We conclude that triamcinolone acetonide palmitate included in liposomes provides a stable carrier system for the efficient treatment of chronic arthritis. This system will have the advantages of intra-articular injection together with an increased efficiency with respect to the use of the free corticoid.

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Conversion of flosequinan to the sulphone metabolite in subcellular fractions of human liver, in-vitro

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Abstract—Flosequinan was metabolized in a NADPH-dependent reaction, by *S*-oxidation to its principal metabolite, the sulphone. Most of the sulphone metabolite was formed within 20 min and reached a plateau by 60 min, following incubations of flosequinan (31, 61 or 122 μM) with the microsomal fraction. Flosequinan was metabolized mainly by the microsomal fraction. The *S*-oxidation of flosequinan in incubations containing mitochondrial and cytosolic fractions was 13 and 5%, respectively, of that detected in the microsomal fraction. The route of metabolism of flosequinan in-vitro correlates closely with that previously observed in-vivo. This model could be useful for studying the potential effects of other drugs on flosequinan metabolism in-vitro.

Flosequinan (7-fluoro-1-methyl-3-methylsulphinyl-4-quinolone) is a new cardiovascular agent which has vasodilatory effects on both arterial and venous vascular beds (Cowley et al 1984). Flosequinan has been shown to be metabolized in man in-vivo by *S*-oxidation to its principal phase I metabolite, flosequinan sulphone (Fig. 1), which is also haemodynamically active (Wynne et al 1985). The aim of the present study was to evaluate the metabolism of flosequinan in-vitro in human liver microsomes and to compare this with in-vivo findings. The extent and route of metabolism of flosequinan in other subcellular fractions were also investigated.

Materials and methods

Chemicals and reagents. Flosequinan (BTS 49 465), the sulphone metabolite (BTS 53 554) and the internal standard for HPLC analysis (BTS 49 037, Fig. 1) were provided by Boots Pharmaceuticals, Nottingham, UK. Reduced nicotinamide adenine dinucleotide phosphate (NADPH) was purchased from Sigma Chemicals, UK. All other chemicals and reagents used were of HPLC or reagent grade.

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Tissue preparation and incubation. Human livers were obtained up to 4 h after death due to cerebral haemorrhage from six donors (four males, two females, age range 32–66 years) with normal liver function. Liver samples were stored at −80°C. Liver homogenates were prepared at 4°C with 4 vols of 0·1 M phosphate buffer, pH 7·4, using a glass–Teflon homogenizer. The mitochondrial fraction was prepared by centrifuging the homogenate at 4°C for 10 min at 700 *g*, followed by centrifugation of the resulting supernatant at 4°C for 15 min at 12 500 *g*, using a Sorval RC-5B (Du Pont) centrifuge. The pellet was washed three times by adding 5 vols of buffer, resuspending and centrifuging at 12 500 *g*. The microsomal and the cytosolic fractions were prepared from the same homogenates by differential centrifugation (Kamali et al 1988), using a Sorval ODT 65B (Du Pont) ultracentrifuge. The protein content of the microsomal fractions was determined according to an established method (Lowry et al 1951).

The time course for the metabolism of flosequinan in the microsomal fraction was assessed by incubating the drug at 37°C, in separate tubes in duplicate, in air at times: 0, 5, 10, 15, 20, 30, 40 and 60 min. The incubation mixture contained in 1 mL (final volume): phosphate buffer, pH 7·4 (6–9 mM), microsomal protein (1 mg mL^{-1}), NADPH (1 mM) and flosequinan (31, 61 or 122 μM). Microsomes were also incubated with flosequinan (122

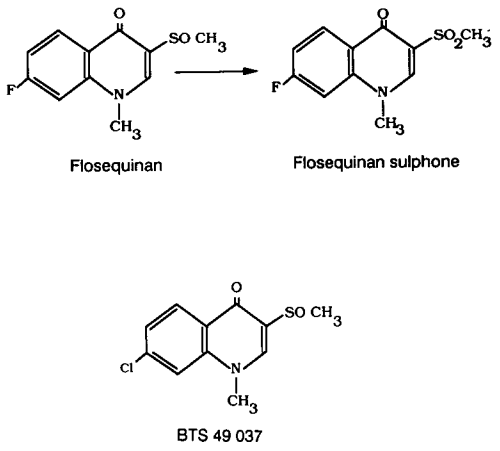

FIG. 1. Chemical structures of flosequinan, flosequinan sulphone and internal standard (BTS 49 037).

μM) at 37°C for 60 min, under an atmosphere of nitrogen. The effect of carbon monoxide on sulphoxidation of flosequinan was also investigated by bubbling carbon monoxide into microsomes for 30 s, in the presence of NADPH, followed by incubation with flosequinan (122 μM) for 60 min.

The ability of other liver subcellular fractions (mitochondria and cytosol) to metabolize flosequinan was investigated by incubating the fractions (equivalent to 300 mg wet tissue) with flosequinan (122 μM) for 30 min.

Sample preparation and analysis. Termination of the reaction and sample extraction were carried out simultaneously, by mixing 100 μL of the incubation mixture with dichloromethane (3 mL) followed by shaking for 10 min. The internal standard (200 μL of a 100 μg mL^{-1} solution) was also added at this stage. The organic layer was separated after centrifugation at 2500 *g* for 5 min and a sample (2·5 mL) evaporated to dryness under an atmosphere of nitrogen at 35°C. Samples were stored at −20°C until analysis by HPLC (Underwood & Hind 1989). Later, samples were reconstituted in 250 μL of the mobile phase and 100 μL was injected onto a C18 reverse phase 25 cm × 0·4 cm (5 μm particle size) column at 40°C. The mobile phase was water:methanol:acetonitrile (65:20:15, v/v/v) at a flow rate of 1·5 mL min^{-1}. Flosequinan, flosequinan sulphone and the internal standard (BTS 49 037) were detected at 254 nm. The intra-assay coefficient of variation for flosequinan and flosequinan sulphone was less than 4%. The limit of detection was 0·05 μg mL^{-1} for both flosequinan and flosequinan sulphone.

Results

Analysis of the extracts by HPLC resulted in two peaks which had the same retention times as those of the authentic reference standards of flosequinan (3·86 min) and flosequinan sulphone (2·84 min). The time course of oxidation of flosequinan to its sulphone metabolite and flosequinan recoveries, at three different concentrations of flosequinan in liver microsomes is shown in Fig. 2. Most of the sulphone metabolite was formed within 20 min and reached a plateau by 60 min, following incubation at 37°C. Flosequinan oxidation measured after 30 min incubation was linear with substrate concentration (Fig. 3) over the concentration range studied (31–122 μM).

Compared with the microsomal fraction, the *S*-oxidation of flosequinan was markedly lower in the mitochondrial and the cytosolic fractions (Table 1). No flosequinan sulphone was detected in the extracts from either the mitochondrial or the microsomal fractions in the absence of NADPH, suggesting that flosequinan metabolism is an NADPH-dependent process. However, the cytosolic fraction was capable of metabolizing flosequinan without added NADPH to a limited extent (Table 1).

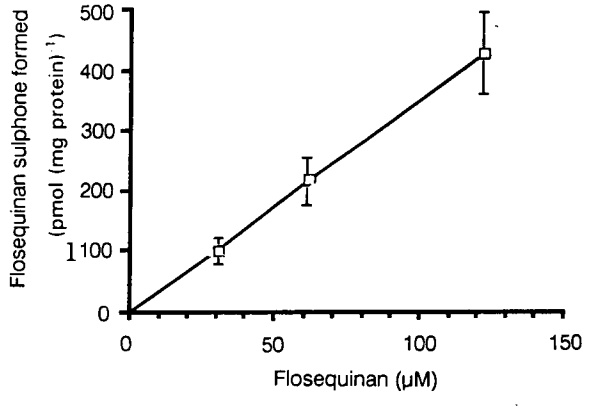

FIG. 3. Dependence of the generation of flosequinan sulphone by human liver microsomes on substrate concentration. Flosequinan was incubated with microsomes under air for 30 min. Values are the mean ± s.e. of six experiments.

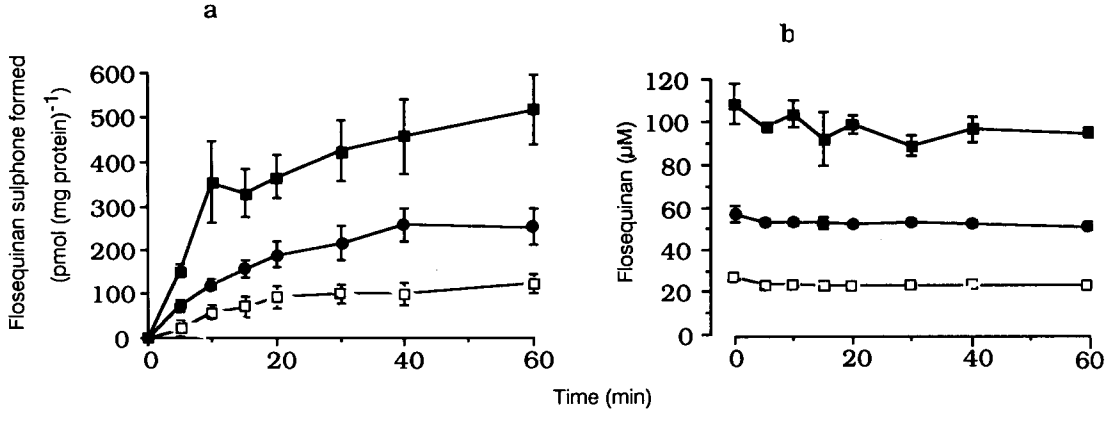

FIG. 2. Time-course of generation of the sulphone metabolite (a) and the disappearance of flosequinan (b) during incubation of flosequinan with human liver microsomes under air. □ 31 μM Flosequinan; ● 61 μM flosequinan, ■ 122 μM flosequinan. Values are the mean ± s.e. of six experiments.

Table 1. Formation of flosequinan sulphone by liver subcellular fractions.

Liver fraction	Flosequinan sulphone formed (mmol (g liver)$^{-1}$)
Microsomes	10·36 ± 1·7
Mitochondria	1·36 ± 0·34
Cytosol	0·59 ± 0·07
[a]Cytosol	0·48 ± 0·26

[a]Flosequinan sulphone formed in the absence of NADPH. The values are the mean ± s.e. of three experiments.

On incubation of flosequinan with microsomes under an atmosphere of nitrogen, no sulphone metabolite was detected. The oxidation of flosequinan to its sulphone metabolite was arrested when samples were pre-treated with carbon monoxide for 30 s before incubation.

Discussion

The present study investigated the metabolism of flosequinan in human liver in-vitro. Flosequinan was metabolized in a NADPH-dependent reaction by *S*-oxidation to its principal metabolite, the sulphone. The microsomal fraction was the major site for the metabolism of flosequinan. The mitochondrial and cytosolic fractions were also capable of metabolizing flosequinan, albeit to a much smaller extent. The cytosolic fraction metabolized flosequinan in the absence of added NADPH. This may have been due to the limited amount of endogenous NADPH present in the cytosolic fraction. Under aerobic conditions, the formation of sulphone metabolite by the microsomal fraction increased with increasing concentrations of flosequinan. Flosequinan oxidation measured by HPLC after 30 min incubation was linear with the substrate concentration range studied (see Results). However, no sulphone metabolite was detected when flosequinan was incubated with microsomes under an atmosphere of nitrogen. This suggests that *S*-oxidation of flosequinan in microsomes is mediated via the oxygen requiring P450 mixed-function oxidase system. This was confirmed when carbon monoxide was shown to inhibit the oxidation of flosequinan to its sulphone metabolite.

The metabolism of flosequinan by human liver microsomes in-vitro reflects that found in man in-vivo (Wynne et al 1985). The results of the present study indicate that the route of metabolism of flosequinan in the in-vitro model of human liver microsomes is a good predictor of the metabolism of flosequinan in-vivo. This model could be useful for studying the potential effects of other drugs on flosequinan metabolism in-vitro and function as an indicator for possible interactions which may be clinically significant.

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