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The effect of intravenous pretreatment with small liposomes on the pharmacokinetics and metabolism of antipyrine in rabbits

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Abstract—The effect of intravenous pre-treatment with empty small liposomes on the pharmacokinetics and metabolism of antipyrine in rabbits has been investigated. The measured half-life of antipyrine was 104 min and the volume of distribution was 830 mL kg^{-1}. The excretion of metabolites in a 24 h urine sample was measured, the main metabolite 4-hydroxyantipyrine was excreted to a level of 10% with the free drug accounting for 4%. The norantipyrine and 3-hydroxymethylantipyrine metabolites were excreted to a level of 8 and 7%, respectively. The intravenous administration of liposomes at a dose equivalent to 8 mg of egg yolk phosphatidylcholine daily for one week, had no significant effect on any of the measured pharmacokinetic parameters. The half-life after liposome treatment was 110 min and the volume of distribution was 790 mL kg^{-1}, the metabolic pattern in the urine was also unaltered. The results suggest that the repeated administration of low doses of liposomes do not affect the pharmacokinetics and metabolism of antipyrine.

It has long been recognized that the simultaneous administration of two drugs to a patient or animal may result in some form of drug interaction (Conney 1967). There are many possible mechanisms by which this interaction can occur (Thomas 1990), ranging from changes in absorption from the gastrointestinal tract (Welling 1984), to increased metabolic clearance due to the induction of drug metabolizing enzymes (Breckenridge & Park 1990).

This latter type of interaction has been reported for a large number of drugs ranging from phenobarbitone with antipyrine (Chambers & Jefferson 1982), to cimetidine and warfarin (Serlin et al 1979). In the former case the clearance of the antipyrine was increased due to the induction of drug-metabolizing enzymes after pretreatment with phenobarbitone. In the cimetidine-warfarin example the clearance of warfarin was reduced by the inhibition of drug metabolizing enzymes due to the cimetidine. In both of these examples the elimination of one drug was affected by the administration of the second drug; however, changes can also occur in the metabolism of a drug without simultaneous changes in the pharmacokinetic parameters (Edeki et al 1990). This type of interaction is an ever present complication of the introduction of new therapeutic agents (Wijands et al 1984).

The increasing research into the use of systemic drug delivery or drug-targeting systems such as liposomes (Lopez-Berestein & Fidler 1989), has demonstrated that these systems can alter the pharmacokinetics and distribution of the entrapped drug. Drug distribution is altered to mirror that of the targeting system (Azmin et al 1985) and usually a prolonged plasma concentration is achieved due to a sustained release effect from the vehicle. Some studies have also indicated that the metabolic profile of the drug may also be altered by entrapment. The levels of adriamycin-7-deoxyaglycone, for example, are higher when adriamycin is administered intravenously in the vesicular form rather than as the free drug (Rogerson et al 1988). Antipyrine metabolism is also altered by entrapment in small vesicular carrier systems (Al-Angary & Halbert 1990), with a decreased quantity of 4-hydroxyantipyrine excreted in the urine but increased amounts of norantipyrine and 3-hydroxymethylantipyrine. The alterations in the pharmacokinetics of the drug are due to sustained drug release from the carrier, but the changes in metabolism may be due to the altered distribution of the drug or to an intrinsic property of the carrier itself.

The studies listed above have all been conducted using a single administration of entrapped drug; however, several liposomal preparations are at the clinical trial stage and will require for clinical use repeated administration. Since these carriers localize in the liver and spleen after intravenous administration (Davis & Illum 1986; Maruyama et al 1990) it is possible that they will also alter the disposition and metabolism of other concurrently administered drugs.

To investigate if liposomes themselves have the ability to alter the pharmacokinetics or metabolism of concurrently administered drugs, we have studied the effect of pretreatment with empty small liposomes on the pharmacokinetics and metabolism of antipyrine in male New Zealand White rabbits.

Materials and methods

Materials. Egg yolk phosphatidyl choline (EYPC), dicetyl phosphate (DCP) and antipyrine were purchased from Sigma Chemical Company, Poole, Dorset, UK. Cholesterol (Biochemical Grade) was obtained from BDH, Glasgow. 4-Hydroxyantipyrine and norantipyrine were purchased from the Aldrich Chemical Company, Dorset, UK. 3-Hydroxymethylantipyrine was a gift from Professor D. Breimer, Leiden University, The Netherlands. All organic solvents used in the assay were of HPLC grade and phosphate buffered saline (PBS) was produced from tablets purchased from Oxoid, UK.

Methods. Preparation of liposomes. A solution of lipids (620 μmol in 10 mL chloroform) was placed in a 50 mL round bottomed flask, and the solvent removed at room temperature (21°C) under reduced pressure using a rotary evaporator. The resultant lipid film was hydrated for 2 h with PBS at 50°C. The large multilamellar vesicles formed were sonicated (at 50°C) for 1 min using an MSE 150 W sonicator with a titanium probe, and power set at 12% of maximum output. The vesicles were kept at the sonication temperature for 30 min, then filtered (0·22 μm) to remove residual titanium. All operations were carried out aseptically using sterile materials under a N_2 gas blanket. The liposomes were stored at 4°C under N_2 and samples were withdrawn aseptically as required. Liposome composition was a 7:2:1 molar ratio of EYPC:cholesterol:DCP.

Photon correlation spectroscopy. A photon correlation spectrometer (Malvern Instruments Model 7027) with 60 channels was used with a He/Ne laser operating at 632·6 nm with a maximal power output of 40 mW. All samples were thermostated to 25°C and the measurements were made at an angle of 90° to the incident beam. Collected data was treated as previously described to obtain the Stoke's radius and polydispersity coefficient (Halbert et al 1984).

Animal model. Male New Zealand White rabbits weighing 2–3 kg were used throughout. Antipyrine solution (6·81% w/v in

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Water for Injections) was injected at a dose of 12·5 mg kg^{-1} via the marginal ear vein. Blood samples were removed from the other marginal ear vein every 10 min for the first hour then after 2, 4 and 6 h. Free access to food and water was allowed before the start of the experiment and after the first hour. A single 24 h urine sample was obtained by the use of a metabolic cage. The free drug was administered first followed by a seven day rest period; liposomes were then administered daily by intravenous injection (equivalent to 8 mg of EYPC/injection) for seven days, followed on the eighth day by a second dose of antipyrine.

HPLC analysis for antipyrine and metabolites. Blood samples. Blood (0·5 mL) was centrifuged, 0·2 mL of plasma collected and 10 μg of phenacetin added (internal standard). The sample was then extracted using a solid phase extraction technique.

Urine samples. Urine was centrifuged, 0·5 mL of the supernatant mixed with 0·5 mL acetate buffer (0·5 M, pH 4·5), containing 6000 units β-glucuronidase (type HP-25 from *Helix pomatia*, Sigma, UK), 10 μg of phenacetin and sodium metabisulphite (16 mg mL^{-1}). The solution was incubated at 37°C in a shaking water bath for 3 h before solid phase extraction.

Solid phase extraction. A C18 reversed phase column (Chromabond) was washed with methanol (2 mL) followed by distilled water (1 mL). The sample (0·2 mL) was applied, washed with distilled water (1 mL), and then eluted with methanol (0·2 mL).

HPLC analysis. Samples were analysed using the method described by Teunissen et al (1983) with minor modifications. A 5 μm MOS-Hypersil (100 × 4·6 mm) column was eluted with 0·02 M phosphate buffer pH 7·2: acetonitrile (85:15, v/v), at a flow rate of 2 mL min^{-1} (Spectra Physics pump SP8810). Samples were injected using a 20 μL loop and detected at 254 nm using an SP8450 UV detector linked to an SP4290 integrator. Concentrations were determined by reference to standard calibration curves.

Pharmacokinetic calculations. Terminal elimination half-lives were calculated using linear regression; the area under the curve (AUC) was calculated using the trapezoidal method with extrapolation to infinity, and was used to calculate the volume of distribution (Vd). A statistical comparison of the results was performed using a two-sample Mann-Whitney test.

Results and discussion

The liposomes produced in this study had an average measured diameter of 170 nm (150 and 190 nm) and showed no size variation on storage at 4°C during the experiment. The size of the liposomes is similar to the values for sonicated liposomes presented in the literature (Machy & Leserman 1983) and these vesicles should on intravenous injection passively target to the reticuloendothelial system in the liver and spleen (Davis & Illum 1986; Maruyama et al 1990). On intravenous administration no overt toxicity was noted and all animals increased in weight during the experiment.

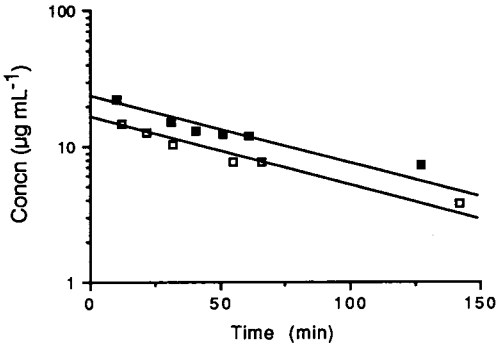

FIG. 1. Antipyrine plasma concentration time plot in Rabbit 2. ■ Control experiment, □ after pretreatment with liposomes.

On intravenous injection antipyrine exhibits classical one-compartment pharmacokinetics (Fig. 1) with a simple monoexponential decline in the plasma concentration with time. The average half-life is 104 min (Table 1) a value which is greater than the reported literature values of 67 (Chambers & Jefferson 1982) and 63 min (Quinn et al 1958). The calculated Vd was 830 mL kg^{-1} a value which is in close agreement with the reported literature values of 600–800 mL kg^{-1} (Shukla et al 1984). It is interesting to note that one of the animals displayed an uncharacteristically long half-life, possibly related to genetic variation in drug metabolism. The metabolites excreted in urine were measured and the results are presented in Table 2. Free antipyrine constitutes approximately 4% of the dosed drug with 4-hydroxyantipyrine and norantipyrine excreted to a level of 10 and 8%, respectively. The 3-hydroxymethylantipyrine metabolite accounts for 7% of the dosed drug and these levels are similar to values already reported in the literature for other species (Danhof et al 1981).

The effect of pretreatment with liposomes on the pharmacokinetics of antipyrine is presented in Table 1 and Fig. 1. No statistically significant change in the measured parameters was found. The average half-life was 110 min with the Vd similar at 790 mL kg^{-1}. The metabolic pattern of the urine sample (Table 2) was also similar to the control and no statistically significant differences were noted.

The results of this study demonstrate that intravenous pretreatment with empty liposomes at a dose of approximately 3–3·5 mg kg^{-1} produces no effect on the pharmacokinetics and metabolism of antipyrine. The results suggest that the metabolic changes noted on the incorporation of antipyrine into small liposomes (Al-Angary & Halbert 1990) are related to the effects of drug entrapment and altered drug distribution and not an intrinsic effect of the liposome on the drug metabolizing

Table 1. Calculated pharmacokinetic values.

	Control			After liposome pretreatment		
	Half-life (min)	Clearance (L min^{-1})	Volume of distribution (mL kg^{-1})	Half-life (min)	Clearance (L min^{-1})	Volume of distribution (mL kg^{-1})
1	169	0·011	1075	192	0·008	776
2	81	0·012	585	69	0·021	814
3	73	0·018	895	90	0·014	872
4	95	0·014	752	88	0·015	687
mean ± s.d.	104 ± 44	0·014 ± 0·003	827 ± 208	110 ± 56	0·014 ± 0·005	787 ± 78

Table 2. Excretion of antipyrine and metabolites in 24 h urine sample.

	Percentage of dose administered			
	Antipyrine	Norantipyrine	4-Hydroxyantipyrine	HMA
Control	4·4±2·1	8·3±7·0	10·5±11·6	7·2±5·7
After liposome pretreatment	4·6±3·5	11·6±6·2	6·2±2·4	5·3±1·5

HMA, 3-hydroxymethylantipyrine. Results, mean±s.d., n=4.

enzymes. However, the dose of liposomes used in this study is relatively low and it is still possible that pharmacokinetic and metabolic changes may be encountered at higher doses of liposomes.

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