used in this study produced a single radiolabelled product which co-chromatographed with 12-HETE. Benoxaprofen at concentrations up to 3×10^{-4} M failed to affect the formation of this radiolabelled product.

Guinea-pig peritoneal cells produce two radiolabelled lipoxygenase products which have been identified by gas chromatography and mass spectrometry (g.c.-m.s.) as 5-HETE (the major product) and 5,12-diHETE. H.p.l.c. analysis revealed a mixture of isomeric 5,12-diHETEs including LTB_4. All of these isomeric products are thought to result from 5-lipoxygenase activity, and depending on the isomeric form are generated from LTA_4 or by a sequential dioxygenation caused by 12- and 5-lipoxygenase. This system did not appear to generate other 12-lipoxygenase products and therefore it was concluded that the 5,12-diHETEs generated within this system had in fact originated from LTA_4. Benoxaprofen ($3 \times 10^{-5} - 2 \times 10^{-4}$ M) caused a dose-dependent inhibition of both 5-HETE and 5,12-diHETE formation (Table 1).

Similarly, differentiated HL60 cells also produced

Table 2. The effect of benoxaprofen on the lipoxygenase activity of HL60 cells. These results represent a typical experiment performed in triplicate. These experiments have been repeated at least 4 times with consistent results.

Concn (M)	5-HETE Radioactivity % ± s.e.m.	Redn %	P	5,12-DiHETE Radioactivity % ± s.e.m.	Redn %	P
Control	12·9 ± 0·6			5·8 ± 0·4		
3×10^{-5}	6·6 ± 0·5	48	<0·001	3·8 ± 0·1	<0·02	34
10^{-4}	2·0 ± 0·12	84	<0·001	1·0 ± 0·2	<0·001	82
2×10^{-4}	0·6 ± 0·1	95	<0·001	0·3 ± 0·1	<0·001	94

two major radiolabelled products. These products were identified by g.c.-m.s. as 5-HETE (the major product) and 5,12-diHETE. Benoxaprofen at concentrations similar to those used in the guinea-pig study caused a similar dose-dependent inhibition of both products (Table 2).

Indomethacin ($10^{-8} - 10^{-6}$ M) caused a significant but concentration unrelated potentiation ($P < 0·05$–$0·01$) in the formation of these lipoxygenase products in both the guinea-pig peritoneal cell and HL60 cell systems.

Benoxaprofen even at high concentrations failed to inhibit the formation of 12-lipoxygenase products from human platelets yet clearly inhibited the formation of 5-lipoxygenase products from both guinea-pig peritoneal cells and HL60 cells at lower concentrations. Previous findings have indicated that benoxaprofen inhibited the formation of 5-lipoxygenase products from rabbit peritoneal cells but failed to inhibit the 15-lipoxygenase activity of soybean. These collective results seem to indicate that benoxaprofen has an unusual and selective effect on lipoxygenase activity. It inhibits the 5-lipoxygenase activity of different cell systems but fails to inhibit the activity of other positional lipoxygenases.

The inhibition of 5-lipoxygenase activity by benoxaprofen is not confined to cells stimulated with ionophore as we have demonstrated that benoxaprofen can reduce the release of leukotrienes from anaphylactic guinea-pig and human lungs (Boot et al 1982).

The results indicate that benoxaprofen can reduce the formation of 5-lipoxygenase products, which possess pronounced biological activity, without affecting the formation of other positional lipoxygenase products which may have a physiological or homeostatic role.

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