J. Pharm. Pharmacol. 1987, 39: 485–487
Communicated November 4, 1986

The influence of drug sorption on pharmacokinetic studies of chlormethiazole and lignocaine

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The influence of drug sorption on the measurement of dose and blood concentrations during pharmacokinetic studies of chlormethiazole and lignocaine in a chronically catheterized sheep preparation has been examined. There was no sorption to soda glass tubes, borosilicate glass volumetric flasks or soda glass microlitre syringes but minor sorption to polypropylene syringes, polypropylene pipette tips and rubber bottle stoppers after 240 min contact. During infusions through administration sets including either polyvinyl chloride or polyethylene catheters, no significant loss of lignocaine occurred, but only 41·7–63·9% of the chlormethiazole dose was delivered. No significant decreases in either drug occurred from blood sampled through an intravascular catheter and stopcock system. There was negligible degradation of the samples over 4 h. Sorption of chlormethiazole or lignocaine to the laboratory equipment used was not a significant source of error but polyvinyl chloride infusion catheters could result in significant reductions in chlormethiazole dose.

As pharmacokinetic studies require the accurate determination of both drug dose and drug blood concentrations, we have examined drug losses occurring due to sorption during the preparation and delivery of the dose, the sampling of blood, and the assay of drug during pharmacokinetic studies of chlormethiazole and lignocaine in a chronically catheterized sheep preparation (Runciman et al 1984a). These drugs were intensively studied because previous reports have indicated that they may be subject to substantial losses under some circumstances (Cossum & Roberts 1981; Lackner et al 1983) and because it was suspected that such losses may have contributed to the unusually high total body clearances of these drugs in the sheep preparation (Mather et al 1986; Runciman et al 1983, 1986).

Materials and methods

The surgical preparation of animals and methodology for pharmacokinetic studies in the sheep preparation have been described by Runciman et al (1984a,b, 1986).

Drug analysis. Aqueous solutions of drugs were assayed using HPLC by direct injection onto a C_{18} reverse phase column (Waters Associates, Milford, P/N 27324) with UV detection at 254 nm. For chlormethiazole, the mobile phase was 60% methanol : 40% 0·5% PICA (Waters) in water at 2 mL min^{-1}; injection volume was 20 μL. Blood and low (μg) concentrations of aqueous solutions were assayed using a modification of the method of Seow et al (1981) (CV 2·5% at 2 μg mL^{-1}). For lignocaine the mobile phase was 65% methanol : 35% 0·1 M sodium dihydrogen phosphate buffer and the injection volume was 20 μL. Blood and low (μg) concentrations of aqueous solutions were assayed using a modification of the method of Mather & Tucker (1974) (CV of 3·5% at 2·1 μg mL^{-1}).

Degradation and sorption from aqueous solutions. Solutions of chlormethiazole edisylate (Astra Pharmaceuticals, North Ryde, NSW) 2 and 8 mg mL^{-1} in dextrose, and of lignocaine hydrochloride (Astra) 6 and 30 mg mL^{-1} in sterile water, were placed in 10 mL soda glass tubes fitted with Teflon-lined screw caps. The degradation of these solutions over 7 h at 4 and 25 °C was determined at 0·5, 10, 240 and 420 min. The original solution was the external standard.

Sorption during contact with laboratory equipment consisting of 100 μL soda glass syringes (Scientific Glass Engineering, Melbourne, VIC), 10 mL borosilicate glass volumetric flasks (Pyrex, USA), 5 mL polypropylene syringes (Terumo, Melbourne, VIC), polypropylene pipette tips (Oxford, St Louis, Missouri) and the rubber stopper of sterile mixing bottles (Pharmacy, Flinders Medical Centre, Bedford Park, SA) (all n = 3) was examined. Drug solutions were left in contact with the equipment, as if in normal use, for 0·5, 10, 240 and 420 min at 25 °C.

Sorption during infusion was examined by simulating infusions with a 50 cc soda glass syringe (Top Surgical, Tokyo, Japan) in a syringe pump (Harvard Apparatus, Millis, Massachusetts) connected to a polypropylene 3-way tap (Top Surgical, Tokyo, Japan), two polyvinyl chloride 30 inch minimum volume extension tubes (Tuta Laboratories, Lane Cove, NSW), a double stopcock (Cobe Laboratories, Lakewood, Colorado) and either a polyethylene intravascular catheter (William A. Cook, Surry Hills, NSW) or the distal lumen of a polyvinyl chloride quadruple lumen thermodilution pulmonary artery catheter (Edwards Laboratories, Irvine, California). The infusion rate was of 10 mL h^{-1} for 240 min at 25 °C. Effluent collected at 15 min intervals was assayed. For chlormethiazole, some samples were also taken at 60 min intervals from sites *between* each component of the infusion systems.

Because of the possibility that drug may diffuse through the septum separating the lumens of the thermodilution pulmonary catheter into the contents of an adjacent lumen, the sampling lumen was filled with sterile water while chlormethiazole or lignocaine solu-

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tions were infused through the other lumen. The water was replaced every 30 min and assayed for the drugs.

Degradation and sorption from blood. The routine blood sampling system consisting of a 100 cm polyethylene intravascular catheter (Cordis Corporation, Miami, Florida), a polypropylene double stopcock (Cobe Laboratories, Lakewood, California), a polyvinyl chloride 30 inch minimum volume extension tube set (Tuta Laboratories, Lane Cove, NSW), another double stopcock, and a 1 mL polypropylene syringe (Terumo, Melbourne, VIC) was assembled for studies in-vitro. Heparinized arterial sheep blood (50 mL) in 250 mL beakers in an oscillating water bath at 37 °C, was spiked with drug solution and mixed to give final concentrations of 0·9 and 2·5 μg mL^{-1} for lignocaine and chlormethiazole, respectively. Over 15 min, five 1 mL aliquots were taken directly into either 1 mL plastic syringes, or 1 mL syringes via the sampling system. Calibration samples were prepared by spiking 1 mL samples of the same blood directly over a 0–2·5 μg mL^{-1} concentration range.

Loss of drug during storage of sheep arterial blood samples was examined by placing 1 mL into heparinized microtubes (Eppendorf, Hamburg, FRG) together with drug solution in internal standard (bromothiazole for chlormethiazole; mepivacaine for lignocaine) to give a range of blood concentrations (n = 9) equivalent to a standard curve. Of these, three were frozen immediately, three were stored on ice for 4 h and then frozen, and three were stored at 25 °C for 4 h and then frozen; drug concentrations were assayed 24 h later.

Statistical analysis. Statistical significance was determined by Student's *t*-test using 95% confidence limits.

Results

Aqueous solutions. Both chlormethiazole and lignocaine solutions were stable at 4 and 25 °C in the glass tubes and there were no losses from the volumetric flasks or microlitre syringes. Contact of chlormethiazole solutions with the 5 mL plastic syringe led to a 7 and 12% increase in concentration of the 2 and 8 mg mL^{-1} solutions, respectively, at 240 min. Their contact with the pipette tip led to a 3% increase in concentration after 240 min. Contact of the lignocaine solutions for extended periods with the rubber stopper led to a 4% decrease at 240 min for the 6 mg mL^{-1} solution and a 14% decrease at 420 min for the 30 mg mL^{-1} solution. However, both chlormethiazole solutions with the rubber stopper led to apparent increases in concentration even after only 0·5 min contact.

Lignocaine infusions resulted in >98% of the dose being delivered, while those of chlormethiazole resulted in 41·7–63·9% of the dose being delivered. The total drug doses delivered during simulated infusions of chlormethiazole are in Table 1. In all cases, the effluent concentration was low initially and slowly increased.

Table 1. The percentage of total dose delivered during simulated infusions of chlormethiazole at 10 mL h^{-1} for 4 hours. The results represent the mean and standard deviation of three infusions.

	Infusion system and catheter	
Drug solution	Thermodilution catheter	Polyethylene catheter
2 mg mL^{-1}	55·4 ± 10·0*	63·9 ± 8·1*
8 mg mL^{-1}	41·7 ± 4·5*	50·9 ± 6·9*

* Statistically different from original solution $P < 0·05$, Student's *t*-test.

From samples of chlormethiazole solution taken between the different components most of the loss was found in the polyvinyl chloride extension tubes. No loss occurred through the polyethylene catheter, but the thermodilution catheter resulted in a 10·3 ± 0% decrease.

Both chlormethiazole (0·22 ± 0·5 μg mL^{-1}), and lignocaine (0·01 ± 0·004 μg mL^{-1}) were detected in the lumen adjacent to the infusion lumen of the thermodilution catheter.

Blood. There was no significant sorption of either drug from blood that had passed through the sampling system. The slopes of the standard curves of chlormethiazole were not significantly different between treatments; those of lignocaine showed a 4% decrease when stored on ice or at 25 °C.

Discussion

No errors in drug dose or blood concentration occurred following contact of chlormethiazole or lignocaine with glass during the preparation of either the infusion solution or the calibration solution, although adsorption to glass can occur at low concentrations of some drugs (Josephson et al 1979). The time- and concentration-dependent sorption of lignocaine by the rubber stopper was consistent with partitioning of the un-ionized form of the drug into the hydrophobic environment of the rubber. This would not cause errors during normal use as contact rarely exceeds 2 min. In contrast, the apparent increases in chlormethiazole concentration with prolonged contact with the 5 mL plastic syringe and pipette tip, and for short periods of contact with the rubber stopper were unexpected. This could have been due to the leaching from the plastic and rubber of a compound(s) with similar chromatographic properties to chlormethiazole. Others have previously noted that contaminants may leach into solution from the rubber seal of plastic syringes (Petersen et al 1981).

The most significant loss that could contribute to errors in dose calculations was the sorption of chlormethiazole into the polyvinyl chloride components of the infusion systems, and this is consistent with other reports (Kowaluk et al 1981; Cossum & Roberts 1981;

Tsuei et al 1980). Since its infusion through polyethylene catheters did result in significant sorption, errors could be avoided by the use of polyethylene infusion catheters and tubing. Correction of these losses is necessary to avoid the over-estimation of dose related pharmacokinetic parameters (Tsuei et al 1980).

Both drugs diffused across the septum separating the lumens of the thermodilution catheter, resulting in concentrations of the same order of magnitude as the blood concentrations.

The authors wish to acknowledge the skilled assistance and helpful advice of Mr C. F. McLean, and the financial support of the National Health and Medical Research Council of Australia. RNU is supported by a National Health and Medical Research Council Biomedical Postgraduate Scholarship.

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J. Pharm. Pharmacol. 1987, 39: 487–490
Communicated December 12, 1986

A gamma scintigraphic evaluation of the precorneal residence of liposomal formulations in the rabbit

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Multilamellar liposomes were prepared from dipalmitoyl phosphatidylcholine or egg lecithin in combination with cholesterol and either dicetyl phosphate or stearylamine. The size and charge of the colloidal preparations were characterized before labelling with [^{111}In]8-hydroxyquinoline. Freshly labelled liposomes were instilled into the eyes of unanaesthetized NZW rabbits and their disposition and drainage followed using gamma scintigraphy. A positive surface charge was found to affect significantly liposomal drainage rate, whereas an increase in size restricted drainage from the inner canthal region. Drainage of the suspending medium was directly compared with liposomes by labelling the medium with [^{99m}Tc] sodium pertechnetate and following the simultaneous change in removal of ^{99m}Tc and ^{111}In from the precorneal area. Slower drainage rates were obtained for the suspending medium compared with solutions of the isotopes suggesting that the liposomes restricted solution drainage.

The most commonly used and convenient route of drug administration to the eye is the topical application of ophthalmic formulations based on aqueous vehicles or petrolatum. Solutions have the disadvantage that most of the instilled drug is lost due to drainage via the nasolacrimal duct within the first 15 to 30 s after instillation (Shell 1982). Conversely, oily bases cause blurring of vision and gumming of the eyelids.

Biodegradable colloids such as liposomes have received attention recently as potential ophthalmic delivery systems. These can be easily prepared from non-toxic materials, which are non-irritant and do not obscure vision. Their surface properties may be altered to confer surface charges or ligands such as lectins to improve adhesion to the cell surfaces (Ketis & Grant 1982). However, there are two possible mechanisms since microparticles above a certain size may physically retard drainage by blocking the inner puncta (Sieg & Triplett 1980).

Schaeffer & Krohn (1982), using an *in-vitro* technique, have demonstrated that positively charged unilamellar liposomes enhance the corneal penetration of penicillin incorporated into the formulation to a greater extent than the drug encapsulated into either negatively charged or neutral liposomes. Positively charged small unilamellar vesicles (SUV) were found to be more effective than positively charged multilamellar vesicles

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