Oxicams: Relative safety and anti-injury effects in rats

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1 Oxicams have certain distinctive properties, which distinguish them from other aryl-alkanoic (carboxylic) acids that show anti-inflammatory/analgesic/antipyretic activities. These include:

- (i) slow rates of metabolism
- (ii) lesser gastro-irritancy/ulcerogenicity
- (iii) preventing fever development
- (iv) some effect on the adaptive responses of the liver to severe inflammation or trauma that compromise normal hormone/xenobiotic metabolism (detoxification).

2 Isoxicam was notably less gastrototoxic than either piroxicam or tenoxicam, when given either orally or parenterally, at equipotent doses in rats.

Keywords isoxicam piroxicam tenoxicam anti-inflammatory gastrototoxicity

Introduction

Isoxicam and piroxicam show most of the spectrum of activities expected of a modern aspirin-like drug, i.e., analgesic, antipyretic and anti-inflammatory activities with relative safety and considerable potency (Downie *et al.*, 1984; Wiseman & Lombardino, 1982). However their unique chemistry confers some distinctive properties in animal systems, notably long elimination half-lives (Table 1).

The acidic character of these drugs is due to the ionisation of an enol group in the benzothiazine ring, facilitated by the adjacent carboxamide moieties. Also because they lack a carboxyl group, they cannot enter one of the principal pathways of drug inactivation, namely, (ester) glucuronide formation. The low pK_a of the enol group protects it from sulphation, (ether) glucuronidation or methylation, the usual fate of phenols, catechols, etc. *in vivo*. Retention within the body is further ensured by their lipophilicity, which minimises both renal and biliary excretion.

Very few of the anti-inflammatory (AI) carboxylic acids have long plasma half-lives (> 12 h), the most notable exceptions being naproxen and benoxaprofen. Such long half-lives permit lower and less-frequent doses to attain stable and sufficient plasma levels of the active drug. This has been one of the great attractions of the oxicams, which need only be prescribed once daily, thus improving the likelihood of patient compliance.

Other consequences of this relative biolongevity are the main subject of this paper. They include:

- (1) potentially greater toxicity, though this is usually recognised in the first studies in animals and only exceptionally in man;
- (2) possible drug effects within the liver and other drug-metabolising tissues that are not demonstrated by more biolabile drugs which are readily inactivated locally.

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Table 1 Half-lives and LD₅₀ values for two oxicams

Parameter	Isoxicam	Piroxicam
$t_{1/2}$ in man (h)	31	45
$t_{1/2}$ in rat (h)	≥ 13	5.8
LD ₅₀ values (mg kg ⁻¹) p.o. for single dose, normal rats	> 5000	≥ 270
multiple doses, arthritic rats	178	21

$t_{1/2}$ = average plasma half-life from single dose studies
Data from Downie *et al.* (1984) and Wiseman & Lombardino (1982)

Methods

Three sets of experiments were carried out, as outlined below. Full details of the methods are given elsewhere (Whitehouse & Brooks, 1986; Whitehouse, 1986).

1. *Gastrotoxicity* of the test drugs was studied in (a) normal rats (both sexes), (b) rats with an acute remitting inflammation induced with oleyl alcohol, and (c) rats with a chronic inflammation, an established adjuvant-induced polyarthritis. All rats were fasted overnight but allowed to drink. Drugs were administered orally as suspensions in 0.02% Tween-20. Sodium salts were prepared for parenteral administration as solutions adjusted to pH < 8.0 and 300 milliosmolar with NaCl. Animals were then chill-stressed (−20°C, for 25 min) to sensitise their gastric mucosa, returned to room temperature for 150 min and sacrificed by cervical dislocation. Visual inspection of the saline-rinsed stomach lining gave a gastric lesion index based on number, severity and frequency of incidence of the haemorrhagic mucosal lesions (Rainsford & Whitehouse, 1978).

2. *Fever development* was studied in rats inoculated in the flank with 2 g kg⁻¹ dried brewer's yeast dispersed in 0.15M NaCl. Drugs were also administered at this time (22.00 h) to determine their ability to reduce or prevent the fever, which was measured with a rectal thermometer 10 and 12 h later.

3. *Altered drug metabolism* in inflamed rats was studied as follows. Rats were injected with 0.1 ml oleyl alcohol (into the tailbase to produce inflammation). Their ability to metabolise pentobarbitone (Pb) was determined 4 days later, by recording sleep times in response to a standard i.p. dose of 20 mg kg⁻¹ in females or, 25 mg kg⁻¹ in males. Test drugs were administered once

daily p.o. for 4 days before determining Pb-sleeping times (PST).

Results

Gastric tolerance to oxicams and phenylbutazone

Table 2 compares the gastric lesion indices obtained when isoxicam, piroxicam, tenoxicam and phenylbutazone were administered in a *single* dose to hooded rats in different states of disease stress. Data are also given for aspirin and indomethacin to permit comparison of their effects in these animals with published data concerning their effects in other rat strains (usually used unstressed). The doses used were the ED₅₀s obtained in hooded rats for inhibition of carrageenin-induced paw oedema (Whitehouse & Brooks, 1985). Thus, these comparisons of gastroirritancy were made at equipotent doses for 'normal' rats).

Table 3 further compares these drugs when given in *multiple* doses to either rats with inflammation or animals with established arthritis, to more nearly simulate the clinical context in which anti-inflammatory drugs are used. These disease-stressed rats are always more susceptible to gastric injury than normal animals (Whitehouse & Rainsford, 1983; Whitehouse *et al.*, 1984).

Altogether, the oxicams and reference drugs were tested for their gastrotoxicity in five experimental contexts (three shown in Table 2, two in Table 3) by two different routes of administration (oral/parenteral, Table 2 only). In every instance, isoxicam was less gastrotoxic than the therapeutically equivalent doses of piroxicam, tenoxicam, phenylbutazone, indomethacin or aspirin.

These results have also to be considered against the much longer half-life of isoxicam in normal rats (Table 1), compared with drugs which include both piroxicam and

Table 2 Gastric irritation by some anti-inflammatory drugs in normal (N), inflamed (I) and arthritic (A) male hooded rats

Drug	Dose (route) (mg kg ⁻¹)		Gastric lesion indices		
			N	I+	A++
<i>Enols:</i>					
Isoxicam	50	p.o.	0	13	25
		i.p.	14	41	68
Piroxicam	10	p.o.	24	60	77
		i.p.	30	67	67
Tenoxicam	20	p.o.	49	99	102
Azapropazone	100**	p.o.	0	0	0
Phenylbutazone	75	p.o.	08	41	51
		i.p.	77	96	80
<i>Carboxylates</i>					
Aspirin	150	p.o.	81	> 100	> 100
		i.p.	27	N.D.	57
Indomethacin	4	p.o.	16	30	> 100
		i.p.	25	60	98

Haemorrhagic gastric lesion indices (GLI) were determined for single dose of drug – ED₅₀ for anti-inflammatory activity* in fasted, chill-stressed rats (*n* = 4/group)

*obtained by inhibition of carrageenin paw oedema (same rat strain)

**less than ED₅₀

ND = not determined

+inflamed rats received 0.1 ml oleyl alcohol in tailbase 4 days previously.

++ arthritic rats received 500 µg dried *M. tuberculosis* in 50 µl squalane in tailbase 17–19 days previously

phenylbutazone. As Table 2 shows, isoxicam was the least toxic drug at approximately 2.5 h. This time span is a considerable fraction (> 0.4) of the half-life of piroxicam or phenylbutazone (as determined in other strains) but not of isoxicam (≤ 0.2).

Prevention of yeast-induced fever

Isoxicam and piroxicam effectively suppressed the development of fever induced by inoculating dried yeast (Table 4). High doses of phenylbutazone and amidopyrine also prevented fever development. Conventional antipyretic drugs (salicylate, paracetamol, antipyrine) were ineffective fever suppressants when given prophylactically. Several anti-inflammatory carboxylic drugs were likewise poor fever suppressants when given at the time fever was initiated, although they were effective antipyretics when tested after the fever was fully established (after 8 h).

The exceptional activity of isoxicam and piroxicam was further indicated by their ability

Table 3 Gastric irritation by some anti-inflammatory drugs given orally in multiple doses to inflamed or arthritic hooded rats

Drug	Inflamed		Arthritic	
	Dose	GLI	Dose	GLI
Isoxicam	50	26	3	05
Piroxicam	10	64	0.75	24
Tenoxicam	20	74	1	74
Phenylbutazone	75	31	40	32
Azapropazone	—	ND	50	07
Indomethacin	4	18	1	13

Animals were fasted after penultimate dose and chill-stressed after final dose (see **Methods**). Doses (mg kg⁻¹) were either ED₅₀ for acute anti-inflammatory activity (Table 2) or ED₅₀ to prevent paw swelling in arthritic rats (when given over days 12–16 post adjuvant). Number of daily doses = 5 for inflamed rats; = 6 for arthritic rats.
GLI = gastric lesion index.
ND = Not determined.

Table 4 Prevention of yeast-induced fever in hooded rats

Drug	Time (h)	Dose (mg kg ⁻¹)	F-P score
Isoxicam	0	100	3+
	-4	100	3+
	-8	100	2+
	0	50	2+
Piroxicam	0	25	+
	0	20	2+
	-4	20	2+
	-8	20	2+
Tenoxicam	0	10	2+
	0	5	+
	0	20	+
	0	200	3+
Amidopyrine	0	160	0
Antipyrine	0	160	0
Paracetamol	0	160	0
Sodium salicylate	0	250	+
Dexamethasone	0	10	2+
Carboplatin (i.p.)	-8	10	+
	0	30	3+
	-4	30	2+
Phenylbutazone	0	150	2+
	0	75	+
Indomethacin	0	10	0
Diffunisal	0	150	0
Oxaprozin	0	150	0

Drugs were given orally (except as noted) at time of yeast inoculation (time 0) or previously as indicated. Untreated animals had fevers $\geq 39.2^\circ$ 12 h later. A fever-prevention (FP) score was assigned = 3+, 2+, + and 0 if the drug reduced the fever by 1.5° , 1.0° , 0.5° or $\leq 0.4^\circ$ respectively. Data were derived from duplicate experiments, each with 3 rats/group.

to suppress fever development, even when given several hours *before* the yeast inoculum. Only two other (and not so well-tolerated) drugs were noted to have this sustained effect, namely, dexamethasone and carboplatin (JM-8): both suppress leukocyte function and induce leukopenia. They also cause involution of the thymus. No evidence was obtained that the oxicams were lymphotoxic in assays of thymic involution.

Effect of oxicams on drug metabolism in inflamed rats

Rats lose some of their capacity to metabolize drugs with inflammation (Beck & Whitehouse, 1974; Mathur *et al.*, 1977) or trauma (Griffith *et al.*, 1983). This deficit, principally in hepatic drug metabolism, is recognised by increased pentobarbitone sleeping times (PST) in inflamed animals (> 100 min) with doses of pentobarbitone that induce sleep times of < 25 min in normal rats, even when co-housed with their inflamed litter mates (Table 5).

Table 5 Effects of oxicams on pentobarbitone sleep times (PST) in hooded rats inflamed with oleyl alcohol (OA)

Treatment*	Dose (mg kg ⁻¹)	PST (min) in	
		males	females
None	—	122 $\pm$ 6	104 $\pm$ 3
Isoxicam	(100)	54 $\pm$ 11	37 $\pm$ 4
	(25)	72 $\pm$ 7	69 $\pm$ 4
Piroxicam	(20)	81 $\pm$ 12	58 $\pm$ 7
	(10)	87 $\pm$ 2	66 $\pm$ 5
Tenoxicam	(20)	90 $\pm$ 4	70 $\pm$ 9

Data are means $\pm$ s.d. from groups of four rats.

PST in non-inflamed rats = 24 $\pm$ 2 min in males, 22 $\pm$ 3 min in females.

*Once daily for 4 days, PST determined on fifth day.

Treatment with oxicams on the previous 4 days significantly reduced the PST in the inflamed animals (Table 5). Parallel studies showed that the same oxicam pre-treatment did not shorten the PST in normal rats, challenged with doses of pentobarbitone sufficient to give PST > 100 min.

Several NSAID drugs failed to normalise PST in the inflamed rats (Table 6a) whilst a few others, notably phenylbutazone and amidopyrine, significantly reduced PST in both normal and inflamed rats (Table 6). Only naproxen at rather high doses and oxaprozin resembled the oxicams in reducing the metabolic deficit and normalising PST in inflamed animals but had no effect on drug metabolism in normal animals.

Discussion

Concerning the stomach toxicity

Gastrotoxicity studies in rats may only have a limited value for predicting drug tolerability in man. Examples are known where an NSAID drug is highly gastrotoxic in rats but relatively harmless in man (e.g., diclofenac). These are probably rare by comparison with the numerous observations that other drugs known to cause gastric bleeding in man (e.g., aspirin, diflunisal, indomethacin, tolmetin) are likewise highly gastro-irritant in stressed rats (Rainsford, 1981; Whitehouse & Rainsford, 1980). The present data show that the drug with the slowest metabolism in rats (isoxicam) was also the least gastrotoxic. That the gastric injury is not due just to oral presentation of the drug is clearly shown by the considerable gastrotoxicity of all the drugs, except aspirin (which is particularly bio-labile), when given parenterally (Table 2).

Table 6 Effect of non-oxicams on pentobarbitone sleep times (PST) in normal (N) and OA-inflamed (I) rats.

Drug	Dose (mg kg ⁻¹)	Reduction in PST in	
		N	I
a			
Sodium salicylate	250	0	0
Meclofenamic acid	50	0	0
Ibuprofen	100	0	0
Flurbiprofen	25	ND	0
Diclofenac	20	ND	0
Benoxaprofen	100	ND	0
b			
Phenylbutazone	150	++	++
Amidopyrine	200	++	++
Dexamethasone	10	+	++
Fenclofenac	200	+	+
Clobuzarit (Closic)	100	+	+
Naproxen	100	0	+
Oxaprozin	100	0	+
Indomethacin	4	—	+

+ = PST 40–70%, 2+ = P.S.T. 10–40% controls.

ND = not determined.

The relationship between drug metabolism and gastro-irritancy may seem remote for drugs ingested orally, where the gastrotoxin is absorbed from the stomach. However, for drugs ingested in delayed-release formulations or presented as suppositories or parenteral formulations, the gastrotoxin is in the circulation and its toxicity may be dependent upon its elimination half-life. Despite its long half-life in rats, isoxicam seems to be remarkably non-toxic to the gastric mucosa, even when presented parenterally—in contrast to other drugs such as phenylbutazone, which are not considered unduly gastro-irritating in man.

Concerning interleukin-1

Fever is undoubtedly an important defence mechanism with survival value to the host, mediated by leukocytic pyrogen (Dinarello, 1982) now identified as interleukin-1 (IL-1), a hormone mediating the host's response to infection and inflammation (Dinarello, 1984a). The profound fever-suppressant activity of isoxicam and piroxicam may reflect either (1) their long effective half-lives or (2) a further activity in counteracting some of the effects of IL-1 including its action on the thermoregulatory centre in the hypothalamus. That this is not a mere tautology is suggested by other data presented in this symposium, specifically (a) the report by Sheppard & Couchman (1986) that oxicams

can suppress the chondrolytic effects of pig IL-1 (catabolin) and (b) the data in this paper on the effect of oxicams on drug metabolism in inflamed rats.

A second property of IL-1, apart from its immuno-regulant role, is triggering the increased hepatic synthesis of fibrinogen and several plasma glycoproteins that accompanies inflammation—the so called 'acute phase reaction' (Dinarello, 1984 a,b). It seems reasonable to hypothesise that the metabolic process that increases fibrinogen synthesis at the expense of albumin synthesis initiated by IL-1 within the liver, may also curtail the synthesis of hepatic drug-metabolising enzymes. The experimental observations that drug metabolism is 'normalised' (or certainly less aberrant) in inflamed animals treated with oxicams may be interpreted as either (1) an anti-IL-1 effect of these drugs or (2) merely a reflection of their long action *in vivo*, suppressing inflammation and preventing IL-1 release from activated monocytes within the inflamed locus. Decisions between these alternative interpretations can probably only be based on further studies of the effects of oxicam on IL-1 systems *in vitro*. This is why Sheppard & Couchman's (1986) report is particularly timely, as it shows the oxicams to be inhibitors of the actions of IL-1 (also see Rainsford, 1985).

In conclusion, the oxicams are not just like all other NSAID drugs: they have additional features

reflecting their longer half-lives and perhaps an extended spectrum of activities (including IL-1 regulation).

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