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COMPARATIVE DISPOSITION OF SULINDAC AND METABOLITES IN FIVE SPECIES

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Abstract—The disposition of the new anti-inflammatory agent, cis-5-fluoro-2-methyl-1-[*p*-(methylenesulfinyl)-benzylidene]-indene-3-acetic acid [sulindac] and its sulfide and sulfone metabolites was studied in five laboratory species. In each, oxidative biotransformation of the parent sulfoxide (sulindac) to sulfone is irreversible, while reduction to sulfide is reversible. The areas under the plasma concentration curves [AUC] of each redox form vary within each species as a function of the form which is administered and, because of differences in the reversible biotransformations and in enterohepatic circulation, vary widely among species for any given redox form administered. Corresponding variations in anti-inflammatory responses, within species, is a monotonic function of the AUC of sulfide, but not of that for sulfoxide or sulfone. A similar correlation is apparent between intestinal toxicity, to the extent that it occurs, and cumulative biliary secretion of the sulfide. Thus, additional evidence is provided for the putative role of the sulfide metabolite as the biologically active form of sulindac.

Sulindac,* cis-5-fluoro-2-methyl-1-[*p*-(methylenesulfinyl)-benzylidene]-indene-3-acetic acid, is a new nonsteroid with a broad spectrum of anti-inflammatory activities in animal models [1] and in man [2, 3].

The only major biotransformations undergone by sulindac in laboratory species [4] and in man [5] are changes in the oxidation state of the sulfinyl group, i.e. oxidation of the parent sulfoxide to sulfone, and reduction to the corresponding sulfide. Several lines of evidence presented elsewhere [1, 6] suggest that the therapeutically relevant responses to sulindac are attributable predominantly, and perhaps exclusively, to the sulfide metabolite. It alone, among the three redox forms of the drug, possesses prostaglandin synthetase inhibitory activity [1], which, according to current consensus, is a common mechanism of activation involved in both the pharmacological responses [7–10] and toxic responses [11] to nonsteroid anti-inflammatory agents.

In the absence of a definitive identification of the anti-inflammatory receptor(s), systemic plasma levels may be assumed to be valid indices of bioavailability with respect to therapeutic response. The most prominent side effects of nonsteroids, in general, is gastrointestinal intolerance. It has been demonstrated in the case of indomethacin that toxicity varies widely among species and is a quantitative function of the total exposure of the intestinal mucosa to the active form of the drug via enteroheptatic recycling [12]. The same causal relationship between biliary secretion and toxicity is implied for flufenamic acid [13].

The present report examines correlations of two aspects of disposition of sulindac and metabolites with appropriate biological responses, viz. anti-inflammatory response with systemic bioavailabilities, and intestinal toxicity with cumulative biliary secretion. The 2-fold purpose is to provide additional evidence for: (1) identification of the biologically active form of sulindac, and (2) a common biochemical mechanism of action for anti-inflammatory response and toxicity.

*Sulindac was formerly designated as MK-231 (see Ref. 4).

MATERIALS AND METHOD

Sulindac and its sulfide and sulfone, all labeled in the methylene group with tritium, were prepared by exchange procedures previously described [14] and diluted with unlabeled carrier to a specific activity of 10 μCi/mg equivalent. Specified dosages and reported concentrations for the sulfide and sulfone metabolites are based upon molar equivalents of parent sulindac.

Sulindac, sulfide and sulfone were determined in plasma, bile and urine by reverse isotope dilution, details of which are presented elsewhere [5, 14]. In the case of urine and bile, samples were pretreated by addition of one-fifth volume of 1 M NaOH which, after 30 min at room temperature, effects complete hydrolysis of the acyl glucuronides of each redox form. Thus, no distinction between free and conjugated forms of drug or metabolites was made in calculations of renal and biliary clearances, nor was any adjustment made for binding to plasma protein. In all samples, radioactivity not accounted for by the sum of sulindac, sulfide and sulfone is designated "unknown metabolite(s)". At the specific activities employed in the present studies, the precision of measurement at the level of 1.0 μg equivalent/sample is ±2 per cent for each redox form.

Calculations of pharmacokinetic parameters were by standard procedures. Areas under plasma concentration vs time curves, [AUC], for the in-

crement actually sampled were calculatyed by log-linear trapezoidal summation, and from the last sampling point to infinity from terminal elimination rates. Plasma clearances, $\dot{V}_{Cl,p}$, were calculated from dose $\div [AUC]_0^\infty$. For material balance calculations, f_u and f_{bile}, the fractions of dosage excreted through infinity in urine and bile, respectively, were calculated by extrapolation of double-reciprocal plots of cumulative recovery vs time. Renal ($\dot{V}_{Cl,r}$) and biliary ($\dot{V}_{Cl,bile}$) clearances were determined incrementally, i.e. $[\text{total excreted}]_{t_1}^{t_2} \div [AUC]_{t_1}^{t_2}$, and/or integrally ($\dot{V}_{Cl,p} \times f_u$; $\dot{V}_{Cl,p} \times f_{bile}$). Conversely, plasma clearances were calculated in shorter term experiments from incremental renal and/or biliary clearances, i.e. $\dot{V}_{Cl,r} \div f_u$ and $\dot{V}_{Cl,bile} \div f_{bile}$. $\Sigma_{bile}^{\%}$ represents cumulative biliary secretion through infinity in the intact animal, expressed as per cent of dose. Details of its derivation and usage are presented elsewhere [12].

In the case of rabbits, dogs and monkeys, crossover experiments were performed in which each redox form was given to at least two animals intact, and subsequently with enterohepatic recycling interrupted as a consequence of total collection of bile. This was via cannulation of the common bile duct with the gall bladder isolated. Simultaneously, renal and biliary clearances of each redox form were determined incrementally and that of the injected redox form, integrally as well.

In rats and guinea pigs, three separate groups of animals were used for determinations of plasma, renal and biliary clearances, respectively—the former after injection of each redox form, and the latter after injection of sulindac only. Mean plasma clearances were calculated from composite plasma profiles for groups of animals sampled alternatively via the ocular sinus, and at sacrifice via the heart and portal vein. Biliary clearances were determined incrementally in conscious rats with bile collected through 3 hr, and in anesthetized guinea pigs for various intervals through 6 hr. Specific experimental conditions are described in the next section.

RESULTS

Rats. A group of fourteen male Sprague–Dawley rats (170–200 g) received sulindac-[^{3}H] at 10 mg/kg, i.v. Animals were sampled three times at various intervals through 72 hr; there were 18 peripheral plasma samples in duplicate and 14 single portal samples (the latter collected at sacrifice) for construction of composite plasma profiles for parent drug and metabolites (Fig. 1). $[AUC]_0^{72}$ values for sulindac, sulfide and sulfone were 19,700, 8,190 and 44,500 $\mu g \cdot min \cdot ml^{-1}$, respectively, with no significant difference between peripheral and portal blood for any redox form. The sum of the three redox forms accounted, within experimental error (± 8 per cent), for all radioactivity in plasma. Based upon the sum of the incremental $[AUC]_0^{72}$ value and $[AUC]_{72}^\infty$ estimated from the terminal elimination rate, plasma clearance was $0.51\ ml \cdot min^{-1} \cdot kg^{-1}$.

To determine whether the oxidative and reductive biotransformations to sulfone and sulfide were reversible, each was administered as such, and composite plasma profiles for all redox forms were determined as described above for dosage with sulindac. After sulfone treatment, it was the only redox form detectable in plasma (Fig. 1, right panel), from which it was slowly eliminated ($\dot{V}_{Cl,p} = 0.150\ ml \cdot min^{-1} \cdot kg^{-1}$). Sulfide, on the other hand, was rapidly oxidized to sulindac and sulfone (Fig. 1, center panel), and was cleared from plasma at a slightly greater rate than sulindac ($\dot{V}_{Cl,p} = 0.73\ ml \cdot min^{-1} \cdot kg^{-1}$). The ratio of $[AUC]_0^\infty$ for sulindac : sulfide : sulfone, which was 1 : 0.4 : 2.4 after i.v. sulindac, was 1 : 1.6 : 6.8 after i.v. sulfide (see Table 6).

Biliary clearances were determined in three separate animals (250–270 g) cannulated via the common bile duct under pentobarbital anesthesia and placed in Bollman restraint devices immediately upon recovery. Bile was collected

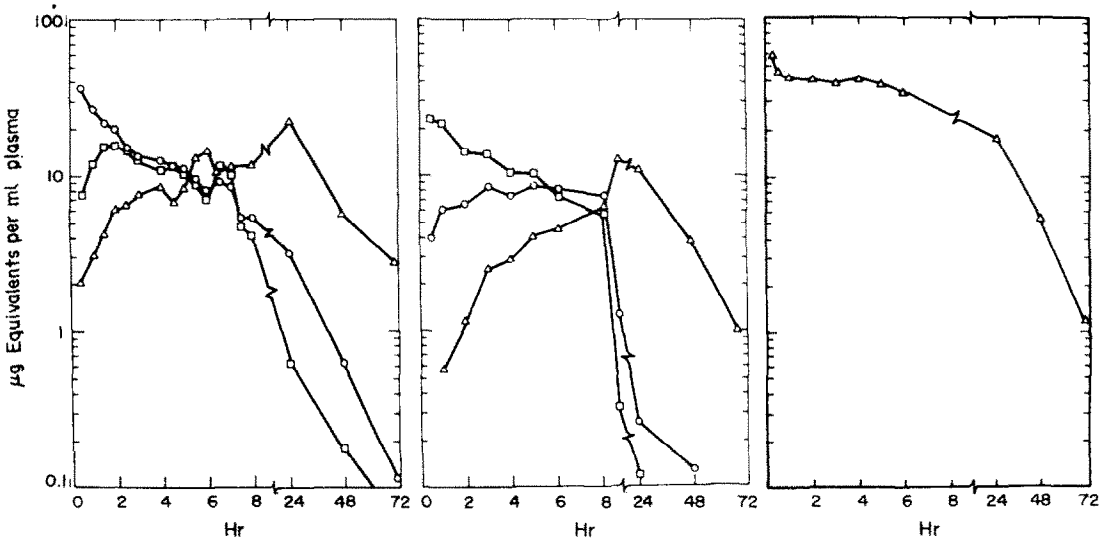

Fig. 1. Composite plasma profiles for sulindac and metabolites in rats after dosage with sulindac at 10 mg/kg (left panel), sulfide at 5 mg/kg (center panel) and sulfone at 5 mg/kg (right panel). Key: sulindac (○), sulfide (□) and sulfone (△).

every 60 min through 4 hr, and plasma was sampled at 90 and 150 min (ocular sinus) and 240 min (heart). For each animal, incremental biliary clearances were reasonably constant over the last 3 hr, and with small interindividual variations in sulindac and sulfide clearances which averaged 1.18 ± 0.18 and 0.47 ± 0.11 ml · min^{-1} · kg^{-1} respectively (Table 1). For each redox form, Σ^{∞}_{bile} values calculated from these mean biliary clearances and appropriate $[AUC]^{\infty}_{0}$ values for each treatment are included in Table 6. After dosage with sulindac itself, the sum of all drug derived material excreted in bile through infinity is equivalent to 643 per cent of the dose, but with only 39.5 per cent as the pharmacologically active sulfide metabolite. With respect to each redox form, their cumulative biliary secretions in the rat are by far the highest of any species examined.

Dogs. Plasma kinetics after injection of sulindac-[^{3}H] and sulfide-[^{3}H] were determined in purebred female beagles prepared with chronic portal fistulas, enabling simultaneous collection of both systemic and portal blood for extended periods without anesthesia.

Typical plasma profiles for the three known redox forms and for unknown metabolite(s) after injection of sulindac are depicted in Fig. 2. Disappearance of the parent drug from the systemic circulation was very rapid ($V_{Cl,p} = 13.0$ ml · min^{-1} · kg^{-1}) and a portal:systemic gradient of about 10:1 became established in 3 hr. Sulfone attained a still higher portal:systemic gradient. Sulfide was detectable in the earliest (10-min) plasma sample, rapidly disappearing below the limit of detection, and reappearing concomitantly with the re-entry of sulindac between 4 and 8 hr, but never attaining a portal:systemic gradient of the magnitude observed for sulindac and sulfone. The relative abundance of unknown metabolites increased steadily with time, accounting for 75 per cent of the circulating radioactivity by 24 hr and 88 per cent by 72 hr. Like the sulfide, unknown metabolites did not appear to be extensively enterohepatically recycled. The dog is the only species examined, including man[5], in which unknown metabolites constitute a significant fraction of the circulating radioactivity.

After sulfide-[^{3}H] treatment, plasma profiles were, after the initial distributive phase, quantitatively similar to those resulting from dosage with sulfoxide. A summary of the areas under the plasma curves for dogs receiving both redox forms is included in Table 6.

Biliary clearances were determined in two dogs after bolus injections of sulindac-[^{3}H]. Detailed data for one are presented in Table 2. Extrapolations of 0–4-hr recoveries to infinity indicated that 20.3 per cent of the dose is ultimately excreted as sulindac, 3.3 per cent as sulfide, 39.4 per cent as sulfone, and 40.5 per cent as unidentified metabolites, thus accounting for 100 per cent of the dose and confirming indirectly the negligible contribution of urinary excretion to the elimination of drug and metabolites in the dog[4]. The means for all incremental biliary clearances of sulindac, sulfide and sulfore in both dogs averaged 9.2 ± 2.0, 5.0 ± 1.5 and 39.0 ± 6.5 ml · min^{-1} · kg^{-1} respectively.

Table 1. Biliary clearances in rats*

	Rat No. 1 (255 g)			Rat No. 2 (270 g)			Rat No. 3 (260 g)		
Time (min)	Sulindac	Sulfone (ml · min^{-1} · kg^{-1})	Sulfide	Sulindac	Sulfone (ml · min^{-1} · kg^{-1})	Sulfide	Sulindac	Sulfone (ml · min^{-1} · kg^{-1})	Sulfide
60–120	1.11	0.46	0.45	1.42	1.76	0.41	1.29	0.55	0.56
120–180	0.97	0.43	0.34	1.23	1.67	0.42	1.02	0.52	0.51
180–240	1.02	0.41	0.35	1.50	0.63	0.54	1.26	0.58	0.70
Mean ± S.D.	1.03 ± 0.070	0.43 ± 0.028	0.37 ± 0.064	1.38 ± 0.14	1.35 ± 0.63	0.46 ± 0.088	1.13 ± 0.044	0.55 ± 0.044	0.60 ± 0.11

*Each rat received sulindac-[^{3}H], 1.0 mg/kg i.v., at zero time.

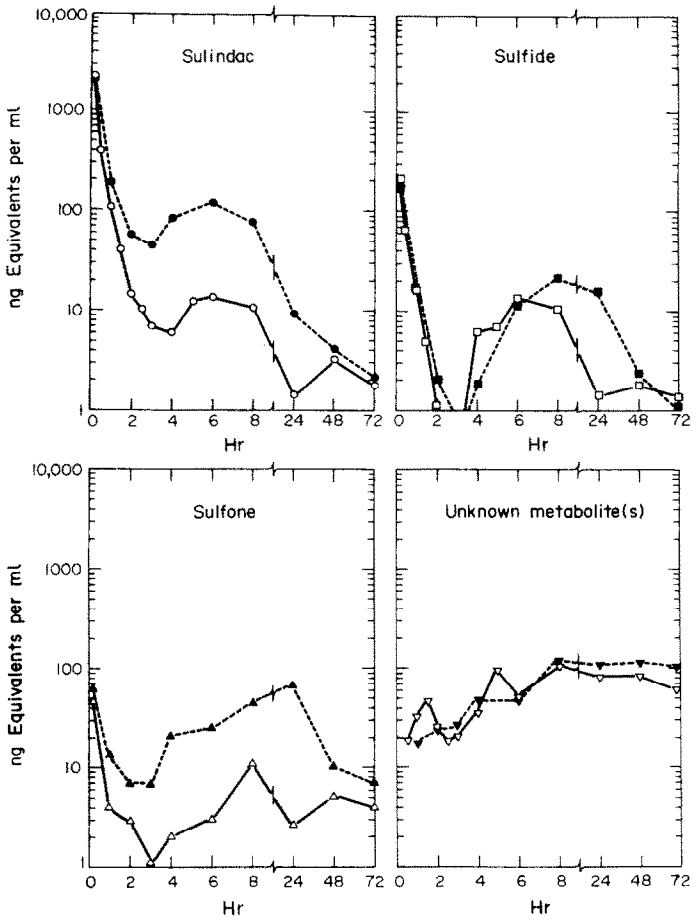

Fig. 2. Plasma profiles of sulindac and metabolites in the dog. A pure bred female beagle (9.6 kg) received sulindac-[^{3}H] at 3 mg/kg i.v. Solid lines represent systemic plasma (femoral vein) and broken lines portal plasma.

Monkeys. Each of three male rhesus monkeys (2.8 to 3.1 kg) received a total of four intravenous treatments with sulindac, sulfide and sulfone according to a crossover design over a total interval of 10 weeks. In the first three treatments, the animals were not anesthetized, and plasma and urine were sampled through 24 hr; in the fourth (terminal) treatment, bile was collected incrementally for varying periods up to 7 hr under phencyclidene–pentobarbital anesthesia.

In Table 3 the plasma, urinary and biliary values are summarized for all twelve treatments. In general, metabolism in the monkey, as evidenced both by the areas under plasma curves and the urinary recoveries of biotransformed products, is not nearly as extensive as in other animal species (see below) and in man[5]. This is especially striking in regard to oxidative metabolism, urinary recoveries of sulfone extrapolated to infinity averaging only 0.57 per cent of the dose after injection of sulfide and 1.12 per cent after sulfoxide. These data reflect both an apparent limited formation of sulfone as well as an unusually low renal clearance in this species. The mean value for $\dot{V}_{Cl,r}$, in three experiments in which sulfone was injected as such and in which observed renal clearance calculations are thus not complicated by possible metabolism within the kidney, varied considerably but averaged only 0.47 ml · min^{-1} · kg^{-1}. Apparent renal clearance for sulfoxide averaged 5.8 ml · min^{-1} · kg^{-1} (mean of six experiments). For sulfide, as in other species[4], renal clearance was negligible.

Comparisons of the [AUC] for each redox form after injection of sulfide confirm the impression that, compared to other species, the capacity for oxidative metabolism is uniquely low in the monkey with respect to the conversion sulfide → sulfoxide, as well as sulfoxide → sulfone.

Biliary clearances varied considerably among the three animals, but in each, sulfoxide clearance exceeded that of sulfide, as seen in other species. The mean values for sulfoxide, sulfide and sulfone were 21.1 ± 17.3, 0.65 ± 0.60 and 5.8 ± 1.8 ml · min^{-1} · kg^{-1} respectively.

Guinea pigs. Three groups of five or six female guinea pigs (350–460 g) received one of the following treatments: (a) 1.0 mg/kg of sulfoxide, with plasma sampled through 48 hr; (b) 1.0 mg equiv. of sulfide-[^{3}H], with sampling of plasma through 24 hr; and (c) 1.0 mg/kg of sulfoxide-[^{3}H] in animals provided with bile cannulas for 30-min collections of bile through 5 hr, and concurrent sampling of plasma. [AUC] values were determined trapezoidally for all experiments.

Plasma and biliary data for guinea pigs are prsented in Fig. 3 and Table 4 respectively. After sulfoxide treatment, $[AUC]_0^\infty$ values for sulfoxide,

Table 2. Biliary secretion of sulindac and metabolites in the dog*

Time (min)	Sulindac				Sulfone				Sulfide				Unknown metabolites		
	Plasma (μg/ml)	Free (μg)	Conj. (μg)	Cum. total	Plasma (μg/ml)	Free (μg)	Conj. (μg)	Cum. total	Plasma (μg/ml)	Free (μg)	Conj. (μg)	Cum. total	Plasma (μg/ml)	(μg)	Cum. total
20	6.1	35	109	146	0.67	3.7	16.8	20.5	0.78	1.99	3.5	5.5	Nil	52	52
40	1.7	262	740	1145	0.46	66	283	368	0.61	22.3	66.4	94.4	Nil	280	332
60	0.66	80	900	2125	0.30	40	1400	1808	0.39	22.4	180	296	0.008	200	532
80	0.41	37	325	2485	0.21	71	1470	3350	0.21	18.6	131	448	0.024	620	1152
100	0.19	24	256	2765	0.15	35	905	4290	0.13	9.87	115	573	0.030	710	1862
120	0.14	13	170	2950	0.098	29	402	4710	0.068	5.26	36.2	614	0.044	780	2642
180	0.12	20	158	3130	0.068	53	580	5340	0.039	6.62	28.0	649	0.058	460	3102
240	0.089	26	124	3280	0.064	70	410	5820	0.020	3.4	8.5	661	0.040	340	3442
∞	—	—	—	4520	—	—	—	8750	—	—	—	745	—	—	9000
$[AUC]_{60}^{240}$ ($\mu g \cdot min \cdot ml^{-1}$)	34.1				20.1				16.36				7.60		
$[Bile]_{60}^{240}$				1155				4012				365			2910
$\dot{V}_{Cl,bile}$ ($ml \cdot min^{-1}$)				33.9				200				22.2			385

*Beagle (7.4 kg) cannulated via common bile duct received 3.0 mg/kg of sulindac-[^{3}H] at zero time.

Table 3. Disposition of sulindac and metabolites in rhesus monkeys

	Redox form measured	Monkey No. 1 (2.8 kg) A Sulfide	B Sulfone	C Sulfoxide	D Sulfoxide* (0–7)	Monkey No. 2 (2.8 kg) A Sulfoxide	B Sulfone	C Sulfide	D Sulfide* (0–6)	Monkey No. 3 (3.1 kg) A Sulfoxide	B Sulfone	C Sulfide	D Sulfide* (0–4)
$[AUC]_{t_1}^{t_2}$	Sulfide	109	0	31.7	33.8	32	0	130	138	44	0	245	191
($\mu g \cdot min \cdot ml^{-1}$)	Sulfone	10	137	7.0	21.8	6.0	163	15.8	1.3	53	316	20.5	3.2
	Sulfoxide	29	0	90.0	223	40.5	0	17.2	7.0	86	0	41.2	8.1
$[Urine]_{t_1}^{t_2}$	Sulfide	Nil	0	Nil	Nil	—	0	Nil		Nil	0	Nil	
(μg equiv.)	Sulfone	5.4	5.7	16.7	84.9	17.1	612	64		21	171	4.0	
	Sulfoxide	98.7	0	386	921	597	0	104		98	0	88	
$\dot{V}_{Cl,r}$	Sulfide	—	—	—	—	—	—	—	—	—	—		
($ml \cdot min^{-1}$)	Sulfone	0.54	0.042	2.40	2.06	2.85	3.75	4.05		0.40	0.54	0.19	
	Sulfoxide	3.40	—	4.30	4.15	14.8		6.0		1.14		2.15	
$[Bile]_{t_1}^{t_2}$	Sulfide				41.3				70.0				798
(μg equiv.)	Sulfone	—	—	—	298				30.5	—	—	—	45
	Sulfoxide				979				691				656
$\dot{V}_{Cl,bile}$	Sulfide				1.22				0.51				4.15
($ml \cdot min^{-1}$)	Sulfone	—	—	—	13.7				23				14.0
	Sulfoxide				4.35				99				81.0
$\dot{V}_{Cl,p}$ (of dosage form only) by													
(1) Dose $\div [AUC]_0^\infty$		25.6	20.2	31	—	69	17.1	21.2	—	36	9.8	12.6	—
(2) $\dot{V}_{Cl,r} \div f_u$		—	21.0	31	—	77	17.9	—	—	29	8.9	—	—
(3) $\dot{V}_{Cl,bile} \div f_{bile}$		—	—	—	10.0	—	—	—	15.7	—	—	—	11.6

*Redox form indicated under Treatments (A–D) indicates dosage form; treatment D was in each case a terminal experiment with bile collected. For treatments A–C, interval $t_1 - t_2 = 0$–24 hr; for treatments D, individual values for $t_1 - t_2$ are indicated under column headings in parentheses.

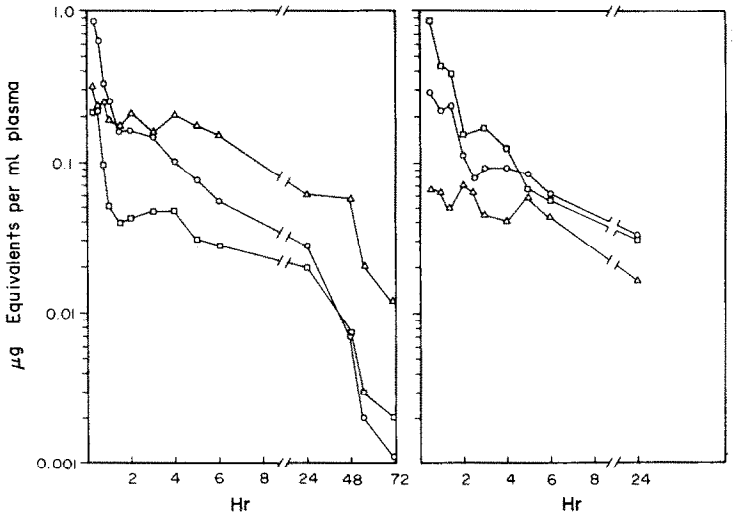

Fig. 3. Composite plasma profiles for sulindac and metabolites in guinea pigs after dosage with sulindac (left panel) and sulfide (right panel), each at 1.0 mg equiv./kg. Key: sulindac (○), sulfide (□) and sulfone (△).

Table 4. Biliary recovery of sulindac and metabolites in guinea pigs*

Hr	N	Total μg equivalents/animal		
		Sulfide	Sulfone	Sulindac
0.25	5	0.72	2.93	63.4
0.5	5	0.83	7.4	57.2
0.75	5	0.60	5.6	23.6
1	5	0.50	4.8	16.4
1.5	4	0.55	7.1	13.7
2	4	0.45	7.2	14.0
2.5	3	0.30	5.5	7.4
3	3	0.10	3.1	6.6
3.5	2	0.10	3.7	2.6
4	2	0.11	3.4	2.0
4.5	1	0.05	3.1	1.8
5	1	0.08	2.1	1.2
$[Bile]_0^5$ (μg)		4.29	55.9	209.3
$[Bile]_0^\infty$ (μg)		9.0	92.0	229
f_{bile}		0.02	0.23	0.51
$[AUC]_0^5$ μg · min · ml^{-1})		11.2	46	52
$\dot{V}_{Cl,bile}$ (ml · min^{-1} · kg^{-1})		0.85	2.7	8.9
$\dot{V}_{Cl,p}$ (by $\dot{V}_{Cl,bile} \div f_{bile}$)		—	17.4	

*A group of five female guinea pigs, average body weight 450 g, received sulfoxide-[^{3}H] at 1.0 mg/kg. Animals were sacrificed at intervals through 5 hr at 60, 120, 180 and 300 min so that all incremental recoveries represent mean for those animals sampled through the period indicated. Each animal was sampled for plasma three times (twice by ocular series, once by heart at sacrifice) for construction of composite plasma profile for calculation of $[AUC]_0^5$.

sulfide and sulfone were 146, 68 and 282 μg · min · ml^{-1} respectively. Collection of bile resulted in approximately 50 per cent decrease in the incremental [AUC] relative to the intact animal (Table 4). Thus, as a consequence of interruption of the enterohepatic cycle, plasma clearance of the parent sulfoxide increased from 6.85 to 17.4 ml · min^{-1} · kg^{-1}, a change of a magnitude previously seen only in dogs. The high biliary clearance required for this result was confirmed directly; $\dot{V}_{Cl,bile} = 8.9$ ml · min^{-1} · kg^{-1} for sulfoxide; mean biliary clearances for sulfide and sulfone were 0.85 and 2.7 ml · min^{-1} · kg^{-1} respectively.

After treatment with sulfide-[^{3}H], $[AUC]_0^\infty$ values were in the expected order—sulfide > sulfoxide > sulfone, i.e. 182, 111 and 68 μg/min ml^{-1}, respectively, for a dosage of 1.0 mg equivalent/kg (Fig. 3, right panel). Plasma clearance of sulfide, 5.5 ml/min^{-1} kg^{-1}, was comparable to that of sulfoxide.

Rabbits. Each of four male New Zealand rabbits (3.6 to 4.0 kg) received either sulindac-[^{3}H] or sulfide-[^{3}H] at 1.0 mg/kg i.v., and plasma and urine were sampled through 48 hr. After a 10-day interval, those receiving sulindac were prepared with bile duct cannulas, injected as before, and bile was collected in 30-min aliquots through 6 hr under halothane anesthesia with concomitant sampling of plasma via cannulation of the marginal ear vein.

Plasma, urine and bile data for all rabbits are summarized in Table 5. Plasma clearance for sulfoxide averaged 4.07 ml/min^{-1} kg^{-1}. Irreversible collection of bile resulted in only about a 10 per cent increase in plasma clearance (4.4 ml · min^{-1} · kg^{-1}), consistent with the low biliary clearance value of 0.60 ml · min^{-1} · kg^{-1}. Mean values for biliary clearance of sulfide and sulfone were 0.97 and 0.56 ml · min^{-1} · kg^{-1} respectively. The rabbit is thus the only species in which the order of apparent biliary clearances is other than sulfoxide > sulfone > sulfide. The unusually low value of $[AUC]_0^\infty$ for sulfide in the rabbit, however, results in a low value for Σ_{bile}^∞ (Table 6).

After sulfide-[^{3}H] treatment, its mean plasma clearance in two intact animals was considerably higher than that of sulfoxide, averaging 10.6 ml · min^{-1} · kg^{-1}. Since its renal clearance is negligible, and its biliary clearance only 0.97 ml · min^{-1} · kg^{-1}, metabolic clearance should account for the predominant fraction of sulfide elimination. This is confirmed by material balance, an average of 60 per cent of the dose being recovered as sulfoxide plus sulfone in urine alone (bile and/or feces were not analyzed). Furthermore, the rabbit is the only one of five species in which the

Table 5. Disposition of sulindac and sulfide in rabbits*

		Sulfoxide-[^{3}H]				Sulfide-[^{3}H]	
		Rabbit No. 1		Rabbit No. 2		Rabbit No. 3	Rabbit No. 4
	Redox form measured	Intact (0–48)†	Bile collected (0–6)	Intact (0–48)	Bile collected (0–5)	(0–48)	(0–48)
$[AUC]_{t_1}^{t_2}$	Sulfide	13.8	27	16.5	14.0	68.5	112
($\mu g \cdot min^{-1} \cdot ml^{-1}$)	Sulfoxide	275	262	189	179	246	243
	Sulfone	490	100	188	120	272	139
$[Urine]_{t_1}^{t_2}$	Sulfide	14.1	10	13.2	4.5	44	16.2
(μg equiv.)	Sulfoxide	745	1203	1970	1260	2750	460
	Sulfone	1203	251	715	775	1090	185
$\dot{V}_{Cl,r}$	Sulfide	0.27	0.10	0.21	0.08	0.16	0.04
($ml \cdot min^{-1} \cdot kg^{-1}$)	Sulfoxide	0.71	1.21	2.76	1.85	2.78	0.53
	Sulfone	0.65	0.66	1.00	1.70	1.0	0.37
$[Bile]_{t_1}^{t_2}$	Sulfide		116		47		
(μg equiv.)	Sulfoxide		520		500		
	Sulfone		193		288		
$\dot{V}_{Cl,bile}$	Sulfide		1.1		0.85		
($ml \cdot min^{-1} \cdot kg^{-1}$)	Sulfoxide		0.50		0.70		
	Sulfone		0.48		0.62		
$\dot{V}_{Cl,p}$‡	by $\dot{V}_1 \cdot K_{1,3}$	2.87	3.20	5.28	5.60	12.6	8.7
($ml \cdot min^{-1} \cdot kg^{-1}$)	by $\dot{V}_{Cl,r} \div f_u$	3.36	4.05	4.60	4.40		
	by $\dot{V}_{Cl,bile} \div f_{bile}$		3.412		5.00		

*Weight of individual rabbits: rabbit No. 1, 3.0 kg; rabbit No. 2, 4.0 kg; rabbit No. 3, 4.0 kg; and rabbit No. 4, 3.6 kg.
†Values for $t_1 - t_2$.
‡$\dot{V}_{Cl,p}$ for injected redox form.

[AUC] for sulfide is less than those for sulfoxide and sulfone after i.v. administration of sulfide, implying that its oxidative metabolism is unusually high.

Biogenic sulindac was isolated from the pooled urines of rabbits receiving a higher dosage of sulfide (20 mg/kg) by preparative thin-layer chromatobraphy (t.l.c.) using the same solvent system employed in the isotope dilution procedure. It had an $[\alpha]_0$ of +11°, compared to a value of ±21° determined for the chemically resolved enantiomers.* Thus, biogenic sulindac is about 66 per cent enriched with respect to the dextrorotatory sulfoxide. Whether this results from absolute or partial stereospecificity with respect to any or all of the three biotransformations, sulfide ⇄ sulindac → sulfone, is not apparent.

DISCUSSION

The material balance in the present studies, in which sulindac is the dosage form, confirms those reported earlier[4]. In addition, it has been demonstrated in all five species that reductive biotransformation to the putative active metabolite is reversible. Oxidation to sulfone, on the other hand, is irreversible and it is a biologically inactive end-product of metabolism.

In Table 6 are summarized two aspects of the foregoing disposition data as they relate to appropriate biological responses, viz. systemic plasma profiles as determinants of the anti-inflammagory response, and cumulative biliary secretions as determinants of intestinal toxicity.

With respect to the anti-inflammatory response, the systemic bioavailability data, $[AUC]_0^\infty$, are subject to the reservation that they represent total plasma profiles through infinity, while the various responses[1, 15] are measured over intervals corresponding to varying segments of the overall time vs plasma concentration curve. Further, the assumption is included that circulating levels of each redox form are valid indices of their concentrations at the receptor site(s). Given these reservations, it is apparent that, within each species for which the respective anti-inflammatory activities of sulindac and sulfide have been determined, responses are direct functions of $[AUC]_{sulfide}$, but not of $[AUC]_{sulindac}$. Detailed regression analyses of response vs concentrations of sulindac and sulfide in appropriate biological fluids provide additional evidence for the same conclusion[6]. Quantitative correlations of availabilities of either redox form among species is precluded, in that different responses are measured in each species.

Interspecies correlations of gastrointestinal toxicity with appropriate disposition parameters are less subject to the reservations stated above, in that a common response can be measured in all, and the temporal relationship between dose and response is such that $[AUC]_0^\infty$, and derived parameters including this term, are most appropriate. Cumulative biliary secretion, $\Sigma^{\%}_{bile}$ is the product of $[AUC]_0^\infty$ and biliary clearance. This parameter has been demonstrated to be a quantitative correlate of indomethacin-related intestinal toxicity which varies over a 40-fold range among five species[12], i.e. toxicity is a function of total exposure of the intestinal mucosa to the active form(s) of the drug as a consequence of enterohepatic recycling.

Values of $\Sigma^{\%}_{bile}$ for sulfide vary widely among species, as do those for indomethacin [12]. In absolute terms, values for the sulfide metabolite are lower than those for indomethacin in each of the five species in which both drugs have been examined and, consequently, intestinal toxicity of

*Dr. M. Jones, personal communication.

Table 6. Correlations of bioavailability with anti-inflammatory response and of cumulative biliary secretion with toxicity

Species	Dosage form	$[AUC]_0^\infty$ (μg equiv. $min^{-1} \cdot ml^{-1}$)*			Anti-inflammatory ED_{50}† ($mg \cdot kg^{-1}$)	$\dot{V}_{Cl,bile}$($ml \cdot min^{-1} \cdot kg^{-1}$)			$\Sigma^{\%}_{bile}$			Intestinal toxicity
		Sulfide	Sulfoxide	Sulfone		Sulfide	Sulfoxide	Sulfone	Sulfide	Sulfoxide	Sulfone	ED_{50} (mg/kg)
Rat	Sulfoxide	830	1970	4830	5.5	0.475	1.18	0.77	39.5	232	372	71
	Sulfide	1400	870	5900	2.3	0.475	1.18	0.77	66.5	102	455	35
	Sulfone	Nil	Nil	6550	Inactive	0.475	1.18	0.77	0	0	505	Inactive
Dog	Sulfoxide	9.7	23	4.8	45	5.0	9.2	39	4.7	21.2	18.4	> 300
	Sulfide	25	2.4	4.2	5.3	5.0	9.2	39	12.5	2.4	16.5	NA‡
Monkey	Sulfoxide	25.1	60	4.5	NA	1.2	21.1	5.8	3.0	127	2.6	⩾ 80
	Sulfide	162	23	21	NA	1.2	21.1	5.8	19.4	48.5	12.2	NA
	Sulfone	Nil	Nil	215	NA	1.2	21.1	5.8	0	0	125	NA
Guinea pig	Sulfoxide	72	149	292	1.7	0.85	8.9	2.7	6.1	133	79	NA
	Sulfide	182	111	68	0.25	0.85	8.9	2.7	15.5	99	18.7	NA
Rabbit	Sulfoxide	15.2	257	285	NA	0.97	0.59	0.56	1.5	15.2	16.0	NA
	Sulfide	90	245	207	NA	0.97	0.59	0.56	8.7	14.4	11.6	NA
Man	Sulfoxide	620	206	510	*ca.* 3.0	NA	NA	NA	NA	NA	NA	NA

*All [AUC] values were normalized on the basis of 1.0 mg/kg, and refer to systemic plasma in all cases except dogs, for which values represent weighted mean of portal and systemic plasma.
†*In vivo* models are as follows: rat, carageenan-induced paw edema; dog, microcrystalline uric acid-induced hindknee joint; guinea pig, inhibition of platelet aggregation; and man, selected rheumatic disorders.
‡NA = not available.

sulindac is markedly less. In each species, $\Sigma^{\%}_{bile}$ for sulindac is at least 5-fold higher than that for the sulfide metabolite and, on the average, is 16-fold higher (Table 6). Thus, it is apparent that the contribution of enterohepatic recycling to the maintenance of sustained systemic levels of active sulfide is achieved predominantly at the level of the inactive precursor, i.e. with a disproportionately lesser exposure of the gut to the active form of the drug.

In the rat, which is the only species for which detailed ED_{50} values for sulindac and sulfide have been determined, toxicity is a direct function of $\Sigma^{\%}_{bile}$ for sulfide, and an inverse one of $\Sigma^{\%}_{bile}$ for parent sulindac. The respective values for $\Sigma^{\%}_{bile}$ for sulfide in five species dosed with sulindac predicts that sensitivity will be in the order: rat $\gg$ guinea pig > dog > monkey > rabbit. The toxicity data currently available are consistent with this extrapolation.

The present correlations of biological responses with appropriate availability values for sulfide, but not with those for parent sulindac, provide additional evidence for the hypothesis developed from other lines of evidence [1, 6] that the biological effects of sulindac are mediated solely via its sulfide metabolite. Thus sulindac is "latentiated" [16] or a "pro-drug" [17], itself devoid of intrinsic pharmacological activity.

It has been suggested that the biochemical mode of action common to non-steroid anti-inflammatory agents (NSAIDs) is inhibition of prostaglandin (PG) synthetase [7–10], and that this same intrinsic property is responsible for the intestinal toxicity characteristic of NSAID's as a group [8, 11]. The present results, considered in the light of the earlier demonstration that sulfide is a potent inhibitor of PG synthetase while sulindac itself is devoid of such activity [18], support both contentions.

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