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Statistical Moments for Placental Transfer of Solutes in Man

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Abstract

The placental transfer of red blood cells and solutes in man has been investigated by statistical moment analysis, using the impulse–response technique. Model compounds of different lipophilicity (sucrose, water, antipyrine, propranolol and labetalol) were injected with a vascular reference (labelled red blood cells) as boluses into either the foetal or maternal circulation of a single-pass perfused placental lobule. Maternal and foetal venous outflow fractions were collected at intervals ranging from 1 to 600 s. Perfusion was conducted at maternal flow rates of 4 and 6 mL min^{-1} and foetal flow rates of 2 and 3 mL min^{-1}, respectively, to yield a constant materno–foetal flow ratio of 2. The outflow concentration–time profile curves were analysed by statistical moment analysis.

The sum of foetal and maternal recovery was close to 100% for red blood cells, sucrose, water and antipyrine, but lower for propranolol and labetalol. The mean transit time (MTT) values ranged from 20 to 500 s. The normalized variance (CV^2) for red blood cells in the foetal and maternal circulation of the placenta were in the ranges 2·31 to 3·86 and 2·00 to 2·03, respectively.

The shape of the outflow concentration–time profiles after bolus input is consistent with that of vascular residence time models such as the dispersion model. The heterogeneity in red blood cell transit times, as defined by CV^2, is greater than in either the perfused leg or perfused liver.

It is now accepted that the placenta is not a significant barrier to the transfer of foreign compounds from the maternal blood to the foetus. Administration of a drug to pregnant women therefore raises the question of potential harmful effects in the foetus. Whereas animal models have been used to study materno–foetal drug transfer, the placentas of experimental animal species are widely different (Faber 1977) and, other than those of the guinea pig and non-human primates, are structurally quite different from the placenta in man.

We have recently developed a multiple indicator dilution approach for study of drug transfer in the perfused placenta in man (Rasiah et al 1997). The use of such methods for accurate prediction of maternal drug disposition across the placenta in man requires knowledge of the pharmacokinetic model which in terms of the organ's anatomy and physiology best describes the drug distribution in that organ. This is currently uncertain.

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The kinetics of placental drug transfer are governed by maternal and foetal blood flow rates, protein binding, placental permeability and the geometry of foetal and maternal placental blood vessels (Chamberlain & Wilkinson 1979; Faber & Thornburg 1983). Several kinetic models, differing in suggested blood-vessel geometry, have been developed to describe placental drug transport; these include countercurrent, concurrent, multivillous, pool flow and double pool flow models (Faber 1977). Attempts to verify these models experimentally (Faber 1977; Schroder et al 1985; Bassily et al 1995) suggest that placentas of some species seem to conform to one or other model but that a different model is needed for the placenta in man, especially for drugs of low permeability (Bassily et al 1995). Over the last 20 years a variety of models has been applied to the liver and kidney, including the ''tube'', ''well-stirred'' (Pang &

Rowland 1977a, b, c; Kakutani et al 1985), ''distributed'' (Bass et al 1978; Forker & Luxon 1978) and ''dispersion'' (Roberts & Rowland 1985, 1986a, b, c) models.

Figure 1 shows the equivalent placental form of a range of clearance models. It must be emphasized that whereas most organs can be described by a single model, the placenta is a dual vascular system model with different heterogeneities in vascular transit time distribution, mean transit time, permeability and binding in the two systems. The potential advantage of the dispersion model is the use of the dispersion number (D_N) to bypass the problem of having to assume a particular vessel geometry. In the placenta the vascular heterogeneity is complicated by the need to define separate D_N values in the two vascular systems (foetal and maternal). In this context, the D_N for a non-eliminated solute can be derived model-independently from the impulse–response relationship of a vascular marker in a given vascular system by statistical moment analysis from the underlying relationship between the normalized variance (CV^2) and dispersion number; $CV^2 = 2D_N$.

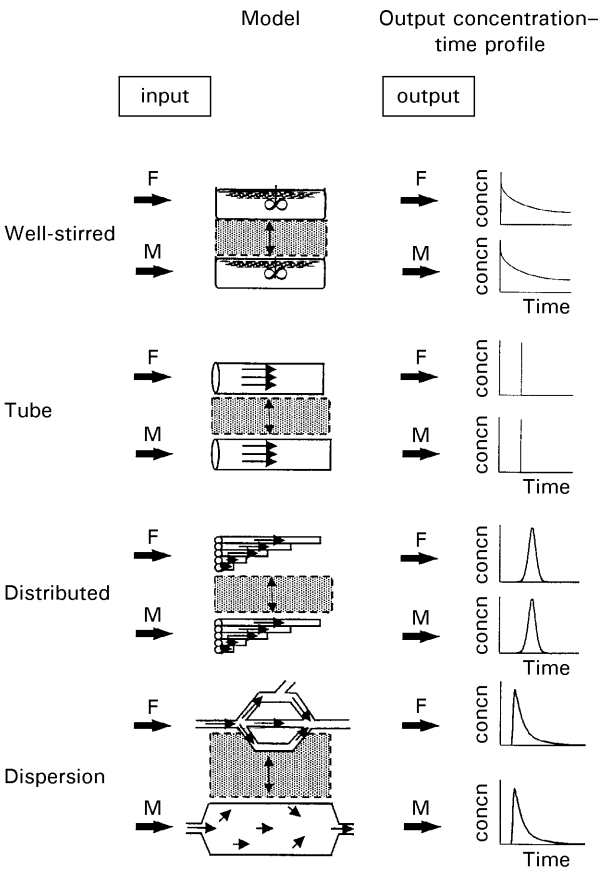

Figure 1. Comparison of different models of placental elimination (F, foetal; M, maternal; T, trophoblast).

The aim of this study was to investigate the disposition of red blood cells and different solutes in the placenta in man by the impulse–response technique. Five model compounds of widely different lipophilicity (sucrose, water, antipyrine and the β-blockers propranolol and labetalol (Table 1)) were injected with a vascular reference (labelled red blood cells) into either the maternal or foetal circulation of a single-pass perfused placental lobule. Outflow fractions were then collected from both the maternal and foetal veins after time intervals ranging from 1 to 600 s (Figure 2).

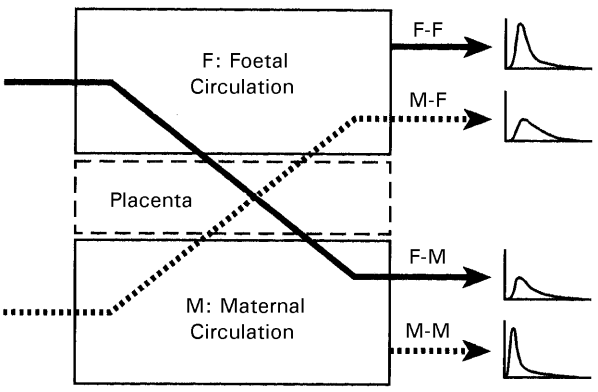

Figure 2. Sampling sites and diagrammatic profiles. Injection into foetal circulation leads to foetal collection (FF) and maternal collection (FM). Injection into maternal circulation leads to maternal collection (MM) and foetal collection MF).

Table 1. Physicochemical properties of the solutes.

Solute	pK_a	Molecular mass	Octanol–water partition coefficient	Fraction unbound[d]
Sucrose	12·7	342·3	0·0014	1
Water	14·8	18	0·0708	1
Antipyrine	1·4	188·2	1·738	0·9
Propranolol	9·5[a]	259·3[a]	3·65[a], 20·2[b], 1230[c]	0·07
Labetalol	7·4, 8·7[a]	364·9[a]	7·08[a], 11·5[b],	0·5

[a]C. Dollery, Therapeutic Drugs, 1991. [b]Database Biobyte, Claremont, CA 91711. [c]J. Sangster, Octanol–Water Partition Coefficients: Fundamentals and Physical Chemistry. [d]Avery's Drug Treatment, Principles and Practice of Clinical Pharmacology and Therapeutics, 1987 (p. 738). Other values are literature values (Wu et al 1995).

Outflow fraction–time profiles for solutes were then analysed by statistical moment analysis and the shapes of the profiles compared with theoretical profiles shown in Figure 1.

Materials and Methods

Statistical moment analysis

The time spent by a blood element in an organ during a single pass is defined as the transit time of the element. Variations in the transit times of elements for a given bolus (impulse) injection of solute result in a transit-time distribution. The first three statistical moments of a transit time distribution are defined as follows (Roberts et al 1990a, b).

The zero moment (area under the curve) is defined as:

$$\mathrm{AUC} = \int_0^\infty \mathrm{C(t)dt} \tag{1}$$

The mean transit time (MTT) for the solute is given by the first moment of the output–time profile:

$$\mathrm{MTT} = \mathrm{AUMC/AUC} = \int_0^\infty \mathrm{tC(t)dt} \Big/ \int_0^\infty \mathrm{C(t)dt} \tag{2}$$

where AUMC is the area under the moment curve and AUC is area under the normalized concentration–time curve.

The spread (or variance, σ^2) of mean transit time distribution is given by the second moment:

$$\sigma^2 = \int_0^\infty \mathrm{t^2C(t)dt} \Big/ \int_0^\infty \mathrm{C(t)dt} - (\mathrm{MTT})^2 \tag{3}$$

$$\mathrm{CV}^2 = \sigma^2/(\mathrm{MTT})^2 \tag{4}$$

where t is time and C(t) is the fraction of the administered dose eluting per second, and MTT, σ^2 and CV^2 are the mean transit time of drug through the tissue, the variance with respect to the mean transit time and the normalized variance, respectively. In this study the areas under the curves from $t = 0$ to infinity were calculated by the parabolas-through-the-origin (PTTO) method (Purves 1992). The frequency output–time profile of drug injected as a bolus into a single-pass isolated perfused placental system is governed by events occurring within: the non-placental regions of the experimental system positioned between the injection site and the beginning of the placental vasculature (tubing, catheter); the placental vasculature; the experimental system positioned between the placental venous exit site and the collection system; and the collection system itself.

MTT and σ^2 for the placenta were accordingly calculated as below:

$$\mathrm{MTT_{out}} = \mathrm{MTT_{pl}} + \mathrm{MTT_{cath}} \tag{5}$$

$$\sigma^2_{\mathrm{out}} = \sigma^2_{\mathrm{pl}} + \sigma^2_{\mathrm{cath}} \tag{6}$$

where ''out'', ''pl'' and ''cath'' refer to the out profile, placenta and catheter, respectively.

Placental perfusion

Peripheral lobules of term placenta in man were perfused using separate maternal and foetal circuits as described elsewhere (Rasiah et al 1997). In brief, a paired chorionic artery and vein feeding a discrete peripheral lobule were cannulated and foetal circuit perfusion commenced. A maternal circuit inflow was established by piercing the basal plate with two catheters. Outflow from the maternal circuit appears through the maternal surface of the lobule and was collected. Foetal and maternal flow rates were 3 and 6 mL $\mathrm{min^{-1}}$, respectively, in four perfusions, and 2 and 4 mL $\mathrm{min^{-1}}$, respectively in four other perfusions. The flow rates were chosen to give a constant maternal–foetal flow ratio of 2. Experiments were conducted at 37°C in a water-saturated atmosphere. The physical integrity and viability of the preparation were assessed by continual monitoring of pressure, pH, temperature and oxygen tension of the maternal ''artery'', foetal artery and foetal vein during the experiment. Foetal arterial pressure was less than 45 mmHg, maternal ''arterial'' pressure was less than 90 mmHg, temperature was 37°C ± 1°C. Oxygen tension was 600 mmHg (approx.), 18 mmHg (approx.) and 90 mmHg (approx.) for maternal ''artery'', foetal artery and foetal vein, respectively, consistent with effective oxygen transfer within the lobule between maternal and foetal circulation (Cannell et al 1988).

After allowing the preparation to stabilize (30 min approx.), a mixture of [^{14}C]sucrose (0·4 μCi), ^{99m}technetium-labelled red blood cells (2·16 μCi), [^{3}H]H_2O (2·0 μCi), [^{14}C]antipyrine (2·0 μCi), unlabelled labetalol (20 mg) and unlabelled propranolol (30 mg) was injected as a 20-μL bolus into either the maternal or foetal circuit with 10 min washing period between injections. Venous effluent from both circulation was then collected every 2 s from 0 to 40 s (foetal collection) or every second from 0 to 10 s (maternal collection), then every 5 s from 40 to 120 s and then every 60 s to the end of collection (540 s). Each collection was followed by a 10-min washout period by the end

of which effluent radioactivity was back to background levels. [^{14}C]Sucrose, [^{3}H]H_2O and [^{14}C]antipyrine were obtained from New England Nuclear (Boston, MA). Red blood cells obtained from the placenta were labelled with ^{99m}technetium by use of a commercially available red blood cell labelling kit (Ultratag RBC; Mallinckrodt Medical, St Louis, MO). Unlabelled propranolol and labetalol were purchased from Sigma (St Louis, MO).

Analysis of solutes

Each sample collected (50 μL) was counted for γ radiation from ^{99m}technetium-labelled red blood cells. Because of the short half-life of ^{99m}Tc (6·02 h) the counts obtained were corrected for decay. Outflow samples of ^{14}C compounds and [^{3}H]H_2O were counted after addition of scintillation fluid (Ultima Gold, Packard, Meridan, CT; 2 mL). Before the measurements tubes were stored for at least 60 h to enable decay of ^{99m}Tc, thus eliminating crossover of γ emission into the β energy window.

Unlabelled propranolol was analysed directly, without extraction, by means of an isocratic HPLC method based on that of Botterblom et al (1993) with some modifications. Labetalol (1 μg L^{-1}; 200 μL) in methanol solution was added to perfusate samples (50 μL) as internal standard. After equilibration and centrifugation (3000 rev min^{-1} for 5 min) the supernatant (60 μL) was injected into the HPLC system, which comprised an LC 1110 pump (ICI Instruments, Australia), an RP-18 Brownlee column (250 mm × 4·6 mm) with HPLC Brownlee NewGuard column (Applied Biosystems, USA), maintained at 37°C by means of a TC 1900 HPLC temperature controller (ICI Instruments, Australia), a model F1000 fluorescence spectrophotometer (Hitachi, Japan), a model LC 1650 advanced autosampler (GBC, Australia) and a Delta Data System computer software package (Digital Solutions, Australia). The mobile phase was 55 : 45 phosphate buffer (0·05 M, pH 2·9)–acetonitrile. The flow rate was 1·3 mL min^{-1}, column eluent was monitored at λ_{ex} 300 nm and λ_{em} 410 nm. Under these conditions, retention times were 3·8 and 5·3 min for labetalol and propranolol, respectively. Standard curves constructed for propranolol (0·1–20 μmL^{-1}) using blank perfusate were linear with correlation coefficients exceeding 0·999. Reproducibility was usually within 2%.

Labetalol was measured under the same conditions with propranolol (4 μg L^{-1}) being used as internal standard. Standard curves constructed for labetalol (0·1–20 μmL^{-1}) were linear with correlation coefficients exceeding 0·999; reproducibility was usually within 2%.

Data analysis

The fraction, y(t), of radiolabelled injected material retrieved, per second, in effluent perfusate at the midpoint sampling time was calculated from output disintegrations min^{-1} values by use of the transformation:

$$y(t) = DPM(t)Q/(\text{dose } p) \quad (7)$$

where y(t) is the fraction of injected material retrieved per second [s^{-1}], DPM(t) is the number of disintegrations min^{-1} of the pipetted volume of the sample collected, Q is the flow rate [μL s^{-1}] and p is the pipetted volume of the sample collected (μL).

For unlabelled material normalization of the concentration–time profile curves was calculated by use of equation 8:

$$y(t) = C(t)Q/DOSE \quad (8)$$

where y(t) is the fraction of injected material retrieved per second [s^{-1}], C(t) is the concentration of the sample collected [μg mL^{-1}], Q is the flow rate [mL s^{-1}], and DOSE is the amount injected [μg].

The moments based on profiles of the fraction of injected material retrieved in a second against time, from time zero to infinity, (determined by parabolas-through-the-origin (PTTO) rule with extrapolation to infinite time) were used to obtain nonparametric estimates of total recovery (availability, F), MTT and CV^2.

Statistics

Data are expressed as means ± s.d. The statistical significance of differences was determined by Student's *t*-test analysis. In each case a *P* value of $< 0·05$ was regarded as indicative of statistical significance.

Results

Typical output concentration–time profiles obtained after bolus injection into the perfused placenta are shown in Figure 3. Comparison of these profiles with the established models of elimination (Figure 1) showed that profiles for both the maternal and foetal circulation are consistent with models, e.g. the dispersion model, used to describe vascular residence time distribution of solutes in organs.

Total recoveries after maternal injection were calculated from the outflow profile of maternal injection–maternal collection (MM) and maternal injection–foetal collection (MF), and after foetal injection from foetal injection–foetal collection (FF) and foetal injection–maternal collection (FM).

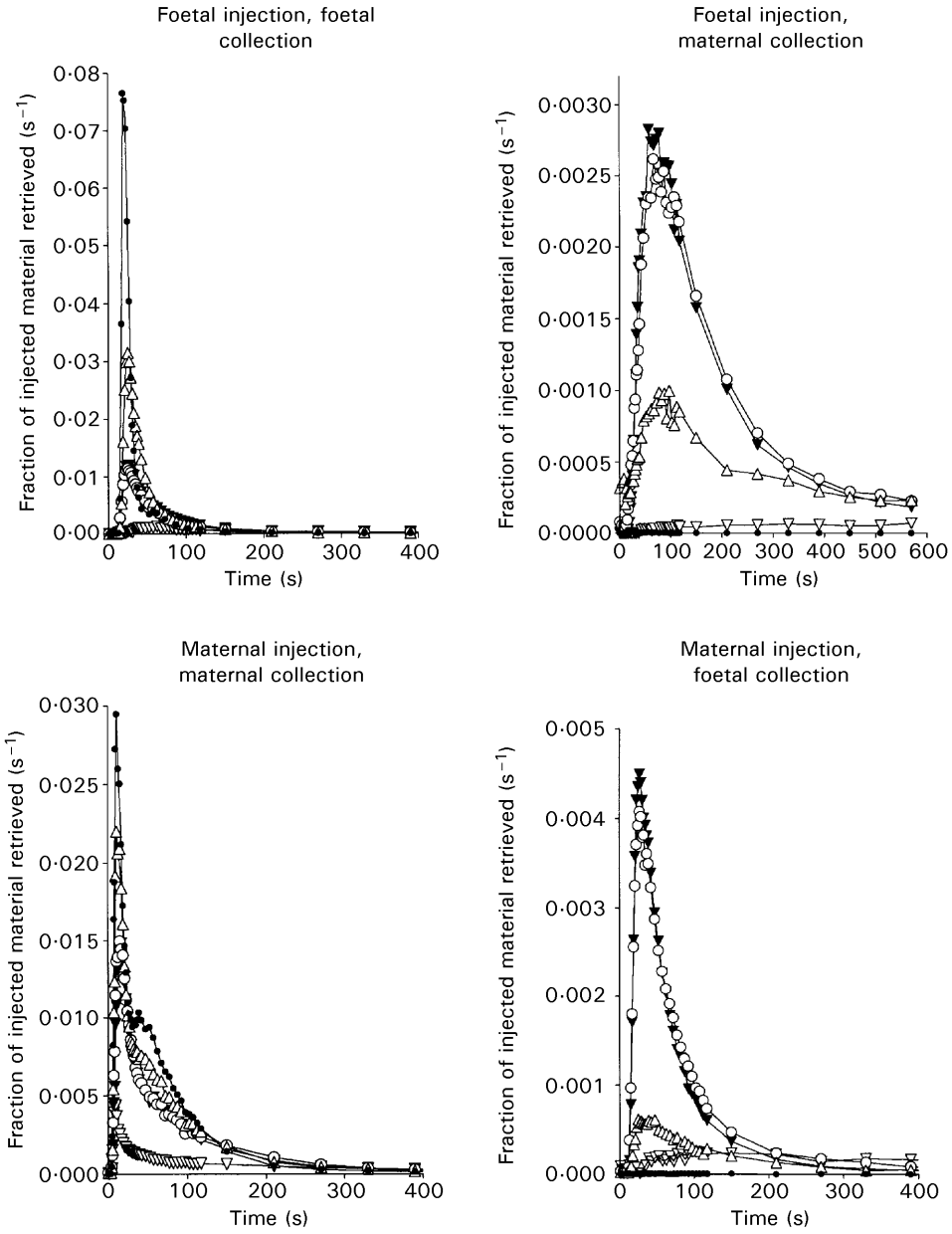

Figure 3. Typical outflow concentration–time profiles of red blood cells (●), sucrose (△), water (○), antipyrine (▼) and propranolol (▽) from impulse–response studies in isolated perfused term placenta from man at a foetal flow rate of 3 mL min^{-1} and a maternal flow rate of 6 mL min^{-1}.

Recoveries of the injected solutes are summarized in Table 2. Total recovery, as the sum of foetal and maternal recoveries, of red blood cells, sucrose, water and antipyrine were close to 100% (range 95·2–119·6%) at both flow rates. Total recoveries of propranolol and labetalol were significantly less, ranging from 37·6 to 93·0%. The total recovery of propranolol after injection into the foetal circuit (43·0 ± 16·9%) was ca half that after injection into the maternal circuit (89·1 ± 19·8%) (difference 46·2%, $P = 0{\cdot}0005$). A potential limitation in the present analysis is the incomplete recovery of some solutes in the maternal circulation after foetal injection. However, the fraction of solute in the outflow of this circulation is 1/200th that in the foetal circulation. There was no statistically significant difference between total recovery after maternal and foetal injection in any other instance. Recovery of solutes on the side of the injection tended to be higher at faster flow rates, but the differences did not reach significance.

Placental transfer of solutes was evaluated from perfusions with collections on the opposite sides (maternal injection–foetal collection (MF) and foetal injection–maternal collection (FM)). Materno–foetal and foeto–maternal transfer of

Table 2. Recovery of solutes.

Solute	Preparation	Flow rate (mL min^{-1})[a]	Recovery (%; mean ± s.d.)					
			Foetal injection			Maternal injection		
			Foetal recovery (FF)	Maternal recovery (FM)	Total recovery (F + M)	Maternal recovery (MM)	Foetal recovery (MF)	Total recovery (M + F)
Red blood cells	P6–9	M: 6; F: 3	99·0 ± 3·7	0	99·0 ± 3·7	95·2 ± 4·8	0	95·2 ± 4·8
	P10–13	M: 4; F: 2	105·3 ± 7·2	0	105·3 ± 7·2	100·6 ± 5·1	0	100·6 ± 5·1
Sucrose	P6–9	M: 6; F: 3	91·5 ± 9·2	28·1 ± 4·4	119·6 ± 13·0	98·0 ± 7·0	6·09 ± 1·30	104·1 ± 8·0
	P10–13	M: 4; F: 2	89·3 ± 7·9	41·8 ± 10·9	131·1 ± 7·2	93·8 ± 5·5	9·03 ± 2·42	102·8 ± 5·1
Water	P6–9	M: 6; F: 3	70·5 ± 15·6	41·5 ± 15·8	112·0 ± 8·4	88·1 ± 5·2	21·5 ± 9·0	109·6 ± 8·2
	P10–13	M: 4; F: 2	57·4 ± 10·2	51·2 ± 8·1	108·6 ± 6·6	79·7 ± 7·0	26·3 ± 10·7	106·0 ± 4·7
Antipyrine	P6–9	M: 6; F: 3	59·4 ± 3·7	43·5 ± 4·9	102·9 ± 4·1	82·6 ± 5·4	26·9 ± 8·1	109·5 ± 4·3
	P10–13	M: 4; F: 2	55·7 ± 7·0	51·2 ± 5·7	106·9 ± 2·3	77·0 ± 4·2	29·8 ± 4·8	106·8 ± 5·2
Propranolol	P6–9	M: 6; F: 3	42·1 ± 21·1	6·18 ± 3·48	48·3 ± 23·8	76·0 ± 35·4	17·7 ± 5·4	93·7 ± 29·9
	P10–13	M: 4; F: 2	31·5 ± 6·3	6·12 ± 3·8	37·6 ± 4·4	48·3 ± 10·4	37·7 ± 4·2	86·0 ± 3·6
Labetalol	P6–9	M: 6; F: 3	37·4 ± 12·8	18·2 ± 13·2	56·6 ± 28·8	68·3 ± 42·9	8·91 ± 3·62	77·2 ± 39·4
	P10–13	M: 4; F: 2	32·2 ± 11·7	17·3 ± 7·8	49·5 ± 17·8	49·4 ± 24·2	11·6 ± 2·4	61·0 ± 25·5

[a]F, foetal; M, maternal.

solutes as a percentage of dose (mean ± s.d.) is shown in Table 2. There was a statistically significant difference between materno–foetal and foeto–maternal transfer of sucrose, water, antipyrine and propranolol (difference and P values: 27·4% and $> 0{\cdot}0001$, 22·5% and 0·0013, 19·0% and 0·0004 and 19·8% and 0·0004 for sucrose, water, antipyrine and propranolol, respectively). Foeto–maternal transfer was significantly higher than materno–foetal transfer for sucrose, water and antipyrine, whereas for propranolol the converse was true. Both foeto–maternal and materno–foetal transfer tended to be higher at the slower flow rate, but differences, except for propranolol, did not reach statistical significance. The materno–foetal transfer of propranolol almost doubled at the slower flow (difference 19·3%, $P = 0{\cdot}0038$). In contrast, the movement of propranolol from the foetal to the maternal circuit was small and was the same at both flow rates (difference 0·06%, $P = 0{\cdot}9813$).

Maternal MTT values were calculated from maternal injection–maternal collection (MM) and foetal MTT values from foetal injection–foetal collection (FF). Values (means ± s.d.) obtained for the solutes are shown in Table 3 together with CV^2 data. The MTT values ranged from about 20 to 500 s. The shortest MTT was found for red blood cells, the highest for propranolol and labetalol. The MTT values for antipyrine and water were similar. There were statistically significant differences between maternal and foetal MTT values for red blood cells (difference 92·2 s, $P = 0{\cdot}002$), sucrose (difference 132·6 s, $P = 0{\cdot}004$, water (difference 153·2 s, $P = 0{\cdot}015$) and antipyrine (difference 115·5 s, $P = 0{\cdot}050$) but not for propranolol (difference 164·0 s, $P = 0{\cdot}146$) and labetalol (difference 90·8 s, $P = 0{\cdot}251$). The ratio of maternal MTT to foetal MTT was ca 5, 3, 2 and 2 for red blood cells, sucrose, water and antipyrine, respectively, consistent with a twofold flow rate difference. CV^2 values for ^{99m}Tc-labelled red blood cells ranged from 2·00 to 3·86. The CV^2 for the polar solutes (sucrose and water) were of a similar magnitude ranging from 1·41 to 3·03. A similar variation in CV^2 was observed for the lipophilic solutes.

Discussion

Analysis of solute outflow patterns by reduction into statistical moments was introduced to pharmacokinetics for in-vivo systems by Cutler (1978) and by Yamaoka et al (1978). The technique enables model-independent characterization of drug disposition by estimation of area under the curve (AUC), mean transit time (MTT) and the variance of transit times (σ^2) (Weiss 1982; Hori et al 1988; Nishida et al 1989).

In this study the foetal circulation MTT values obtained for solutes in the placenta after foetal injection tended to increase with lipophilicity in a manner similar to that of reported in the isolated perfused rat liver for solutes in albumin-free

Table 3 Estimated mean transit time and normalized variance from a non-parametric analysis of the out-flow profile from the vasculature into which the solute was injected.

Solute	Preparation	Flow rate ($mL\,min^{-1}1$)[a]	Mean transit time (s; mean ± s.d.)		Normalized variance (mean ± s.d.)	
			Foetal injection–foetal collection (FF)	Maternal injection–maternal collection (MM)	Foetal injection–foetal collection (FF)	Maternal injection–maternal collection (MM)
Red blood cells	P6–9	M: 6; F: 3	19·5 ± 7·5	109·9 ± 47·8	2·31 ± 1·54	2·00 ± 0·80
	P10–13	M: 4; F: 2	25·1 ± 15·1	119·1 ± 14·0	3·86 ± 1·20	2·03 ± 0·46
Sucrose	P6–9	M: 6; F: 3	52·1 ± 9·7	147·5 ± 57·7	3·03 ± 1·32	1·80 ± 0·72
	P10–13	M: 4; F: 2	62·6 ± 21·4	232·4 ± 63·2	2·03 ± 0·87	1·72 ± 0·32
Water	P6–9	M: 6; F: 3	90·5 ± 13·3	188·0 ± 83·4	2·23 ± 0·18	1·62 ± 0·61
	P10–13	M: 4; F: 2	90·5 ± 30·7	299·4 ± 72·0	2·27 ± 0·27	1·41 ± 0·39
Antipyrine	P6–9	M: 6; F: 3	79·2 ± 25·8	197·4 ± 146·4	1·52 ± 0·24	1·54 ± 0·73
	P10–13	M: 4; F: 2	110·6 ± 43·8	223·5 ± 45·8	1·52 ± 0·13	1·66 ± 0·65
Propranolol	P6–9	M: 6; F: 3	401·6 ± 60·7	211·0 ± 209·4	1·08 ± 0·07	4·49 ± 2·82
	P10–13	M: 4; F: 2	370·6 ± 96·2	508·0 ± 220·5	1·01 ± 0·22	1·17 ± 0·41
Labetalol	P6–9	M: 6; F: 3	179·8 ± 74·8	291·0 ± 156·9	2·08 ± 0·61	1·81 ± 0·29
	P10–13	M: 4; F: 2	397·5 ± 174·5	327·1 ± 206·8	1·42 ± 0·36	1·55 ± 0·60

[a]F, foetal; M, maternal.

perfusate (Chou et al 1993). The MTT were 22·3 s for red blood cells, 57·4 s for sucrose and 90·5 s for water on the foetal side. These solutes are normally distributed in the vascular, extracellular and total water space, respectively. The MTT for antipyrine was 94·9 s, similar to that of water. Its outflow concentration–time profile was also similar to that of water. The high MTT for propranolol and labetalol are consistent with delayed distribution as a result of tissue binding.

The foetal circulation MTT observed in this study for red blood cells, sucrose, water, antipyrine and propranolol are similar to those reported in isolated perfused rat hind limb—Wu et al (1995) reported MTT values of 17 s for albumin, 83 s for sucrose, 128 s for water, 103 s for antipyrine and 238 s for propranolol for a flow rate of $4\,mL\,min^{-1}$. The MTT values were found to be slightly lower in rat liver (13 s for Evans blue, 10 s for sucrose, 34 s for water (Roberts et al 1990b)).

The low availabilities found for propranolol and labetalol in this study are consistent with low availabilities reported for propranolol in the leg—Wu et al (1995) showed that the low recovery reflected high tissue binding and slow efflux of solutes from the tissue. Schneider & Prögler (1988) also found 52% recovery for labetalol and 38% recovery for propranolol from dual in-vitro perfused placental tissue from man. Metabolites were not found for either propranolol or labetalol in our single-pass experiments, suggesting that the low recovery was not because of placental metabolism. It has been suggested that the concept of flow-dependence of clearance at high permeability and flow independence at low permeability is not applicable to placental clearance (Bassily et al 1995). This was based on the flow independence of placental transfer irrespective of the permeability of the placenta to the drug in constant materno–foetal flow ratio experiments, in which Q_M and Q_F were changed simultaneously. In contrast, placental transfer of drugs was altered by changing the materno–foetal flow ratio irrespective of the placental permeability of the solutes (Schneider et al 1985; Schroder et al 1985; Bassily et al 1995). In our experiments the Q_M was 6 and $8\,mL\,min^{-1}$ and Q_F was 3 and $4\,mL\,min^{-1}$, giving a materno–foetal flow ratio of 2·0, a value consistent with the situation in-vivo. Both materno–foetal and foeto–maternal transfer of solutes were similar at the two different flow rates but identical flow rate ratio for all solutes (sucrose, water, antipyrine, propranolol, labetalol). There was, however, a striking difference between materno–foetal transfer of propranolol at different maternal and foetal flow rates, despite the identical materno–foetal flow ratio (2·0). Higher materno–foetal placental transfer occurred at the lower absolute flow rates, as predicted by all organ clearance models. At a lower flow rate the solute has a longer transit time through the placenta (transit time = volume of distribution divided by flow rate). Given that the

extraction is a function of mean transit time (proportional to 1/Q), extraction is higher on the lower flow rates.

A model is required for interpretation of statistical moments in terms of underlying physiological processes so that all phenomena, such as diffusion, binding, distribution and elimination, which might take place in the tissue, can be described. The mathematical model best describing drug disposition in the placenta in man has been uncertain. Models used to describe disposition of drugs in the liver and kidney include the ''tube'', ''well–stirred'', ''distributed'' and ''dispersion'' models. We found that the shape of the outflow concentration–time profile from the placenta in man after bolus administration of a solute (Figure 3) seems to be consistent with that of a model describing a vascular transit-time distribution, such as the dispersion model, rather than ideal models (Figure 1). Such models describe the variation in transit times in terms of a single parameter such as in the dispersion model or a number of parameters as in more complex models (Weiss et al 1997).

The dispersion model for the placenta is characterized by two dispersion numbers ($D_N: 0-\infty$), each defining the variation of transit times of solute molecules in the two vascular beds (foetal and maternal) with limits of no variation (tube model, $D_N = 0$) and infinite variation (well-stirred model, $D_N = \infty$) and by two efficiency numbers (R_N), one for each vascular bed. R_N is defined by the intrinsic clearance of the solute from the vascular bed (CL_{int}), the blood flow (Q), the protein binding of solute in the blood (f_u) and the permeability surface area product of vascular wall (P) i.e.:

$$R_N = f_u CL_{int} P/Q(P + CL_{int}) \qquad (9)$$

Hence in the placenta CL_{int} is defined by the sum of metabolism (CL_{int}^{m}) and clearance from the maternal to foetal ($CL_{int}^{M\to F}$) or foetal to maternal circulation ($CL_{int}^{F\to M}$), i.e.:

$$CL_{int}(F) = CL_{int}^{m} + CL_{int}^{F\to M} \qquad (10)$$

for foetal injection in a single-pass preparation or

$$CL_{int}(M) = CL_{int}^{m} + CL_{int}^{M\to F} \qquad (11)$$

for maternal injection in a single-pass preparation. Given that metabolism in the placenta is usually negligible in a single pass, $CL_{int}^{m} \to 0$ so that $CL_{int}(F) \approx CL_{int}^{F\to M}$ and $CL_{int}(M) \approx CL_{int}^{M\to F}$. Hence:

$$R_N(F) = f_u CL_{int}^{F\to M} P/Q(P + CL_{int}^{F\to M}) \qquad (12)$$

or

$$R_N(M) = f_u CL_{int}^{M\to F} P/Q(P + CL_{int}^{M\to F}) \qquad (13)$$

for foetal and maternal injections, respectively. The overall recovery (F) for the dispersion model (with mixed boundary conditions), the well-stirred model (F_{ws}) and the tube model (F_T) for a given bed is therefore defined as:

$$F_{disp} = \exp[(1 - (1 + 4D_N R_N)^{1/2})/2D_N];$$
$$F_{ws} = 1/(1 + R_N); \; F_T = \exp(-R_N) \qquad (14)$$

Consistent with these models, lower recoveries (F) were observed for the more lipophilic solutes (higher P, $CL_{int}^{F\to M}$ and $CL_{int}^{M\to F}$) and lower flow rates (Table 2).

We recognize that the statistical moments analysis of outflow concentration data has significant limitations. Fuller pharmacokinetic information might be obtained by non-linear regression of impulse–response data using appropriate vascular residence time distribution models. Our preliminary analysis of placental data obtained so far suggests that more complex models (Weiss et al 1997) might be required for adequate description of the tail of data plotted on a semilogarithmic scale. Accordingly, we have limited the presentation of findings in this paper to model-independent statistical moments and observations on the shape of the impulse–response profiles for red blood cells and various solutes in the maternal and foetal circulation. In conclusion, we have used statistical moment analysis to describe solute distribution in the isolated perfused term placenta in man after impulse–response studies. The shape of the outflow concentration–time profile curves seems to be consistent with vascular transit time distribution models such as the dispersion model. The CV^2 values for red blood cells in the placenta in man are indicative of higher dispersion than is reported for the leg and liver.

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