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SHORT COMMUNICATIONS

Influence of ibuprofen on drug-metabolizing enzymes in rat liver *in vivo* and *in vitro**

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Ibuprofen [2-(4-isobutylphenyl)propionic acid] is a non-steroid anti-inflammatory drug [1] introduced into therapy of rheumatic diseases few years ago [2]. Ibuprofen toxicity was studied in detail by Adams *et al.* [3]. In rats, the primary toxic effect is damage of the gastrointestinal tract; moreover, repeated drug administration was found to cause enlargement of several organs, including the liver. Signs of hepatotoxicity were not observed. The authors attributed the organ enlargement to hypertrophy [3]. It seemed possible to us that liver enlargement was due to enzyme induction [4].

MATERIALS AND METHODS

Male Wistar rats (Uje: Wist) aged 30–35 days were used as in previous investigations [5] for determination of ascorbic acid, hexobarbital sleeping time and aminopyrine *N*-demethylation. Animals were divided into groups of 7 or 8 rats each and pretreated for 3 consecutive days p.o. or s.c. with ibuprofen.† One ml/100 g body wt methylcellulose (Tylose®) solution was used as drug vehicle. Control groups received vehicle only. The daily s.c. dose of ibuprofen was 650 mg/kg, corresponding to 50 per cent of the acute s.c. LD_{50} [3]. In another experiment, comprising 4 groups of rats, ibuprofen was given by gastric intubation at a dose of 400 mg/kg, i.e. 25 per cent of the acute p.o. LD_{50} [3]. For comparison, one group of rats received 40 mg/kg phenobarbital sodium daily, and another received 400 mg/kg ibuprofen and 40 mg/kg phenobarbital sodium together. Time of administration was about 8·00 a.m.. On the third day, ascorbic acid excretion in urine was determined after i.p. injection of 2 ml saline per 100 g body wt to ensure a sufficient urine flow.

Animals were placed into funnels with wire screen floors from 3·00 to 8·00 p.m., and urine excreted during this time was collected. Ascorbic acid was assayed as described earlier [6]. On the morning of the fourth day, hexobarbital sleeping time was measured, i.e. time from injecting 100 mg/kg hexobarbital sodium i.p., dissolved in 1 ml saline per 100 g body wt, until reappearance of righting reflexes; environmental temperature was 28°. Following awakening, the animals were anesthetized with ether and killed by decapitation. After bleeding, the livers were removed and homogenized in 2 vols of ice-cold 1·15% KCl solution. Aminopyrine *N*-demethylation by 20 min × 15,000 *g* liver supernatant was determined as described in a former paper [5], measuring the formaldehyde released during incubation.

Liver supernatants of untreated rats were used as enzyme sources for *in vitro* experiments. Ibuprofen was added to the complete incubation mixtures as the sodium salt, giving final concentrations of 10^{-5}–10^{-3} M. Type of inhibition and apparent K_i value were determined graphically according to Lineweaver–Burk and Dixon [7], respectively.

RESULTS AND DISCUSSION

As shown in Table 1, pretreatment with ibuprofen doubled hexobarbital sleeping time, which is considered to be a measure of enzymic hexobarbital metabolism [8]. Aminopyrine *N*-demethylation rate by liver 15,000 *g* supernatant and ascorbic acid excretion in urine following p.o. administration of ibuprofen were found to be reduced to less than half of control values. Liver weight did not change significantly. When ibuprofen was given s.c. ascorbic acid excretion was observed to be increased. Phenobarbital shortened hexobarbital sleeping time and increased both aminopyrine *N*-demethylation and ascorbic acid excretion. When phenobarbital was given together with ibuprofen, sleeping due to hexobarbital was prevented in 6 of 8 animals, and aminopyrine *N*-demethylation was stimulated additionally. The phenobarbital-induced rise of ascorbic acid excretion was not significantly changed by ibuprofen.

In an attempt to clarify the nature of the above-mentioned inhibitory effects of ibuprofen, we studied the influence of this drug on aminopyrine *N*-demethylation by 15,000 *g* liver supernatant *in vitro*. Ibuprofen proved to be an inhibitor of microsomal *N*-demethylase *in vitro*. Inhibition was of a mixed type, the apparent K_i value was 5×10^{-4} M (Fig. 1).

There are at least two possibilities for explanation of the inhibitory action of ibuprofen. First, the ibuprofen effects might be due to hepatotoxic properties. The yellowish colour and soft consistency of the organ somewhat resembled the effect of CCl_4 poisoning. Also CCl_4 causes a depression of hepatic drug metabolizing activity [9]. A CCl_4-like mode of action could provide an explanation for the opposite influence of ibuprofen on ascorbic acid excretion following p.o. or s.c. administration of the drug. At the lower p.o. dosage, ibuprofen decreased excretion, probably by an inhibition of ascorbic acid synthesis, whereas the increase following s.c. administration of a higher dose was possibly due to leakage through the injured cell membrane [10].

However, the analogy between ibuprofen and CCl_4 was not a thorough one. CCl_4 is known to inhibit microsomal enzyme induction [11]. Ibuprofen even reinforced the phenobarbital effect to some extent. Adams *et al.* observed toxic alterations of the gastrointestinal tract and kidneys,

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Table 1. Influence of pretreatment with ibuprofen and/or phenobarbital sodium on hexobarbital sleeping time, ascorbic acid excretion and aminopyrine *N*-demethylation in rats. Results of 2 separate experiments with different routes of drug administration

Pretreatment and dose administered daily for 3 days	Route of administration	Hexobarbital sleeping time (min)	Ascorbic acid excretion in urine (mg/5 hr) per 100 g body wt	Aminopyrine *N*-demethylation by 15,000 *g* liver supernatant (μmoles formaldehyde g protein^{-1} min^{-1})
Control (1 ml 1% Tylose®/100 g)	s.c.†	59·0 ± 6·3 (7)	0·053 ± 0·011 (7)	35·8 ± 1·8 (7)
Ibuprofen (650 mg/kg)	s.c.†‡	118·0 ± 11·7 (5)*	0·162 ± 0·039 (5)*	18·8 ± 1·3 (5)*
Control (1 ml 1% Tylose®/100 g)	p.o.	30·4 ± 2·7 (8)	0·36 ± 0·06 (5)	48·2 ± 2·5 (8)
Ibuprofen (400 mg/kg)	p.o.§	74·2 ± 14·6 (5)*	0·10 ± 0·02 (5)*	17·1 ± 4·1 (6)*
Phenobarbital-sodium (40 mg/kg)	p.o.	8·4 ± 0·8 (8)*	1·94 ± 0·35 (5)*	107·5 ± 6·4 (8)*
Ibuprofen (400 mg/kg) plus phenobarbital sodium (40 mg/kg)	p.o.	3·8 ± 2·4 (8)‖	1·45 ± 0·18 (5)*	133·2 ± 7·9 (8)**

Means ± S.E.M., numbers of animals in parentheses.
* Significantly different from controls at $P \leq 0{\cdot}05$.
** Significantly different from all above values in the same column at $P \leq 0{\cdot}05$. Statistical comparison was carried out using multiple *t*-test.
† This experiment was carried out 5 months prior to the p.o. experiment.
‡ Three of 8 animals died.
§ Two of 8 animals died.
‖ Only 2 of 8 animals had a total loss of righting reflexes (14 and 16 min, respectively).

but did not report signs of hepatotoxicity in rats following 4 days pretreatment with 540 mg/kg ibuprofen daily [3]. Therefore, the observed ibuprofen effects cannot be attributed solely to toxic injury of the liver, though toxic actions may participate in the inhibitory influence on drug metabolism.

Second, ibuprofen might possess properties like SKF 525-A [12]. Ibuprofen proved to be an inhibitor of microsomal aminopyrine *N*-demethylation *in vitro*; the type of inhibition was a mixed one. Drugs may remain in microsomes for a time following treatment *in vivo*, and these residual amounts can account for later changes in microsomal properties *in vitro* [13]. It seems likely that ibuprofen was present in microsomes of pretreated animals at the time of enzyme assay *in vitro* in quantities sufficient for inhibition of aminopyrine *N*-demethylation.

No inhibition was observed, when phenobarbital-treated rats received ibuprofen simultaneously. Ibuprofen concentration probably did not reach levels sufficient for enzyme inhibition in these animals; this was possibly due to a phenobarbital-induced acceleration of ibuprofen metabolism. In the rat, three metabolites of ibuprofen were found [3]. Eventual inhibitory or inducing effects of these metabolites should be negligible, for the concentrations attained may be expected to be very low. Lower mortality in the group treated with ibuprofen and phenobarbital together

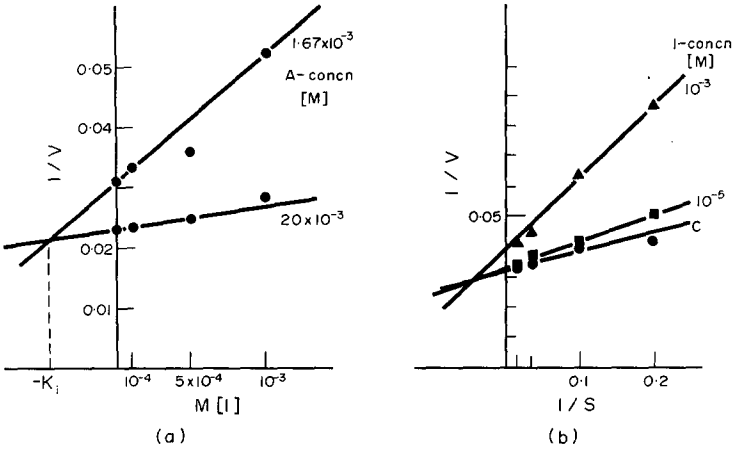

Fig. 1. Inhibitory effect of ibuprofen on aminopyrine *N*-demethylation by 15,000 *g* liver supernatant. (a) Dixon plot. *v* is given as μmoles formaldehyde min^{-1} g protein^{-1}. (b) Lineweaver–Burk plot. S is given as mM aminopyrine; for *v* see above.

favours the assumption of an enhanced metabolism of ibuprofen, although the difference is not statistically significant.

Liver enlargement, as observed by Adams *et al.* [3] after many weeks treatment, might indicate an inducing property of ibuprofen, since many inducers increase liver weight [14]. This is true even for typical microsomal inhibitors like SKF 525-A [15] or phenylbutazone [16].

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Institute for Pharmacology and Toxicology, Friedrich Schiller University, Jena, GDR

CLAUS REINICKE*
WOLFGANG KLINGER

* Present address: Medizinische Universitätsklinik Jena, 69 Jena, Bachstr. 18, GDR.

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Drug-induced lesions in trypanosome fine structure: a guide to modes of trypanocidal action

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Trypanocidal drugs have incompletely known modes of action derived mainly by surmise from their effects on non-trypanosomal systems [1, 2]. In the present study, representative drugs wre chosen from the main classes of trypanocide: diamidine (pentamidine, hydroxystilbamidine, Berenil); adenine nucleoside (Puromycin, Puromycin aminonucleoside, Cordycepin(3′-deoxyadenosine), Nucleocidin); aminoacridine (acriflavine); amino-phenanthridine (Ethidium); aminoquinaldine (Antrycide); naphthylamine sulphonate (suramin) and arsenical (tryparsamide, Mapharside). Most of them were assayed under standard conditions *in vitro*, using only one monomorphic strain of trypanosome (*Trypanosoma rhodesiense* (N) [3]), so that comparative inhibition of parasite motility, infectivity, respiration and glycolysis could be recorded (Table 1). As the bloodstream form of this trypanosome does not multiply *in vitro*, infectivity was used as an index of cell division; motility correlates well with respiration and glycolysis, so that simple *in vitro* tests of effects on infectivity and motility with this strain can show if the primary action of a trypanocidal drug is likely to be on macromolecular synthesis or on energy-yielding reactions.

The trypanocides were also used to treat the same trypanosome strain *in vivo*, and the ultrastructural lesions produced were analysed as a further guide to drug action. Infected mice were treated with curative intraperitoneal drug doses, and blood samples were taken 5–6 hr after treatment and processed for electron microscopy as described elsewhere [3]; in the case of Mapharside, which acts rapidly, the sample was taken 1 hr after treatment. All fixation and preparative procedures were rigidly standardized, and as none of the lesions to be described were found in the normal untreated trypanosome, these lesions have been ascribed to drug action; the lesions have also been found to be detectable and reproducible with other fixation and preparative methods (unpublished work with Dr. D. J. McLaren, to whom we are indebted for Figs. 1, 2a and 3).

In vitro, only Ethidium, Antrycide and suramin affected trypanosome infectivity (cell division) more than respiration or glycolysis (Table 1). Like the highly active acriflavine and the thiol-reactive phenylarsenoxide Mapharside, Cordycepin affected all four activities equally but was even more strongly inhibitory; its rapid cytotoxicity may be due to incorporation into and inactivation of adenine nucleotide coenzymes as occurs with the related Tubercidin [4]. The remaining drugs were not markedly selective, but nitrofurazone, an active electron-acceptor [5], inhibited respiration much more strongly than glycolysis, and pentamidine showed preferential inhibition of glycolysis. Puromycin and Puromycin aminonucleoside tended to affect infectivity more than respiration or glycolysis; like Ethidium [6] and Antrycide[7], both are powerful inhibitors of macromolecular biosynthesis[8,9]. Acriflavine and Cordycepin are also known to inhibit nucleic acid synthesis [10, 11], but strong inhibition of energetic processes is obviously an additional, if not a primary cause of rapid cytotoxicity.

Further insight into trypanocidal action was provided by study of characteristic drug-induced lesions in fine structure. Various cytotoxic agents produce injuries in the mammalian cell which can be correlated with their biochemical properties. For example, Puromycin [12] and ethionine [13] are protein synthesis inhibitors and cause changes in polyri-