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An experimental animal model for studying the effects of a novel lymphatic drug delivery system for propranolol

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Summary

An experimental model using the anaesthetised pig is described which allowed the simultaneous collection of mesenteric lymph, hepatic portal blood and systemic blood samples. The levels of propranolol observed in lymph reached 103 ng/ml after intraduodenal administration of a standard preparation of the drug at a dose of 2 mg/kg. Dosing with a novel lymphatic drug delivery system containing an equivalent dose of the drug resulted in a lymph propranolol concentration of 278 ng/ml. Peak lymph propranolol levels with each delivery system occurred within 15 min of dosing and then rapidly declined. The hepatic portal blood concentrations of propranolol were initially lower than those observed in lymph in all cases and only equalled or exceeded peak lymph levels 30 min after dosing. The early appearance of propranolol in the systemic circulation is consistent with direct delivery from the lymphatic system.

Introduction

Propranolol is a widely accepted and clinically effective cardiovascular drug used in both human and veterinary medicine. Although almost completely absorbed from the gastrointestinal tract after oral administration, only a small percentage of the drug is usually systemically available. The poor bioavailability results from 'first-pass' clearance and metabolism of as much as 80% of the propranolol reaching the liver via the hepatic portal vein. Avoidance of the first-pass effect improves the systemic bioavailability of propranolol. Rectal administration and absorption avoids the hepatic first-pass effect since the haemorrhoidal veins drain directly into the cordal vena cava, and therefore the systemic circulation (Pletscher et al., 1960). For example, intra rectal administration increases propranolol systemic bioavailability from less than 10% to greater than 60% in rats (Iwamoto and Watanabe, 1985) and from an average of 18% to 41% in human subjects (Cid et al., 1986).

A further pathway by which orally administered drugs may avoid the hepatic first-pass effect is by absorption into the lymphatic system.

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Materials which are absorbed via the lymphatic system, which include long-chain fatty acids, cholesterol and fat soluble vitamins, enter the systemic circulation directly via the jugular vein. Also found in lymph are drugs absorbed from the gastrointestinal tract. The extent to which drugs enter the lymph has generally been found to relate to the lipid solubility of the drug and the concomitant absorption of lipids (De Marco and Levine, 1969; Sieber et al., 1974).

The present investigation describes a study in which propranolol contained in a novel lymphatic drug delivery system is compared with a standard preparation of the drug. The investigation was conducted in an anaesthetised pig model, specifically designed to assess lymphatic drug delivery systems in which simultaneous collection of peripheral and hepatic portal blood samples and lymph was possible.

Materials and Methods

Gilts weighing 30–40 kg were used. In this study pigs were kept in individual pens and fed a conventional weaner diet with milk powder containing 30% fat for at least 5 days; 100 g of butter containing 5 g Sudan Black was fed to the pigs on the evening before and on the morning of surgery. Anaesthesia was induced with azaperone and metomidate and maintained with halothane and nitrous oxide given with oxygen via an endotracheal tube. Hartmann's solution was given via a cranial vena cava catheter (Angiocath, Deseret) at the rate of 10 ml/kg per h. Following a mid-line laparotomy incision from the xiphisternum to the brim of the pelvis, the splenic vein running along the mesenteric attachment to the spleen was dissected out for 5 mm as near to the tip of the spleen as possible. A catheter made from Tygon tubing (o.d. 3.2 mm, i.d. 1.6 mm) was passed via the splenic vein and mesenteric veins into the hepatic portal vein, being directed by manipulation of the veins within the abdomen. A ligature was placed either side of the entry point of the catheter into the splenic vein to control reflux haemorrhage and to anchor the catheter in place. Furthermore, a cuff was placed 300 mm from the tip (this measurement had been determined from previous studies) which aided in fixing the catheter in place. The hepatic portal vein catheter was kept patent by a slow infusion of saline. To facilitate propranolol administration a section of the proximal duodenum was located and catheterised using a through-the-needle catheter (o.d. 1.34 mm). This was retained in place using a simple purse-string suture.

The Sudan Black was used to facilitate lymphatic catheterization since it gave the chyle a pale blue colour which could be seen through the transparent lymphatic walls against the darker coloured intestines. By careful dissection, a lymphatic vessel as near as possible to the mesenteric root was isolated and ligated. The vessel proximal to the ligature was left for a short time to become distended and then catheterised using either a through-the-needle catheter (o.d. 1.34 mm) or an over-the-needle catheter (o.d. 1.2 mm) directed towards the intestine and a ligature placed around the catheter and vessel wall.

Blood samples (volume 4 ml) were collected simultaneously from the hepatic portal and cordal vena cava catheter in cold (4° C) heparinised tubes. Initial samples were collected 30 and 15 min before dosing. Further samples were taken immediately after dosing, 5 min post-dose, and then at 15, 30, 75, 120, 180 and 240 min. Lymph was collected continuously into pre-weighed sample tubes, containing 50 IU heparin in 50 μl saline, which were replaced at the same time as each blood sample was taken. After collection, blood samples were prepared for analysis by centrifugation to remove red blood cells. Plasma samples were subsequently stored at 4° C. Lymph flow was determined gravimetrically assuming a density of 1 g/ml.

Three pigs (A–C) were used in this study. Each pig was given a different formulation of propranolol in 20 ml of buffer pH 7.4 via the duodenal catheter. Pigs A and B received two separate formulations of propranolol contained in a unique drug delivery format. The two separate formulations administered to pigs A and B contained, in addition to propranolol, a mixture of bile acids from the following list: taurocholate, taurochenodeoxycholate, taurodeoxycholate and cholic acid,

cocrystalized from an alcoholic solution. The dose administered was in each case 12 mg/kg body weight with a propranolol dose equivalent of 2 mg/kg. Pig C received a standard preparation of propranolol at a dose of 2 mg/kg body weight.

Propranolol and its metabolites were detected in pig plasma and lymph using a modified method based on that described by Harrison et al. (1985). Solid-phase extractions of pig plasma were carried out on C_{18} Bond-Elute^R columns, capacity 1 ml and containing 100 mg of solid phase column packing material, using a Vac-Elute^R system under a vacuum of 250–500 mmHg. The columns were initially activated by sequentially washing with two 1 ml aliquots of 0.1 M HCl, acetonitrile, and double distilled water. Extraction was carried out using 1 ml of lymph or pig plasma followed by two washes with 0.5 ml of water:acetonitrile (9:1). After releasing the vacuum, the Vac-Elute^R needles were wiped and standard 1.1 ml conical glass autosampler vials were placed under the columns. Propranolol glycol (a neutral metabolite of propranolol) was eluted from the column using two 0.5 ml aliquots of acetonitrile under vacuum. Fresh sample vials were used to collect propranolol together with its basic metabolites 4-hydroxypropranolol and *N*-desisopropylpropranolol which were eluted under vacuum using 0.5 ml of acetonitrile:0.1 M HCl (1:1) twice.

Conjugates in plasma were extracted using the same procedure described above following hydrolysis. Incubations carried out at 37°C contained 0.5 ml of plasma or standard, 0.1 ml of 1 M sodium acetate solution containing 20 mg/ml (w/v) ascorbic acid (as anti-oxidant), pH 4.8 and 0.1 ml of enzyme solution containing 10000 U/ml β-gluconidase and 370 U/ml of sulphatase (Sigma G0876). After 1 h, 0.2 ml of stopping reagent (0.1 M sodium hydroxide) was added, vortexed and centrifuged for 5 min at $1600 \times g$. A 0.7 ml aliquot of the supernatant was subjected to the Vac-Elute^R extraction described earlier.

Propranolol and its metabolites extracted from pig plasma and lymph were quantified using reverse phase high-performance liquid chromatography using a Milton Roy^R Constametric 3000 solvent delivery system, Fluromonitor III fluorescence detector, fitted with a 214 nm excitation filter and a 254 nm emission filter, and a CI4000 computing integrator. Samples were injected using a Milton Roy automatic sample injector, model 713, fitted with a 50 μl sample loop. Separations were carried out, in duplicate or triplicate, on a Varian Micropak^R 150 mm $\times$ 4 mM MCH-5-N-CAP analytical column. For the chromatographic separation of propranolol and its basic metabolites a mobile phase containing acetonitrile:0.1% v/v orthophosphoric acid (23:77) at a flow rate of 2 ml/min was used. For the metabolites propranolol glycol and naphthoxylitic acid, a mobile phase containing acetonitrile:water (30:70) was used.

A series of propranolol standards were prepared of known concentration, range 10–1000 ng/ml, and their resulting integrated peak areas determined by the HPLC method described earlier. A standard curve was constructed using the least squares method and the correlation coefficient found to be 0.998. A further series of standards were prepared in pig lymph and pig plasma, extracted and analysed. Recoveries were found to be in excess of 99%. The levels of propranolol in the samples were therefore determined by comparison of their integrated peak areas to those of the standards.

Results

The surgical procedure was well tolerated by the pigs. No problems were seen during the course of the experiment.

After dosing, lymph flow initially declined in all of the test animals compared to that recorded in the pre-dose period (Table 1). In the pig receiving the standard formulation of propranolol (pig C), lymph flow declined to 39 μl/min by 15 min and continued close to this level for the remainder of the experiment. In contrast, lymph flow, having initially decreased in both pig A and B steadily increased after 30 min until the end of the experimental period.

The peak secretory rates of propranolol in lymph occurred within the first 15 min in all three pigs. The secretory rate of propranolol was observed to be highest in pig A where the level was

TABLE 1

Comparison of lymph flow in pigs A, B and C

Pig	−15–0 min	0–5 min	5–15 min	15–30 min	30–75 min	75–120 min	120–180 min	180–240 min
A	175	150	104	120	78	112	149	155
B	224	150	132	119	138	154	203	292
C	96	61	39	40	40	35	40	27

The data represent the total lymph flow during the experimental period from each of the pigs. Lymph flow rates in μl/min were determined gravimetrically assuming a density of 1 g/ml. Pigs A and B received propranolol (2 mg/kg) in two different forms designed to enhance lymphatic drug delivery. Pig C received a standard preparation of the drug (2 mg/kg).

more than 2-fold greater than the control (pig C; Table 2), whereas pig B demonstrated a lower secretory rate than the control. The increase in lymph flow observed after 30 min with both pigs A and B did not correspond to any noticeable increase in propranolol secretion rate. HPLC analysis detected only free propranolol in pig lymph; no trace of any metabolites was observed.

Samples of hepatic portal blood plasma were observed to contain measurable amounts of propranolol within five minutes of drug administration. The control, pig C, achieved peak hepatic

TABLE 2

Secretion of propranolol into pig lymph

Pig	0–5 min	5–15 min	15–30 min	30–75 min	75–120 min	120–180 min	180–240 min
A	ND	2.89	0.17	0.01	0.19	0.10	0.03
		(278)	(22)	(8)	(78)	(39)	(10)
B	0.78	0.82	0.14	0.06	0.10	0.06	0.09
	(26)	(62)	(17)	(20)	(30)	(18)	(18)
C	1.26	0.20	0.14	0.03	0.02	0.01	0.01
	(103)	(52)	(52)	(36)	(19)	(19)	(22)

Values are either secretion rate in ng/min, or concentration (in parentheses) in ng/ml of propranolol in lymph and are means of triplicate determinations. In all cases the relative standard deviation of the values was less than 3%. Pigs A and B received propranolol (2 mg/kg) in two different forms designed to enhance lymphatic drug delivery. Pig C received a standard preparation of the drug (2 mg/ kg).

TABLE 3

Hepatic portal plasma concentrations of propranolol

Pig	5 min	15 min	30 min	75 min	120 min	180 min	240 min
A	16	23	18	125	360	206	184
B	4	21	35	32	15	46	108
C	75	88	273	96	89	235	162

Values represent the concentration of propranolol in ng/ml measured in hepatic portal blood and are expressed as means of duplicate or triplicate determinations. In all cases the relative standard deviation of the values was less than 3%. Pigs A and B received propranolol (2 mg/kg) in two different forms designed to enhance lymphatic drug delivery. Pig C received a standard preparation of the drug (2 mg/kg).

TABLE 4

Systemic plasma concentrations of propranolol

Pig	5 min	15 min	30 min	75 min	120 min	180 min	240 min
A	36	57	51	17	31	32	23
B	21	13	23	24	46	13	16
C	20	28	34	23	33	38	26

Values represent the concentration of propranolol measured in the systemic blood supply in ng/ml, and are means of duplicate or triplicate determinations. In all cases the relative standard deviation between determinations was less than 3%. Pigs A and B received propranolol (2 mg/kg) in two different forms designed to enhance lymphatic drug delivery. Pig C received a standard preparation of the drug (2 mg/kg).

portal plasma propranolol concentration after 30 min (Table 3). In comparison the onset of high hepatic portal propranolol was delayed in both pigs A and B until 75 and 240 min post-dose, respectively (Table 3). In all three pigs only traces of propranolol metabolites were detected in the hepatic portal plasma samples.

Propranolol was detected in systemic blood plasma samples 5 min after administration in pigs A–C. Plasma propranolol levels were generally higher in pig A than pig B or C (Table 4). Peak plasma propranolol concentration in the systemic circulation was achieved after 15 min with pig A, 120 min with pig B and 180 min with pig C (Table 4). All of the major propranolol metabolites were detected in the systemic blood samples collected from each pig.

Discussion

The surgical procedure described enabled concomitant collection of lymph, hepatic portal and systemic blood samples from anaesthetised pigs. The method used to collect hepatic portal blood requires little dissection and is associated with minimal blood loss. However, it does require care to achieve accurate placement of the catheter in the hepatic portal vein. Also the tip of the spleen is infarcted which is well tolerated without any detectable systemic effects.

Markowitz et al. (1959) have described a method for draining the lymphatics of dogs by surgically isolating the drainage of the thoracic duct into a jugular fistula. This option was rejected because (a) it is a chronic preparation, (b) in the pig it would require a thoracotomy, and (c) the method we used selectively collects intestinal lymph. This experimental model is ideally suited to the study of the distribution of drugs and their metabolites through these compartments after their absorption from the gastrointestinal tract. In the present situation this model system has been used to assess the function of a novel drug delivery system designed to enhance the absorption of propranolol through the lymphatic system as opposed to the hepatic portal blood supply. However, a model of this type could be equally well suited to assessing (i) factors affecting lymph formation, or (ii) absorption through the hepatic portal system (in which case lymphatic cannulation may not be required).

The present study has demonstrated that formulations of propranolol presented within a novel drug delivery system containing a mixture of bile acids can result in an enhanced lymphatic absorption of this drug. A noticeable characteristic of the lymphatic absorption of propranolol is the rapid appearance of the drug in lymph (Table 1) after dosing. In the case of pig A during the first 30 min, the systemic propranolol concentration was at least 2-fold greater than that measured in the hepatic portal blood supply (cf. Tables 3 and 4). Given that the surgical procedure used ensured only a proportion of the lymph draining from the duodenum was collected, the high levels of propranolol observed in the systemic circulation of pig A during the first 30 min suggest a significant contribution from the lymphatic system. Since the systemic blood levels of propranolol can only be

derived from the hepatic portal blood supply or the lymphatic system, it is likely that the lymphatic system was responsible for the delivery of sufficient propranolol to maintain levels above that found in the hepatic portal system. In the case of pigs B and C, this effect was less apparent, an observation which is consistent with the lower levels of lymphatic propranolol delivery measured.

When the propranolol metabolite content of the samples was assessed, it was observed that only propranolol was present in lymph together with only trace amounts of metabolites in the hepatic portal system. In contrast, large amounts of the propranolol metabolites, particularly 4-hydroxy propranolol conjugates, were found in the systemic circulation. Taken together, these observations are indicative that propranolol in lymph and hepatic portal blood are probably en route from the absorptive sites of the gastrointestinal tract.

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