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The effect of some benzimidazoles on the disposition of antipyrine and tolbutamide from the rat isolated perfused liver

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The anthelmintic benzimidazoles, mebendazole, albendazole and flubendazole have been screened for any propensity to alter the disposition of antipyrine and tolbutamide in the rat isolated perfused liver preparation. The benzimidazoles were added as a 2.5 mg bolus dose into the perfusate reservoir 5 min before the administration of either antipyrine or tolbutamide. Neither mebendazole or albendazole produced any significant effect on the pharmacokinetics of either of the substrate drugs. In contrast, flubendazole significantly decreased the clearance of antipyrine (by 40%) indicating inhibition of mixed function oxidase activity. However, flubendazole did not alter the disposition of tolbutamide. The results suggest that not all benzimidazoles inhibit hepatic drug metabolizing enzymes and that different forms of cytochrome P-450 are involved in the metabolism of antipyrine and tolbutamide.

Clinical and experimental studies revealed the potential of drugs containing the imidazole moiety to inhibit mixed function oxidase (M.F.O.) activity (Puurunen & Pelkonen 1979; Desmond et al 1980; Little et al 1981; Mihaly et al 1982) and the clinical significance of such hepatic drug–drug interactions is recognized (Serlin et al 1979; Klotz et al 1979). The benzimidazoles have widespread therapeutic applications; however the ability of these substituted imidazoles to inhibit drug metabolism has received little attention (Dickins & Bridges 1982; Murray & Ryan 1983; James & Little 1983).

The aim of this study was to screen the anthelmintic benzimidazoles, mebendazole, albendazole and flubendazole for any propensity to influence the disposition of antipyrine or tolbutamide, which are two model substrates used to evaluate M.F.O. activity. The experimental model chosen was the isolated perfused rat liver preparation (IPRL). This model has previously been used to investigate hepatic drug–drug interactions for the imidazoles, cimetidine and ranitidine (Mihaly et al 1982) the benzimidazole omeprazole (Webster et al 1984) and the 8-aminoquinoline primaquine (Mihaly et al 1984). The results obtained from these experimental studies correlated well with the results obtained from investigations in man (Henry et al 1980; Gugler & Jensen 1984; Back et al 1983).

Methods

Male Wistar rats were anaesthetized with sodium pentobarbitone (60 mg ml^{-1}; 60 mg kg^{-1} i.p.), their livers were isolated using standard techniques and then perfused in a constant flow (15 ml min^{-1}) recirculating system at 37 °C as previously described (Mihaly et al 1982). The principal indices of liver viability were steady oxygen consumption (1·5–2·0 μmol g liver^{-1} min^{-1}), sustained bile production (0·4–0·6 ml h^{-1}), constant perfusion pressure (6–8 cm of water), reproducible liver function test results and normal visual appearance.

The elimination of a 2·5 mg bolus dose of the test substrates, antipyrine and tolbutamide (as 10 mg ml^{-1} solutions), were each studied in the presence or absence of one of the three benzimidazoles, mebendazole, albendazole and flubendazole. The benzimidazoles were added as a 2·5 mg bolus dose into the perfusate reservoir (25 μl of a 100 mg ml^{-1} solution in formic acid as the vehicle) 5 min before administration of either antipyrine or tolbutamide. Eight experimental groups were studied (n = 5 in each group), (a) antipyrine (i.e. control group; 25 μl of formic acid was administered at time minus 5 min) (b) antipyrine plus mebendazole (c) antipyrine plus albendazole (d) antipyrine plus flubendazole (e) tolbutamide (i.e. control group; 25 μl of formic acid was administered at time minus 5 min) (f) tolbutamide plus mebendazole (g) tolbutamide plus albendazole (h) tolbutamide plus flubendazole.

Samples (1·0 ml) were taken from the perfusate reservoir immediately before administration of the test substrate and at 30, 60, 90, 120, 150, 180, 210 and 240 min for the measurement of antipyrine or tolbutamide levels. An equal volume of fresh perfusate was added to the reservoir to replace that removed by sampling. Perfusate antipyrine and tolbutamide levels were determined by high performance liquid chromatography using the methods by Danhof et al (1979) and Nation et al (1978) respectively.

Pharmacokinetic parameters for antipyrine and tolbutamide were calculated using standard model independent formulae (Gibaldi & Perrier 1982). Statistical comparison was made using the Student's non-paired *t*-statistic, accepting $P < 0.05$ as the level of significance. Data are presented as mean ±s.d.

Results and discussion

In all eight experimental groups, the perfusate levels of either antipyrine or tolbutamide followed a monoexponential decline. Due to the poor solubility of the benzimidazole compounds in water, it was necessary to prepare solutions of these substances using formic acid as the vehicle. The administration of formic acid however, did not affect any of the indices of liver

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Table 1. Pharmacokinetic parameters for antipyrine and tolbutamide in the IPRL in the absence of any other drug (i.e. control) and after administration of mebendazole, albendazole or flubendazole to the perfusate (n = 5 in each group).

Test substrate	Pre-treatment	Clearance (ml min $^{-1}$)	Half-life (min)	Volume of distribution (ml)
Antipyrine	Control	0·63 ± 0·16	146·9 ± 36·0	128·2 ± 8·2
	Mebendazole	0·50 ± 0·06	179·4 ± 12·9	128·7 ± 12·9
	Albendazole	0·50 ± 0·04	186·0 ± 12·7	132·9 ± 10·8
	Flubendazole	0·38 ± 0·10*	216·8 ± 55·6*	113·6 ± 9·5*
Tolbutamide	Control	0·50 ± 0·10	214·5 ± 55·9	151·5 ± 26·1
	Mebendazole	0·53 ± 0·15	226·8 ± 62·6	162·2 ± 14·2
	Albendazole	0·61 ± 0·13	180·9 ± 26·6	156·2 ± 16·1
	Flubendazole	0·58 ± 0·09	207·9 ± 31·7	170·3 ± 5·2

*$P < 0·05$ compared with control.

viability in these studies, furthermore antipyrine elimination from the IPRL has previously been investigated (Mihaly et al 1982; Webster et al 1984); in these studies the pharmacokinetics of antipyrine, in the absence of any other compound, were in accordance with the values reported here for control preparations.

In the present study flubendazole significantly decreased the clearance of antipyrine by 40% compared to control values (Table 1). This was translated into a 50% increase in the elimination half-life and was accompanied by a slight (11%) although significant reduction in the volume of distribution of antipyrine. The reduced clearance and prolonged half-life of antipyrine suggests an inhibition of this drug's metabolism by flubendazole. However, this benzimidazole also appeared to produce a small effect on the distribution of antipyrine, presumably within the liver. By contrast, flubendazole had no significant effect on the disposition of tolbutamide, implying that in the IPRL model, this benzimidazole exhibits substrate specificity in its potential to inhibit drug metabolism. In addition, neither mebendazole nor albendazole produced any significant effect on the pharmacokinetics of either antipyrine or tolbutamide. This indicates that the presence of the benzimidazole nucleus in a molecule may not necessarily confer an inhibitory capacity against the mixed function oxidase system.

Furthermore, it has been suggested that imidazole substances, require an unsubstituted and sterically unhindered C-2 position, for binding with cytochrome P-450 (Wilkinson et al 1974). In this vein it is interesting to note that all three benzimidazoles carried substitutions in this position. In spite of this, flubendazole *did* inhibit antipyrine metabolism, illustrating that for this benzimidazole, the presence of other structural components, were important in determining the inhibitory effect observed in this study.

The disposition of test substances in the IPRL has previously been used to examine hepatic drug–drug metabolism interactions. In the case of the H_2-receptor antagonists, ranitidine and cimetidine (Mihaly et al 1982), the benzimidazole gastric acid secretion inhibitor, omeprazole (Webster et al 1984) and the antimalarial, primaquine (Mihaly et al 1984), the results derived from this experimental model have agreed qualitatively with the conclusions of clinical drug interaction studies (Henry et al 1980; Gugler & Jensen 1984; Back et al 1983).

The findings from the experiments described here indicate that the propensity to inhibit drug metabolism is not universal for all benzimidazoles. In the case of flubendazole the inhibitory effect seen in the IPRL may be manifested clinically in which case only specific metabolic pathways are likely to be affected.

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Effect of implanted ethylene-vinyl alcohol copolymer matrices containing 5-fluorouracil on Ehrlich ascites carcinoma

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The antitumour activity of ethylene-vinyl alcohol (EVAl) copolymer matrices containing 5-fluorouracil (5-FU) was evaluated against Ehrlich ascites carcinoma in mice. A prolongation of the life-span of tumour-bearing mice following intraperitoneal implantation of therapeutic matrices was noted. These results indicated that EVAl matrices containing 5-FU may be effective in cancer chemotherapy. Matrices composed of EVAl copolymer could be useful vehicles for implanted, inserted, or surface-applied delivery systems for anticancer agents.

To maximize the effectiveness of anticancer agents and to minimize their toxic side effects, topical administration of a controlled release preparation into cancerous lesions has been attempted. Unlike conventional routes of drug administration, controlled release systems that use implanted, inserted, or surface-applied polymeric vehicles can deliver a steady quantity of drug to a target area over long periods of time. A variety of synthetic polymer membranes or matrices have been employed as rate-controlling barriers in such systems, including silicone rubber (Rosenblum et al 1973), hydro-gels (Arlen et al 1972), polyethylene (Sato et al 1975), polylactic acid (Yolles et al 1975), vinyl polymers (Kaetsu et al 1980), and ethylene-vinyl acetate (Miyazaki et al 1982). Only limited work has been reported with the use of implantable polymer/drug composites in the treatment of tumour-bearing animals.

Ethylene-vinyl alcohol (EVAl) copolymers prepared from ethylene-vinyl acetate (EVAc) copolymers are non-toxic, flexible, and heat-processable. The unique characteristic of this copolymer, different from EVAc copolymer, is its hydrophilicity (Yamashita et al 1979). The safety and biocompatibility of the copolymer are reflected in its use as a haemodialysis membrane (Hoshino et al 1978). Physicochemical properties of the EVAl copolymer can be varied over a wide range by means of changes in the comonomer ratios (Iwasaki & Hoshino 1977). EVAl can be applied for the controlled release of hydrophilic drugs because of its hydrophilic character. However, no attention appears to have been directed to EVAl copolymer as a drug carrier except for our studies (Miyazaki et al 1981; 1983a, b).

In the previous paper (Miyazaki et al 1983b), it was demonstrated that the release rate for a potent anticancer agents, 5-fluorouracil (5-FU), could be easily controlled by modifying ethylene/vinyl alcohol ratios in the EVAl copolymer matrices. The present investigation was undertaken to evaluate the antitumour activity of implanted EVAl copolymer matrices containing 5-FU against Ehrlich ascites carcinoma in mice.

Materials

5-Fluorouracil (5-FU) was obtained from Sigma Chemical Co., St Louis and used without further purification. Ethylene-vinyl alcohol (EVAl) copolymers with 43, 54 and 60 mol% of ethylene unit were gifts from Kuraray Co., Tokyo.

Methods

Preparation of the EVAl copolymer matrices

Controlled release 5-FU copolymer matrices were prepared based on the method of Miyazaki et al (1983b). They were fabricated in the shape of a disk, 1 cm diameter and 0·2 cm thick. The EVAl copolymer (2 g) and the required amount of drug were dissolved in 40 ml of solvent (n-propanol–water, 3 : 1) at 80–85 °C. This mixture was poured onto a polyester film and the solvent was allowed to evaporate. The residue obtained was melt-pressed in a conical mould to produce a disk of

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