RÔLE OF BILE IN THE ELIMINATION OF DIPROPYL SULPHONE FROM THE MALE RAT

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SUMMARY

Following oral administration of [^{35}S]-dipropyl sulphone to male Wistar rats (4.24 mmol/kg body wt), the only radioactive component subsequently found in the blood and bile was the sulphone. Biliary excretion played an important rôle in the elimination of this compound, with 16% of the dose excreted during the first twenty-four hours and 33% passing through the bile duct over a two day period. Bile/plasma concentration ratios remained constant during the first day (*c.* 46-fold), suggesting that a concentration process was taking place and that active transport of this low molecular weight compound (150 Da) into the bile was occurring.

KEY WORDS

dipropyl sulphone, bile, rat, molecular weight threshold

INTRODUCTION

The potential contribution of the hepatic biliary system in the distribution and elimination of xenobiotics of low molecular weight is an area which has received relatively little attention since the generalised acceptance that a minimum molecular weight is necessary before significant biliary secretion can occur. Recent investigations into the fate of orally administered radiolabelled dipropyl sulphone, a symmetrical unbranched dialkyl compound with a low molecular weight, have shown that although urine was the major route of excretion with small

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amounts appearing in the faeces, a substantial amount of radioactivity had made its way into the bile /1/. This is unusual for a compound of such low molecular weight. The present paper reports a detailed investigation into the role of biliary secretion following dipropyl sulphone administration to the rat.

MATERIALS AND METHODS

Chemicals

Dipropyl sulphide, dipropyl sulphoxide, dipropyl sulphone and 1-bromopropane were obtained from Aldrich Chemical Co. Ltd. (Dorset, UK) and sodium sulphide from Sigma Chemical Co. Ltd. (Dorset, UK). All other chemicals were of analytical grade and readily available within the laboratory. The purity of dipropyl sulphone was increased from 97.2% to 99.5% by dissolving it in a minimum quantity of cold diethyl ether (1 vol) and extracting the filtered ether with ice-cold water (3 vol). Evaporation at 40°C of the separated ether layer permitted the dissolved sulphone to crystallize before being dried over anhydrous silica gel. Capillary gas-chromatography (see below) indicated that dipropyl sulphoxide was the major impurity.

Dipropyl-[^{35}S]-sulphone was synthesized from sodium [^{35}S]-sulphide (Amersham International plc, Bucks, UK) via dipropyl [^{35}S]-sulphide as previously described in detail /2/. Briefly, 1-bromopropane was refluxed with sodium [^{35}S]-sulphide in aqueous ethanol (70% v/v) for 7-8 hours /3/. After 'salting out', the upper oily layer of dipropyl [^{35}S]-sulphide was purified by distillation (142°C) and then refluxed for 2 hours with aqueous hydrogen peroxide (30% v/v) in an equal mixture of acetic acid and acetic anhydride /4/. Following extraction with petroleum ether (b.p. 40-60°C) and distillation at 119°C, the residue was recrystallised from ethanol/water. Slow cooling to -20°C precipitated crystals of dipropyl-[^{35}S]-sulphone which were removed and dried in a vacuum dessicator over phosphorus pentoxide at 4°C in the dark.

The translucent plate-like crystals (m.p. 29-30°C uncorr.; lit. value 28-30°C, /4-6/) were collected and stored at -20°C in nitrogen flushed amber vials. Chemical and radiochemical purities (by gas chromatography-mass spectrometry [g.c.-m.s.] and t.l.c. /1/) were in excess of 99.5% with a specific activity of 23.6 mCi/mol (radiochemical yield 60-70%). As expected, a strong I.R. absorption band at 2970 cm^{-1} was

observed (n-propyl chain) together with strong to medium-strong bands at 1280 and 790 cm^{-1}, characteristic of the sulphone moiety /7, 8/. A molecular ion at m/z 150 was discernable (0.5% abundance) with a base peak at m/z 43 (propylene) and a disintegration pattern dominated by alkyl chain fragments, many retaining the intact sulphone moiety /2,9-11/.

Animal dosing

Dipropyl [^{35}S]-sulphone, dissolved in corn oil, was administered orally (4.24 mmol/4 ml/kg body weight; 25 μCi per rat) to adult male rats (Wistar strain, 250 g; National Institute of Medical Research, London, UK) following an overnight fast. Until cannulation, animals were permitted free access to food ('Lab Sure' rat pellets; K.K. Greef Ltd., Croydon, UK) and water.

Bile studies

After administration of the radiolabelled compound as described above, rats were anaesthetized with sodium pentobarbitone (60 mg/kg body weight, i.p.; 'Sagatal', Rhône Poulenc, Essex, UK). A ventral mid-line incision was made just caudal to the xiphoid cartilage and the common bile duct located beneath the right lobe of the liver, lying within the duodenal loop. Two lengths of cotton thread were passed beneath the duct, one being tied around the duct at its junction with the duodenum to occlude biliary flow and thus distend the vessel. Polythene tubing (0.61 mm o.d., 0.28 mm i.d.; Portex Ltd., Kent, UK) was introduced into the bile duct through a small puncture and the second thread tightened to secure the cannula. The body wall and overlying skin were sutured separately, the cannula emerging at the caudal end of the incision after being anchored to the muscular abdominal wall with thread. Anaesthesia was augmented where necessary (24 mg/kg body weight) so that all of the rats remained unconscious throughout the study which continued for up to six hours before the rats were killed. Bile was collected from a large series of animals, so that at least three separate samples were obtained for each hour period. Varying the time intervals between dosing and surgery permitted a complete 0-48 hour bile collection to be obtained.

Blood studies

After administration of [^{35}S]-dipropyl sulphone as previously described, rats were anaesthetized with sodium pentobarbitone (60 mg/kg body weight, i.p.; 'Sagatal'). A curved incision into the ventral side of the neck permitted the exposure of the left carotid artery lying adjacent to the vagus nerve, beneath the digastric and omohyoid muscles and parallel with the trachea. The isolated artery was supported on three lengths of cotton thread, that distal to the heart formed a ligature around the artery which was then occluded with an artery clamp proximal to the heart. A polythene cannula (1.8 mm o.d., 0.4 mm i.d.; Portex Ltd.) was inserted through a puncture just caudal to the ligature and secured with the remaining two cotton threads before removal of the artery clamp. Blood samples (1.0 ml) were collected into heparinised tubes and a solution of heparin (10% v/v in isotonic saline) was employed to prevent clotting within the cannulae. The samples were centrifuged to obtain plasma. Anaesthesia was augmented when necessary (24 mg/kg body weight) so that all the rats remained unconscious throughout the study. A maximum of three consecutive blood samples was taken from a single animal which remained under anaesthesia for no more than four hours before being killed. As with the bile study, varying the time intervals between dosing and surgery permitted a complete two-day profile to be constructed.

Quantification of radioactivity

Aliquots (0.2 ml) of bile and plasma were added directly to vials containing scintillation fluid (10 ml, "Ecoscint'; National Diagnostics Ltd., NJ, USA) and counted by liquid scintillation spectrometry (Packard Tricarb 4640; Ambac Industries Inc., IL, USA), internal standards being used for quench correction.

Sample extraction and chromatography

Pooled plasma and bile samples were extracted with toluene (3 x vol) and centrifuged. The toluene layers were separated, combined and evaporated to dryness under a stream of nitrogen. Following reconstitution to a minimal volume (c. 10 µl) with toluene, they were examined by g.c.-m.s. Extraction of known amounts of dipropyl sulphide, dipropyl sulphoxide and dipropyl sulphone from control biological material

permitted the establishment of lower detection limits (less than 0.3% administered dose) for these compounds.

Gas chromatography - mass spectrometry was carried out on a Hewlett Packard 5890 II series gas chromatograph fitted with a 5971 series mass selective detector (Hewlett Packard, Stockport, UK). The fused-silica capillary column (30 m x 0.25 mm i.d.) was coated (film thickness 0.25 μm) with cross-linked phenyl-methyl silicone (5%) with a helium gas flow rate of 1 ml/min. The column oven was initially held at 70°C for 2 min, then raised at 10°C/min until a temperature of 270°C was reached, which was maintained for a further 2 min. The injection port was held at 250°C. The g.c.-m.s. interface temperature, the ionization energy and the ion source temperature of the electron impact mass spectrometer were 280°C, 70eV and 185°C respectively. During instances when the mass selective detector was not activated, a flame-ionization detector held at 280°C was employed.

Spectrometric methods

Infrared spectra were obtained by injecting samples in the liquid state into a sodium chloride cell and placing in the scanning chamber of a Varian Fourier Transform I.R. (Varian Associates, Surrey, UK) where all water vapour was removed prior to recording spectra from 800 to 4000 cm^{-1}. Mass spectrometry was carried out using a Hewlett Packard 5971 series mass selective detector attached to the capillary gas chromatograph as described above.

RESULTS AND DISCUSSION

Metabolite identification

Examination of toluene extracts of bile and plasma by g.c.-m.s. showed only the presence of dipropyl sulphone (Rt = 10.6 min) in all samples, with no detectable (less than 0.3% administered dose) dipropyl sulphoxide (Rt = 9.6 min) or dipropyl sulphide (Rt = 4.6 min). Mass spectral examination of the appropriate peak confirmed the identity, with a small molecular ion (M^+) at *m/z* 150 ($C_6H_{14}SO_2$), a major peak at *m/z* 43 (C_3H_9) for the propyl side-chain and a fragmentation pattern identical to that of authentic dipropyl sulphone /2/. During initial studies with non-radioactive dipropyl sulphone, the sulphoxide was identified in both plasma and bile samples and accounted for about

2% of the administered dose. However, this minor component disappeared when purified material, both non-radioactive and radioactive, was employed, suggesting that the sulphoxide was an impurity in the purchased material, a point verified by g.c.-m.s. examination (97.2% sulphone, 2.3% sulphoxide, 0.5% unknown; lot no. KT 01905BM). Consequently, levels of radioactivity within the plasma and bile were taken as being directly indicative of dipropyl sulphone levels.

Biliary excretion of dipropyl sulphone

Biliary excretion was shown to play an important role in the distribution of this compound. A cumulative plot showed that 16% of the dose was excreted during the first twenty-four hours with 33% passing through the bile duct over a two day period (Fig. 1). A noticeably constant biliary excretion rate (0.87% dose/hour) was maintained between 12-38 hours following oral administration and this was reflective, in part, of steadily climbing plasma levels which peaked at the end of the first day. Calculated bile/plasma concentration ratios remained remarkably constant over the first twenty-four hours following dosing (46.3 ± 12.1; mean ± s.d.), although there was a significant increase ($p<0.1$, *t*-test) from the first twelve hour period (36.9 ± 6.0) to the next (58.0 ± 8.7) (Fig. 2). Ratios then increased dramatically after 30 hours (peaked at 42-46 hours, *c.* 390) owing to a rapid decrease in plasma levels. These high bile/plasma ratio values suggested that a concentration process was taking place and that active transport into the bile may be occurring in addition to simple diffusion.

General considerations

From early investigations undertaken with compounds which could readily ionize to yield strongly anionic polar groups (pK_a5 or less) /12/ it has been generally (perhaps erroneously) assumed that for extensive biliary excretion of any xenobiotic to occur there is a requirement for a minimum threshold molecular weight (rat 325 ± 50 Da) below which little (not more than 5-10% of the dose) biliary elimination can be expected to take place. The imprudent application of these initial stringent observations to all xenobiotic compounds may generate unsound conclusions.

A prolonged secretion, albeit of smaller amounts, can still lead to a substantial proportion of an administered dose passing through the biliary system and the absence of an initial surge cannot be taken as an

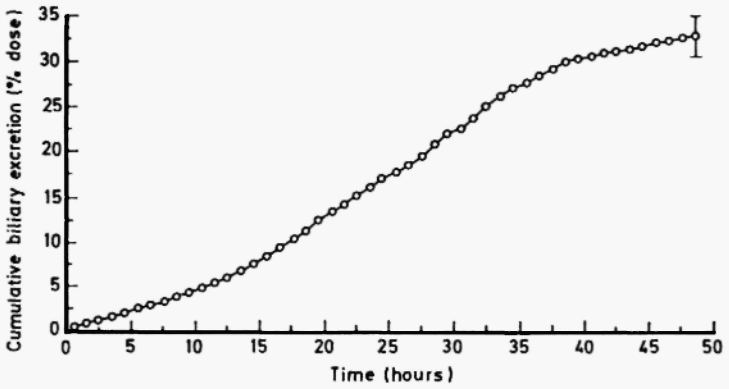

Fig. 1: Cumulative biliary excretion of [^{35}S]-dipropyl sulphone following its oral administration (4.24 mmol/kg body wt) to male Wistar rats. Each data point is based on at least 3 animals, the largest standard deviation being given at the point it was observed.

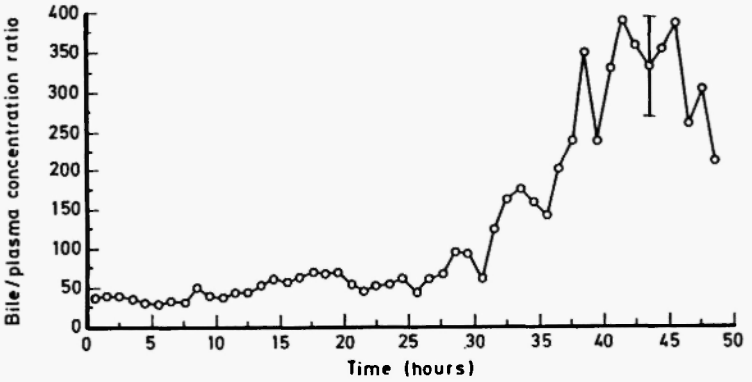

Fig. 2: Calculated bile/plasma [^{35}S]-dipropyl sulphone concentration ratios following the oral administration of [^{35}S]-dipropyl sulphone (4.24 mmol/kg body wt) to male Wistar rats. Each data point is based on at least 3 animals, the largest standard deviation being given at the point it was observed.

indication of the non-involvement of the biliary system in the distribution of a xenobiotic around the body. Dipropyl sulphone, with a molecular weight of only 150 Da, is excreted in the bile at a remarkably constant rate for at least 24 hours following a single oral administration. Therapeutic applications of such a maintained plateau concentration could include the delivery of anti-cancer drugs to the biliary system without the necessity of constant infusion.

Compounds containing a sulphoxide or sulphone moiety may interact strongly with their surrounding environment (via hydrogen bond formation, etc.) and thereby delay their excretion /13/. Enterohepatic circulation may be one of the processes which assist in this retention within the body. Indeed, initial observations suggested that the presence of a polar group in a molecule appeared to be a requirement for extensive biliary excretion to occur, but the exact definition of polar was elusive /12/. Dipropyl sulphone may be excreted in the bile because of one or more of a variety of factors. Charge separation existing around the S-O bond (more pronounced in sulphoxides) may result in molecular stacking or clusters, thereby increasing the apparent molecular weight. This is similar to an idea advanced to explain the lack of excretion of certain azo dyes such as congo red (697 Da) in the bile; its existence in solution as an aggregate of apparent molecular weight of about 8000 Da would be too large for biliary removal to occur /12/. Dipropyl sulphone may also undergo electrostatic 'piggy-backing' with other molecules being excreted or become accidentally integrated into complex molecular transport packages. The large bile/plasma concentration ratios observed, indicative of a major concentration gradient (plasma to bile), suggest the presence of active transport systems operating at one or both poles of the hepatocyte /14/ and imply that some organic ion transporter for such small molecular weight polar compounds is available /15-17/. Now that a suitable candidate has been uncovered, more detailed investigations are required to elucidate the molecular mechanisms involved in the biliary transport of such low molecular weight xenobiotics.

REFERENCES

1. Nickson RM, Mitchell SC, Zhang AQ. Fate of dipropyl sulphone in rat. Xenobiotica 1995; 25: 1391-1398.
2. Nickson RM, Mitchell SC. Fate of dipropyl sulphide and dipropyl sulphoxide in rat. Xenobiotica 1994; 24: 157-168.

3. Bost RW, Conn MW. Preparation of n-propyl sulphide. Organic Syntheses 1935; 15: 72-73.
4. Cumper CWN, Reid JF, Vogel AI. Physical properties and chemical constitution, Part XLIV: Electric dipole moments of some dialkyl sulphides, disulphides, sulphoxides and sulphones. J Chem Soc 1965; 5323-5330.
5. McAllan DT, Cullum TV, Dean RA, Fidler FA. The preparation and properties of sulfur compounds related to petroleum; I. The dialkyl sulfides and disulfides. J Am Chem Soc 1951; 73: 3627-3631.
6. Spring W, Winssinger C. Einwirkung von Chlor auf Sulfonverbindungen und Organische Oxysulfide. Bericht der Deutschen Chemischen Gesellschaft 1883; 16: 326-330.
7. Bellamy LJ. The infra-red spectra of complex molecules. London: Methuen, 1954; pp. 295-297.
8. Brunn J, Doerffel S. Die Schwingungsspektren einfacher sulfide, sulfoxide und sulfone im Bereich 500-1500 cm^{-1}. Wissenschaftliche Zeitschrift, Leuna-Merseburg 1971; 13 ser 2: 101-108.
9. Aplin RT, Bailey K. The mass spectra of alkyl sulphones. J Chem Soc 1967; B: 513-516.
10. Bowie JH, Williams DH. Studies in mass spectroscopy XV. Tetrahedron 1966; 22: 3515-3525.
11. Smakman R, DeBoer ThJ. The mass spectra of some aliphatic and alicyclic sulphoxides and sulphones. Organic Mass Spectrometry 1970; 3: 1561-1588.
12. Smith RL. The Excretory Function of Bile. London: Chapman and Hall, 1973; pp. 16-34.
13. Mitchell SC. Biological consequences of drug sulphoxidation. Drug Metab Drug Interact 1988; 6: 245-252.
14. Coleman R, Chipman JK. Factors governing biliary excretion. In: Hill MJ, ed. Role of Gut Bacteria in Human Toxicology and Pharmacology. London: Taylor and Francis, 1995; pp. 231-243.
15. Coleman R. Biochemistry of bile secretion. Biochem J 1987; 244: 249-261.
16. Klaasen CD, Watkins JB. Mechanisms of bile formation, hepatic uptake and biliary secretion. Pharmacol Rev 1984; 36: 2-67.
17. Nathanson MH, Boyer JL. Mechanisms and regulation of bile secretion. Hepatology 1991; 14: 551-566.