SULPHINPYRAZONE METABOLISM DURING LONG-TERM THERAPY

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- 1 The plasma concentrations of sulphinpyrazone and four of its metabolites are reported together with the amounts excreted in urine. Eight insulin-requiring diabetics were investigated, all treated with sulphinpyrazone 600–800 mg day⁻¹ for 2.5 years or more.
- 2 Blood samples were drawn before the first morning dose and 2 h later. The mean plasma concentrations were ($t = 0$ h – $t = 2$ h): sulphinpyrazone 7.1–16.0 $\mu\text{g ml}^{-1}$; sulphide 2.8–4.3 $\mu\text{g ml}^{-1}$; sulphone 1.7–4.8 $\mu\text{g ml}^{-1}$; *p*-OH-sulphide 0.67–0.89 $\mu\text{g ml}^{-1}$; *p*-OH-sulphinpyrazone 0.10–0.16 $\mu\text{g ml}^{-1}$. Statistically significant correlations were found between the plasma concentrations at $t = 0$ of the sulphide and the *p*-OH-sulphide and that of sulphinpyrazone.
- 3 In urine, a very wide range in excretion of unconjugated compounds was observed. Sulphinpyrazone were excreted in amounts corresponding to 1–30% of the daily dose. The metabolites were generally excreted in amounts corresponding to less than 1% of the daily dose; however, up to 3% was found as the sulphone.
- 4 Increases of the concentrations of all compounds in urine were found after treatment with β -glucuronidase indicating O-conjugation with glucuronic acid.
- 5 Since both the sulphide and the sulphone were found more active as inhibitors of platelet function *in vitro* than their parent compound, they may together constitute the major part of the platelet inhibitory drug activity in plasma during long-term therapy with sulphinpyrazone.

Introduction

During the past few years, sulphinpyrazone has been extensively investigated for its use in prophylaxis and therapy of thromboembolic disorders. Recently, the drug has been shown significantly to reduce the incidence of sudden death during the first half year after acute myocardial infarction (Anturane Reinfarction Trial Research Group, 1980). *In vitro*, sulphinpyrazone inhibits platelet cyclooxygenase thus inhibiting platelet thromboxane A₂ synthesis (Ali & McDonald, 1977). Vessel wall prostacyclin synthesis is left relatively intact (Westwick, Webb & Lewis, 1979a). The clinical effects of the drug are probably related to these actions, although other mechanisms have been proposed (Braumwald, 1980).

Attention was drawn to the metabolism of sulphinpyrazone by the finding of a prolonged and biphasic effect of sulphinpyrazone in the rabbit (Buchanan, Rosenfeld & Hirsh, 1978). This effect was described as an immediate concentration-related platelet suppressive action followed by a late and more pronounced depression of platelet function with a maximum about 18 h after administration. Since the late depression was found after sulphinpyrazone had disappeared from plasma it was suggested that this was due to an as yet unknown but slowly eliminated and

very potent metabolite. The biphasic pattern was later reported in guinea-pigs (Butler, Pay, Wallis & White, 1979) and in humans (Maguire, Pay, Turney, Wallis, Weston, White, Williams & Wood, 1979). Recently, a reductive metabolic pathway of sulphinpyrazone in the rabbit was demonstrated (Kirstein Pedersen & Jakobsen, 1979). Two metabolites, a sulphide and a *p*-OH-sulphide, were present in plasma with maximum concentrations corresponding to the late secondary depression of platelet function. The sulphide metabolite was *in vitro* at least 3 times as potent, and the *p*-OH-sulphide at least as potent as sulphinpyrazone in inhibition of collagen-induced platelet aggregation. With this in mind, it was obviously necessary to reinvestigate the metabolism of sulphinpyrazone in humans, since the metabolites probably contribute to the clinical effects of the drug. No data were available concerning the plasma levels of sulphinpyrazone or its metabolites during long-term therapy. This paper reports plasma and urine concentrations of sulphinpyrazone and its metabolites in a group of diabetic patients on chronic oral sulphinpyrazone therapy. A scheme of the possible metabolic pathways of sulphinpyrazone in man are shown in Figure 1.

Figure 1 Possible metabolic pathways of sulphinpyrazone in man.

Methods

Eight patients (three male and five female) with insulin-requiring diabetes (age 35–67 years) were studied. Hepatic and renal function (as judged by serum creatinine levels and liver-specific enzyme levels) were normal. Three patients received other drugs (frusemide, propranolol, atenolol and antacids). The duration of sulphinpyrazone therapy was in all patients 2.5–3 years. The dose given was 200 mg four times daily (one patient 200 mg three times daily). Heparinized blood samples (10 ml) were drawn at 08.00 h (about 10 h after the previous dose) immediately before ingestion of the next 200 mg tablet ($t=0$), and again 2 h later ($t=2$). Samples from 24 h urine collections obtained the previous day were kept at -20°C together with the plasma samples until analysis.

The relative *in vitro* activity of all compounds were assessed by their ability to delay arachidonic acid

(2 mM) induced aggregation of human platelets. Platelet rich citrated plasma was incubated for 15 min with different concentrations of the compounds and the time required for 50% of maximum aggregation after challenge with arachidonic acid was measured on a Payton® aggregometer.

Determination of sulphinpyrazone and the well known metabolites the sulphone and the *p*-OH-sulphinpyrazone was carried out as previously described (Jakobsen & Kirstein Pedersen, 1979). The compounds were extracted from acidified plasma with dichloromethane, methylated and gas chromatographed on 2% OV-17 (100 cm). The new metabolites (S and SOH) were identified, and quantitated as described in details elsewhere (Jakobsen & Kirstein Pedersen, 1981). In brief, methylated extracts from patient plasma samples were analysed on a Jeol D-100 GC-MS instrument. Mass spectra at 24 eV and retention times on 3% OV-1 (100 cm) of the new metabolites S and SOH were identical to

those obtained from authentic samples (G 25671 and G 33378 obtained from Ciba-Geigy, Basel). Quantitation of the methylated compounds was carried out by GC and selective ion monitoring of their base peaks ($m/z = 266$ for S and 296 for SOH). As internal standard butylated G 25671 (S) (base peak: $m/z = 308$) was used. Incubation of the urine samples with β -glucuronidase (Sigma S g-0251) 2000 u/ml was performed at pH 5 for 24 h at 37°C .

Results

The amounts of sulphinpyrazone and four metabolites excreted by each patient are shown in Figure 2. The plasma levels of all compounds are listed in table 1 together with the patient data.

Figure 3 shows the relationship between the plasma concentrations of sulphinpyrazone and two of the

metabolites, the sulphide and the *p*-OH-sulphide at $t = 0$. Statistically significant positive correlations were found ($r = 0.888$, $P < 0.005$ and $r = 0.864$, $P < 0.005$, respectively). There was no correlation between the plasma concentrations of the sulphone or of *p*-OH-sulphinpyrazone and the concentrations of sulphinpyrazone.

Figure 4 shows the log dose-response curves for sulphinpyrazone and its four metabolites as inhibitors of platelet aggregation.

Discussion

The metabolism of sulphinpyrazone in man after single dose administration has been investigated by Dayton, Sicam, Landrau & Burns (1961) and Dieterle, Faigle, Mory, Richter & Theobald (1975). These investigators recovered more than 95% of a

Figure 2 The excreted amounts of sulphinpyrazone and four of its metabolites in urine. The white columns indicate the amounts of unconjugated compounds, and the dark columns indicate the amounts liberated after β -glucuronidase treatment of the urine. (SO₂ = the sulphone, S = the sulphide, S-OH = the *p*-OH-sulphide).

Figure 3 The correlation between the plasma concentration of the sulphide (●), the *p*-OH-sulphide (○) and sulphinpyrazone at 08.00 h.

Figure 4 The influence of sulphinpyrazone and its metabolites on the time required for 50% aggregation of human platelets after incubation with 2 mM arachidonic acid. Each point is the mean of 3–6 determinations. (○) = the sulphide, (▽) = the sulphone, (□) = the *p*-OH-sulphide, (●) = sulphinpyrazone and (△) = the *p*-OH-sulphinpyrazone.

single oral 200 mg dose of [14 C]-sulphinpyrazone in urine and identified two oxidative metabolites: a sulphone (3%) and *p*-hydroxylated sulphinpyrazone (9%). The highest plasma levels of the sulphone and of *p*-hydroxy-sulphinpyrazone were only about 1/20 of that of sulphinpyrazone. A major metabolic pathway appeared to be C-glucuronidation of sulphinpyrazone (28%) and of the sulphone (2%), while more than 50% was excreted unchanged. C-

glucuronidation involves covalent binding of glucuronic acid to a carbon atom in the central dioxo-pyrazolidine ring structure. O-glucuronides were not found.

The demonstration of a reductive metabolic pathway of sulphinpyrazone in the rabbit (Kirstein Pedersen & Jakobsen, 1979) lead to the investigation of the presence of reductive metabolites in humans during sulphinpyrazone therapy. The results from the present study show that all four metabolites, in unconjugated form, are excreted in urine during long-term therapy, the sulphone being the predominant product. An unexpected finding is the increase in the urinary concentration of both sulphinpyrazone and the metabolites after β -glucuronidase treatment. This indicates excretion of O-glucuronides since C-glucuronides are not cleaved by this enzyme (Richter, Alt, Dieterle, Faigle, Kriemler, Mory & Winkler, 1975).

In another single dose study (Jakobsen & Kirstein Pedersen, 1979) no increase in the urinary concentrations were found after glucuronidase treatment. This suggests a difference in the pattern of metabolism between single dose administration and chronic administration, involving formation of O-conjugated products after prolonged treatment.

The excretion pattern of unconjugated metabolites does not reflect the principal metabolic pathways in the body and seems unrelated to the plasma levels of the metabolites. In plasma, the sulphide and the sulphone metabolite are found in concentrations of approximately 25% of the sulphinpyrazone concentrations as well 2 as 10 h after administration.

Table 1 Details of patients and plasma concentrations of sulphinpyrazone and its metabolites

Patient		A	B	C	D	E	F	G	H	
Weight (kg)		74	72	63	61	75	80	72	73	
Sex		M	F	F	F	M	F	F	M	
Age (years)		35	47	67	61	43	67	64	38	
Dose of sulphinpyrazone (mg kg ⁻¹ day ⁻¹)		10.8	11.1	12.7	9.9	10.6	10.0	11.1	10.9	
	Time (h)	Plasma concentration (mg/ml)								Mean $\pm$ s.e.mean
Sulphinpyrazone	t = 0	5.3	8.0	13.2	7.6	5.6	10.8	4.0	2.5	7.1 $\pm$ 1.3
	t = 2	9.1	20.0	15.1	16.1	15.0	23.2	20.0	9.1	16.0 $\pm$ 1.8
Sulphide	t = 0	3.6	2.3	6.0	2.7	1.7	4.2	1.0	1.0	2.8 $\pm$ 0.6
	t = 2	8.6	2.6	6.4	5.7	2.0	5.4	2.5	1.5	4.3 $\pm$ 0.9
<i>p</i> -OH-sulphide	t = 0	0.76	0.82	0.99	0.65	0.37	1.1	0.29	0.37	0.67 $\pm$ 0.11
	t = 2	1.0	0.83	1.2	1.1	0.35	1.6	0.61	0.45	0.89 $\pm$ 0.15
Sulphone	t = 0	3.8	0.46	4.2	0.70	0.45	3.0	0.25	0.49	1.7 $\pm$ 0.60
	t = 2	5.5	2.6	2.4	4.2	3.0	8.4	6.8	5.7	4.8 $\pm$ 0.76
<i>p</i> -OH-sulphinpyrazone	t = 0	0.16	0.03	0.20	0.08	0.05	0.13	0.05	0.06	0.10 $\pm$ 0.02
	t = 2	0.17	0.04	0.19	0.07	0.09	0.32	0.25	0.11	0.16 $\pm$ 0.03

In order to evaluate the formation rate of the metabolites from plasma concentration measurements, it is necessary to know the plasma clearance (i.e. the distribution volume and the elimination rate) of each metabolite. No data concerning the sulphone are available from literature, but for the sulphide some pharmacokinetic data are available. Brodie, Yü, Burns, Chenkin, Paton, Steele & Gutman (1954) found an average elimination constant of 0.20 h⁻¹, corresponding to a T_{1/2} of 3.5 h. The apparent volume of distribution for the sulphide can be estimated as approximately 6 litres (calculations are based on data from a plasma concentration curve shown in the above mentioned publication). Both values are comparable to those of sulphinpyrazone. Lecaillon, Souppart, Schoeller, Humbert & Massias (1979) reported the plasma half-life of sulphinpyrazone to be about 3.8 h and the distribution volume to be 60 ml kg⁻¹. Having these data in mind, the reductive pathway seems to be of major quantitative importance in the elimination of sulphinpyrazone during long-term therapy. Burns, Yü, Ritterband, Perel, Gutman & Brodie (1957) calculated plasma half-lives for the *p*-hydroxylated metabolites to be about 1 h (the *p*-OH-sulphinpyrazone) and 17 h (the *p*-OH-sulphide). Assuming a similar volume of distribution, the *p*-hydroxylated products seem to be of minor quantitative importance. This is in contrast to the elimination pattern of phenylbutazone during long-term therapy, where the *p*-hydroxylation to oxyphenbutazone is predominant (Aarbakke, Bakke, Milde & Davies 1977).

An example of a metabolic change in the oxidation state of a sulfoxide occurs with another anti-inflammatory agent, sulindac which also is transformed into a sulphide and a sulphone. Metabolic

reduction of sulindac to the sulphide is reversible, whereas oxidation to the sulphone is irreversible (Hucker, Stauffer, White, Rhodes, Arison, Umbenhauer, Bower & McMahon, 1973). Whether this is also the case for sulphinpyrazone remains to be answered, but since this compound originally was isolated as a urinary metabolite of the sulphide (Burns *et al.*, 1957), reduction is probably a reversible process. The *p*-OH-sulphide can arise from either hydroxylation of the sulphide or from reduction of the *p*-OH-sulphinpyrazone; the short plasma half-life and the high polarity of the latter compound, however, make this possibility unlikely. As the dioxypyrazolidine ring structure is not involved in the metabolic changes, C-glucuronidation is likely for all compounds.

All metabolites are active with respect to inhibition of platelet function. Westwick, Webb & Lewis (1979a, 1979b) investigated the effect of the oxidative metabolites upon platelet TXB₂ synthesis compared to that on vascular wall prostacyclin synthesis. Sulphinpyrazone was found to be the most active in the inhibition of platelet function while the sulphone was the most active in the inhibition of vessel wall PGI₂ synthesis; the *p*-OH-sulphinpyrazone possessed no activity. All three compounds were, however, reported equally active in the inhibition of acute thrombocytopenia of rabbits following induction of Arthus reaction (Margulies & White, 1979). The sulphide is a potent inhibitor of collagen induced platelet aggregation (Kirstein Pedersen & Jakobsen, 1979). In this test system, the sulphide was found to be three times as active, the sulphone about twice as active and the *p*-hydroxylated metabolites about as active as sulphinpyrazone. The results in Figure 4 show that the sulphide is about 10 times as active and

the sulphone about 5 times as active as sulphinpyrazone in inhibition of arachidonic acid induced platelet aggregation, probably a more specific test of platelet cyclooxygenase inhibitors.

The relatively high activity of the two predominant metabolites in plasma, the sulphide and the sulphone, make plasma concentration measurements of sulphinpyrazone itself questionable in the evaluation of concentration-effect relationships of the drug. Oxidation into the sulphone seems to be subject to large interindividual variations, especially reflected in the morning plasma levels. This variation could partly be due to variation in the rate of elimination. On the other hand the concentrations of the sulphide and the *p*-OH-sulphide correlated significantly to that of sulphinpyrazone, indicating a similarity or constancy of the distribution and elimination mechanism of these compounds.

In conclusion, we find a reappraisal of the pharmacological profile of sulphinpyrazone necessary.

References

- AARBAKKE, J., BAKKE, O.M., MILDE, E.J. & DAVIES, D.S. (1977). Disposition and oxidative metabolism of phenylbutazone in man. *Eur. J. clin. Pharmac.*, **11**, 359–366.
- ALI, A. & MCDONALD, W.D. (1977). Effects of sulfinpyrazone on platelet prostaglandin synthesis and platelet release of serotonin. *J. Lab. clin. Med.*, **89**, 868–875.
- ANTURANE REINFARCTION TRIAL RESEARCH GROUP (1980). Sulfinpyrazone in the prevention of sudden death after myocardial infarction. *New Engl. J. Med.*, **302**, 250–256.
- BRAUWALD, E. (1980). Treatment of the patient after myocardial infarction. *New Engl. J. Med.*, **302**, 290–293.
- BRODIE, B.B., YÜ, T.F., BURNS, J.J., CHENKIN, T., PATON, B.C., STEELE, J.M. & GUTMAN, A.B. (1954). Observations on G-25671, a phenylbutazone analogue (4-(phenylthioethyl)-1, 2-diphenyl 3, 5-pyrazolidinedione). *Proc. Soc. exp. Biol.*, **86**, 884–887.
- BUCHANAN, M.R., ROSENFELD, J. & HIRSH, J. (1978). The prolonged effect of sulfinpyrazone on collagen-induced platelet aggregation *in vivo*. *Thrombos. Res.*, **13**, 883–892.
- BURNS, J.J., YÜ, T.F., RITTERBAND, A., PEREL, J.M., GUTMAN, B. & BRODIE, B.B. (1957). A potent new uricosuric agent, the sulfoxide metabolite of the phenylbutazone analogue, G-25671. *J. Pharmac. exp. Ther.*, **119**, 418–426.
- BUTLER, K.D., PAY, G.F., WALLIS, R.B. & WHITE, A.M. (1979). Biphasic prolonged inhibition of platelet prostaglandin biosynthesis induced by sulphinpyrazone. *Thrombos. Haemostas.*, **42**, 101.
- DAYTON, P.G., SICAM, L.E., LANDRAU, M. & BURNS, J.J. (1961). Metabolism of sulfinpyrazone (anturane) and other thio analogues of phenylbutazone in man. *J. Pharmac. exp. Ther.*, **132**, 287–290.
- DIETERLE, W., FAIGLE, J.W., MORY, H., RICHTER, W.J. & THEOBALD, W. (1975). Biotransformation and pharmacokinetics of sulfinpyrazone (Anturan®) in man. *Eur. J. clin. Pharmac.*, **9**, 135–145.
- HUCKER, H.B., STAUFFER, S.C., WHITE, S.D., RHODES, R.E., ARISON, B.H., UMBENHAUER, E.R., BOWER, R.J. & MCMAHON, F.G. (1973). Physiologic disposition and metabolic fate of a new anti-inflammatory agent cis-5-fluoro-2-methyl-1 (*p*-methyl-sulfinyl)-benzylidene acid in the rat, dog, rhesus monkey and man. *Drug Metabolism Disp.*, **1**, 721–736.
- JAKOBSEN, P. & KIRSTEIN PEDERSEN, A. (1979). Determination of sulfinpyrazone and two of its metabolites in human plasma and urine by gas chromatography and selective detection. *J. Chromatogr.*, **163**, 259–269.
- JAKOBSEN, P. & KIRSTEIN PEDERSEN, A. (1981). Identification and determination of two new metabolites of sulfinpyrazone by mass spectrometry. *J. Pharm. Pharmac.*, **33**, 89–92.
- KIRSTEIN PEDERSEN, A. & JAKOBSEN, P. (1979). Two new metabolites of sulfinpyrazone in the rabbit. A possible cause of the prolonged *in vivo* effect. *Thrombos. Res.*, **16**, 871–876.
- LECAILLON, J.B., SOUPPART, C., SCHOELLER, J.-P., HUMBERT, G. & MASSIAS, P. (1979). Sulfinpyrazone kinetics after intravenous and oral administration. *Clin. Pharmac. Ther.*, **26**, 611–617.
- MAGUIRE, E.D., PAY, G.F., TURNER, J., WALLIS, R.B., WESTON, M.J., WHITE, A.M., WILLIAMS, L. & WOOD, H.F. (1979). Inhibition of human platelet function induced by sulphinpyrazone. *Thrombos. Haemostas.*, **42**, 101.
- MARGULIES, E.H. & WHITE, A.M. (1979). Sulfinpyrazone. In *Pharmacological and biochemical properties of drug substances*, Goldberg, M.E. American Pharmaceutical Association, Washington.
- RICHTER, W.J., ALT, K.O., DIETERLE, W., FAIGLE, J.W.,

The finding of a reductive metabolic pathway, giving rise to two new circulating and active metabolites, together with the demonstration of significant steady state plasma levels of the sulphide metabolite and the sulphone metabolite, make it likely that the metabolites also contribute to the *in vivo* effects of the drug. With respect to 'platelet inhibitory drug activity', the sulphide and the sulphone constitute a major part of the activity during chronic therapy. In addition, these metabolites may exert other actions (for example antiarrhythmic?) which might also contribute to the preventive effect of sulphinpyrazone therapy upon sudden death after acute myocardial infarction.

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- KRIEMLER, H.-P., MORY, H. & WINKLER, T. (1975). 272. C-glucuronides, a novel type of drug metabolites. *Helv. Chim. Acta*, **58**, 2512–2517.
- WESTWICK, J., WEBB, H. & LEWIS, G.P. (1979a). The effect of sulphinpyrazone, aspirin and their metabolites on prostacyclin production by rabbit aortic rings. *Thrombos. Haemostas.*, **42**, 99.
- WESTWICK, J., WEBB, H. & LEWIS, G.P. (1979b). Comparison of sulphinpyrazone and its metabolites on $T \times B_2$ and PGE_2 generation by human platelets. *Thrombos. Haemostas.*, **42**, 402.

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