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Placental Passage of Benzoic acid, Caffeine, and Glyphosate in an Ex Vivo Human Perfusion System

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Ex vivo perfusion of the human term placenta is a method to study placental transfer without extrapolation from animal to human and with no ethical concerns for mother and child. However, ex vivo placenta perfusion has a limited potential within chemical screening and testing as the method is time-consuming. This study was an attempt to construct data needed to develop quantitative structure–activity relationship (QSAR) models that are able to predict placental transfer of new compounds. Placental transfer is a biological activity that statistically may be related to the physiochemical properties of a given group of compounds. Benzoic acid, caffeine, and glyphosate were chosen as model compounds because they are small molecules with large differences in physiochemical properties. Caffeine crossed the placenta by passive diffusion. The initial transfer rate of benzoic acid was more limited in the first part of the perfusion compared to caffeine, but reached the same steady-state level by the end of perfusion. The transfer of glyphosate was restricted throughout perfusion, with a lower permeation rate, and only around 15% glyphosate in maternal circulation crossed to the fetal circulation during the study period.

The trophoblasts in the placenta serve as a membrane regulating the passage of substances between the fetal and maternal compartments. The human placenta ex vivo perfusion technique enables kinetic studies on the passage across the term placental membrane. Using human placentas, no extrapolations from animal to human are needed and the studies cause no ethical concerns to mother or child (Lind et al., 2007). However, the results are only valid for the term placenta, and extrapolation from ex vivo to in vivo situation is necessary. The placenta perfusion method is time-consuming and associated with some technical and logistic challenges. Thus, the need of fresh placental tissue creates logistical challenges, while the use of term and discarded tissue necessarily means that some placentas are unusable. Employing quantitative structure–activity relationship (QSAR) processes on chemical structure in relation to transplacental transfer provides a method to predict the placental transfer of new compounds. QSARs are theoretical computational models that can be used to predict the physiochemical and biological fate of a molecule (http://ecb.jrc.it/QSAR). The promotion of QSARs is integrated in the new European chemical legislation REACH (Registration, Evaluation and Authorization of Chemicals). The aims are to predict toxicological responses, provide new knowledge for regulatory purposes, and reduce in vivo toxicological testing. To apply QSAR in relation to placental transfer, high-quality placenta perfusion data are needed for chemicals possessing a wide variety in chemical structure and properties. Hewitt et al. (2007) modeled ex vivo placenta perfusion data collected from the literature. The models include descriptors that relate to hydrogen bonds, molecular size, and hydrophobicity (number of ethyl groups, halogen atoms, aliphatic rings). The best performing models are constructed from single-source data sets with homogeneous compounds. This article provides single-source data from placenta perfusions with the three small size heterogeneous compounds: benzoic acid, caffeine, and glyphosate.

BENZOIC ACID

Benzoic acid is a weak acid and is used as a pH adjuster and a preservative in the food, beverage, and cosmetic industry. Furthermore, benzoic acid is a precursor in many chemical

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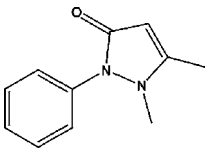
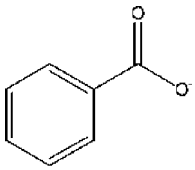
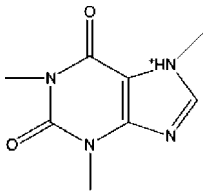
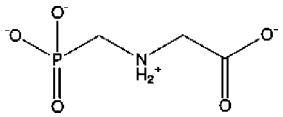
TABLE 1
Charactersitics of the Study Compounds

Parameter	Antipyrine	Benzoic acid	Caffeine	Glyphosate
Structure				
Empirical formula	$C_{11}H_{12}N_2O$	$C_7H_6O_2$	$C_8H_{10}N_4O_2$	$C_3H_8NO_5P$
Systemic name (IUPAC)	Phenazone, 1,2-dihydro-1, 5-dimethyl-2-phenyl-3H-pyrazol-3-one	Benzoic acid Benzene carboxylic acid	1,3,7-Trimethy lxanthine	*N*-(Phosphonomethyl) glycine
CAS number	60–80–0	65–85–0	58–08–2	1071–83–6
Molecular weight (g/mol)	188.2	122.1	194.2	169.1
Solubility in water (g/L, 25°C)	51.9	3.4	10–50	12
pK_a	1.4	4.2	10.4 (40°C)	0.8 (first phosphonic), 2.34 (carboxylate), 5.73 (second phosphonic), 10.2 (amine)
Partition coefficient log $K_{oc/w}$	0.38	1.88	0.63	–3.2 (25°C)
LD50 (mg/kg bw/d, oral, human)	1700 (rat)	0–5 (ADI)	170	5000

Note. Glyphosate: Danish Environmental Protection Agency (2000a, 2000 b); benzoic acid: WHO (2005); caffeine: Merck Index, Chemfinder; antipyrine: Chemfinder, Merck Index.

syntheses. Benzoic acid has a molecular weight of 122 g/mol, is slightly soluble in water (3.4 g/L, 25°C), and is ionized at physiological pH (Table 1; WHO, 2005). Benzoic acid is rapidly absorbed from the gastrointestinal tract and conjugated with glycine in the liver, forming hippuric acid that within 6 h from absorption is almost entirely excreted in the urine (Quick, 1931; Kubota & Ishizaki, 1991). The acceptable daily intake (ADI) established by the World Health Organization (WHO) is <5 mg/kg, and the maximal level of benzoic acid, including benzoates measured as the free acid, is 0.2% in food and beverage and 5% in cosmetics (WHO, 2005). Monocarboxylic acids may cross the human placenta using monocarboxylic transporters (MCT). Carstensen et al. (1983) found that lactate, a monocarboxylic acid, crossed the human perfused placenta by a bidirectional carrier-mediated mechanism. A study using the human monolayer forming choriocarcinoma cells (BeWo), a representative of the human trophoblast, confirmed that an asymmetric carrier-mediated transport system for monocarboxylic acids exist using benzoic acid as a monocarboxylic acid (Utoguchi et al., 1999). The permeation of benzoic acid was greater in the apical-to-basolateral direction compared to the basolateral-to-apical direction. The transfer was dependent upon a proton gradient and the transfer rate increased with decreasing pH (Utoguchi et al., 1999).

CAFFEINE

Caffeine, a trimethylxanthine alkaloid, is the psychoactive stimulant present in coffee, tea, cola, and energy soft drinks. Caffeine has a molecular weight of 194 g/mol, is hydrophilic, and is ionized at physiological pH (Table 1). Caffeine is rapidly and completely absorbed from the gastrointestinal tract, enters all body tissues, and freely crosses the placenta (Abdi et al., 1993; Grosso et al., 2006). The elimination half-life ($t_{½}$) of caffeine ranges from 2 to 4.5 h but is decreased by concomitant cigarette smoking ($t_{½}$ = 1–3 h) and increased during late pregnancy ($t_{½}$ = 11–18 h) (Aldridge et al., 1981; Parsons & Neims, 1978). Frequent caffeine intake during the third trimester may therefore produce accumulation of caffeine. Caffeine is mainly metabolized to the dimethylxanthines paraxanthine (84%), theobromine (12%), and theophylline (4%) by cytochrome P-4501A2 (CYP1A2) in the liver (Callahan et al., 1982). Further metabolism is accomplished by xanthine oxidation (oxidation in the purine) and *N*-acetylation (Tsutsumi et al., 2001).

Because caffeine freely crosses the placenta and the placenta and fetus lack or are deficient in CYP1A2 expression, the fetus is exposed to the same level of caffeine as the mother (Sesardic et al., 1990; Arnaud, 1987). Caffeine affects the central nervous system (CNS) and the endocrine system (Pollard, 1989), and is a teratogen (Scott, 1983) in high doses. However, a recent meta-analysis, summarizing published data from 1974 to 2001 on caffeine intake and adverse reproductive effects in humans, concludes that no reproductive dysfunctions such as delayed conception, low birth weight, spontaneous abortion, fetal death, and abnormality were associated with caffeine consumption (Leviton & Cowan, 2002). Many of the reviewed studies were flawed due to inefficient assessment of caffeine exposure, residual confounding, and suboptimal statistical analysis to deal with confounders (Leviton & Cowan, 2002).

GLYPHOSATE

Glyphosate is the active compound in Roundup, which is the most widely used herbicide. Glyphosate is a nonselective herbicide that inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase, essential for the production of chorismate, an intermediate in the production of aromatic amino acids such as phenylalanine, tyrosine, and tryptophan (Danish EPA, 2000a). As mammals do not synthesize these amino acids, glyphosate exclusively affects plants. Glyphosate has a molecular weight of 169 g/mol, is hydrophilic, and is ionized at physiological pH (Table 1). Glyphosate is partly (15–36%) absorbed from the gastrointestinal tract, has a rapid excretion, and has no accumulative tendencies (Williams et al., 2000). The main metabolite is aminomethylphosphoric acid (AMPA). However, in rats nearly 100% of the absorbed glyphosate remains as unmetabolized glyphosate (Brewster et al., 1991). The Danish Environmental Protection Agency (EPA) has concluded that glyphosate and its metabolites impose no particular risk to health. Glyphosate has a low oral acute toxicity (LD_{50} = 5 g/kg) and is not a mutagen, carcinogen, teratogen, or reproductive toxicant (Danish EPA, 2000b).

MATERIALS AND METHODS

Placentas

Placentas from uncomplicated pregnancies and births were obtained immediately after vaginal birth or elective caesarean section at the Danish University Hospital, Rigshospitalet. Informed written consent was given before birth or in relation to the birth. The project was approved by the Ethical Committees in the Communities of Copenhagen and Frederiksberg (KF 01-145/03 + KF(11) 260063) and the Danish Data Protection Agency.

Perfusion Model

The perfusion model is described elsewhere in detail (Mose et al., 2007; Mose & Knudsen, 2005). Briefly, one vascular unit in the placenta was perfused by cannulation (Flocare Pur Sondes-MP, Ch 5/50 + Ch6/60) of the fetal circulation in one villous tree and simultaneous supply of maternal perfusion medium to the intervillious space. The fetal and maternal perfusion media (2 × 100 ml) consisted of Krebs Ringer buffer with added dextran, glucose, and heparin. The perfusion media were recirculated by roller pumps to attain a flow rate of 3.5 ml/min and 12 ml/min in fetal and maternal circulation, respectively. The maternal perfusion medium was aerated with oxygen and the fetal one with nitrogen. A preperfusion period was used to stabilize the placenta before addition of test compound and the positive control compound antipyrine.

Compounds

After preperfusion, 97 ml maternal perfusion medium was added and 1 ml antipyrine (10 g/L; Aldrich-Chemie, Steinheim, Germany) together with labeled and unlabeled benzoic acid (1), caffeine (2), or glyphosate (3). (1) One milliliter labeled benzoic acid (1 ml, 0.33 m*M*, 1.54 μCi/ml) diluted from American Radiolabeled Chemicals (ARC) 187 benzoic acid ([ring-^{14}C(U)], 60 mCi/mmol, 0.1 mCi/ml) and 1 ml unlabeled benzoic acid (19.67 m*M*; Acros Organics (423470250)). (2) One milliliter labeled caffeine (18.18 μ*M*, 1 μCi/ml) diluted from ARC 235 caffeine ([1-methyl-^{14}C], 55 mCi/mmol, 1 mCi/ml) and 1 ml unlabeled caffeine (19.98 m*M* or 9.99 m*M*; Acros Organcs (ACR108160100)). (3) One milliliter labeled glyphosate (18 μ*M*, 1 μCi/ml) diluted from ARL 1312 14C-glyphosate ([glyphosate-glycine-2-^{14}C], 55 mCi/mmol, 50 μCi/ml) and 1 ml unlabeled glyphosate (19.98 m*M*; Dr. Ehrenstorfer GmbH, Germany (EHRC14050000)). The total concentration in maternal perfusion media from the beginning of the perfusions was 0.1 mg/ml antipyrine, 200 μ*M* benzoic acid, 200 μ*M* glyphosate, 200 μ*M*, or 10 μ*M* caffeine. Standard solutions with caffeine and glyphosate were diluted in water and standards with benzoic acid diluted in ethanol:water (1:10).

Samples

Samples (0.5 ml) were collected from the maternal and fetal perfusion medium before and 2, 5, 10, 15, 30, 45, 60, 90, 120, and 150 min after addition of antipyrine and test compound and centrifuged 5 min at 4000 × g. Two hundred microliters supernatant was transferred to counting vials and 2 ml scintillation liquid (Ecoscint XR LS-372; BN instruments A/S) added. The remnant supernatant was stored at –20ºC for antipyrine analysis. Tissue samples were taken before and after perfusion. Before perfusion the tissue samples were taken from different locations surrounding the area removed for perfusion and after perfusion the cotyledon was cut through and a slice collected. The samples were cut from full thickness tissue without amnion into smaller pieces and 1 g were isolated and extracted in 3 ml acetonitrile for a minimum of 72 h. After extraction, tissue was removed and 2 ml scintillation liquid was added before scintillation counting. Oxygen tension and pH was

measured regularly in the perfusion media and fetal venous outflow by an ABL5 blood and gas analyzer (Radiometer, Denmark).

Binding to the Perfusion System

The binding of benzoic acid, glyphosate, and caffeine to the perfusion chamber, tubing and fittings was studied. One hundred milliliters perfusion buffer was circulated in the maternal circulation and through an empty perfusion chamber. Benzoic acid, caffeine, or glyphosate was added and samples collected as described for the perfusion studies. The percent added compound not accounted for in the perfusion medium after end study was used as a measure of binding to the tubes and the perfusion system.

Analysis

Benzoic acid, caffeine, and glyphosate were measured by liquid scintillation counting and a calibration curve was included in each test round. The five calibration curve samples were diluted in Krebs Ringer buffer without dextran and blood cells. The scintillation counter was programmed to count twice and maximum 10 min per sample. The second counting was used. Antipyrine was analyzed on a LaChrom high-performance liquid chromatography (HPLC) system equipped with a C-18 column and a SecurityGuard precolumn as described elsewhere (Mose et al., 2007; Mose & Knudsen, 2005).

Fetal–Maternal Concentration Ratio, Mass Balance, and Permeability Rate

The fetal–maternal concentration (FM) ratio is the concentration in fetal circulation at end perfusion divided by the concentration at end perfusion in maternal circulation (Figure 1). The FM ratio enables comparison between perfusions and between studies using different placentas and concentrations. An indicative qualitative measure of the permeability coefficient was estimated from the initial linear part of the relationship between perfusion time and the fetal/maternal concentration ratio (FM ratio). The indicative permeability rate illustrates the initial rate of transfer from maternal to fetal circulation, whereas the FM ratio rather illustrates the final level of exchange at end perfusion.

The mass balance after perfusion was calculated as a sum of counts per minute (cpm) in perfusion media, tissue, and samples

$$\text{FM ratio} = [C]_F/[C]_M$$

FIG. 1. Fetal–maternal concentration ratio (FM) is the ratio between the concentration in fetal circulation and the concentration in maternal circulation when equilibrium between the two circulations is reached. Equilibrium occurs after approximate 150 min. $[C]_F$ = the concentration in fetal chamber after 150 min of perfusion; $[C]_M$ = the concentration in maternal circulation after 150 min of perfusion.

$$\frac{C_M \times V_M/0.2 + C_F \times V_F/0.2 + (C_M + C_F)/2 \times V_S/0.2 + C_C \times M_C + C_T \times M_T}{C_I \times 100/0.2} \times 100\%$$

FIG. 2. Mass balance equation to calculate the mass balance after end perfusion. The sum of counts per minute (CPM) in maternal circulation, fetal circulation, samples collected, and in tissue placed in perfusion chamber in relation to the initial theoretical number of counts added to the maternal circulation. C_M = cpm, maternal 150 min, V_M = volume, maternal 150 min, C_F = cpm, fetal 150 min, V_F = volume, fetal 150 min, V_S = total sample volume, 0.2 = sample volume, C_C = cpm, Cotyledon (1 g), M_c = mass, cotyledon, C_T = cpm, Tissue around cotyledon, M_T = mass, tissue around cotyledon, C_I = cpm, initial number of counts added to the maternal circulation (taken from standard curve).

in relation to the theoretical amount of radioactivity in maternal circulation from the beginning of perfusion (Figure 2). The initial number of counts added to the maternal circulation was taken from the standard curve as no sample was collected at time 0.

RESULTS

Sixteen placentas from uncomplicated deliveries resulted in 19 perfusions: 7 with glyphosate given as 1A, 1B, 2, 3, 4, 5, and 6; 7 perfusions with caffeine shown as 7A, 7B, 8, 9, 10, 11, and 12; and 5 perfusions with benzoic acid termed 13, 14A, 14B, 15, and 16 (Table 2). Perfusions with the same number but different letter are from the same placenta using separate cotyledons simultaneously. The average maternal age was 33 ± 5 yr. Mothers 1 and 11 were smokers; mothers 10 (antibiotics), 14 (antidepressant SSRI), and 16 (heparin) ingested prescription drugs; and mother 13 underwent fertility treatment to become pregnant. All but mothers 2, 6, 7, 8, and 10 delivered by elective caesarean section. Mother 12 delivered twins.

Antipyrine (100 mg/L) rapidly crossed from maternal to fetal circulation. The fetal FM ratio was 0.97 ± 0.03 after 150 min of perfusion (n = 18) (Figure 3). One perfusion is only usable up to 120 min because the fetal volume loss was too large during the last 30 min of perfusion, which indicates leakage within the system. The FM ratio after 120 min of perfusion was 0.94 ± 0.07 (n = 19). The indicative permeability coefficient was 0.92/h, calculated as the slope of linear part of the curve in Figure 3 (Table 3). The recovery of antipyrine in perfusion media after end perfusion was 82.2 ± 9.8%.

Glyphosate (200 μ*M*) had a restricted transfer across the placenta as the FM ratio was 0.34 ± 0.14 after 150 min of perfusion (n = 6) and 0.24 ± 0.05 after 120 min of perfusion (n = 7) (Figure 4). The indicative permeability coefficient was 0.11/h (Table 3). A small amount (0.5 ± 0.2%) of the added [^{14}C]glyphosate accumulated in the tissue during perfusion and approximately 5% was bound to the perfusion system. The mass balance of glyphosate in perfusion media and tissue after end perfusion was 67.8 ± 18.5% (n = 7) when calculated in relation to added radioactivity.

TABLE 2
Data from the Individual Perfusion

	Glyphosate							Caffeine							Benzoic acid				
Parameter	1A	1B	2	3	4	5	6	7A	7B	8	9	10	11	12	13	14A	14B	15	16
Volume loss (M, ml)	–3.7	–0.9	11.7	8.8	11.8	1.7	4.4	14.0	11.0	2.6	–1.2	3.8	7.8	5.9	6.8	5.3	–16.1	–3.7	–2.6
Volume loss (F, ml)	9.6	4.7	–1.0	2.2	7.8	10.5	5.7	7.3	7.5	7.2	4.2	1.1	7.1	5.3	6.8	2.8	12.2	9.2	10.0
Flow (fetal, ml/2min)	7.7	7.6	8.0	7.2	7.0	7.1	7.5	7.6	7.2	6.7	7.1	6.8	7.5	6.9	6.8	7.1	7.0	7.1	7.0
Time (birth to lab, min)	33	33	—	34	27	21	38	24	24	45	24	29	–	39	29	35	35	30	31
Preperfusion (min)	54	60	43	25	34	38	48	47	36	45	30	34	35	22	38	26	19	40	60
Maternal age (yr)	34	34	—	27	42	35	32	33	33	28	30	34	25	36	39	37	37	35	26
Placenta weight (g)	804	804	980	685	690	700	547	624	624	980	878	620	560	1660	730	987	987	700	888
Cotyledon weight (g)	35	25	17	16	19	20	38	16	18	36	16	23	17	20	28	12	14	25	15
Total perfused (g)	76	58	74	65	68	48	74	50	50	100	53	64	60	66	79	63	80	75	65
Cesarean section	Y	Y	N	Y	Y	Y	N	N	N	N	Y	N	Y	Y	Y	Y	Y	Y	Y
Gestation age (wk + days)	37 + 4	37 + 4	—	—	—	—	40 + 6	41	41	41 + 5	38 + 4	40	—	37 + 1	—	38 + 5	38 + 5	—	37 + 1
Smoker	Y	Y	—	N	N	N	N	N	N	N	N	N	Y	N	N	N	N	N	N
Medicine	N	N	—	N	N	N	N	N	N	N	N	Y	N	N	Y	Y	Y	N	Y

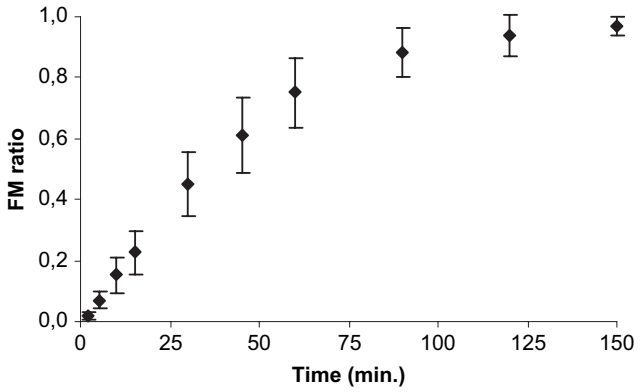


FIG. 3. Placental transfer of antipyrine in an ex vivo placental perfusion system. Data are the means ± SD of 19 ex vivo human placenta perfusions. The time is the number of minutes counting from the addition of 1 ml antipyrine (10 g/L) to maternal circulation and the FM ratio is the concentration in fetal circulation divided by the concentration in maternal circulation.

TABLE 3
Indicative Measure of Permeability Coefficients (per Hour) for the Four Model Compounds

Antipyrine	Caffeine	Benzoic acid	Glyphosate
0.92	1.03	0.60	0.11

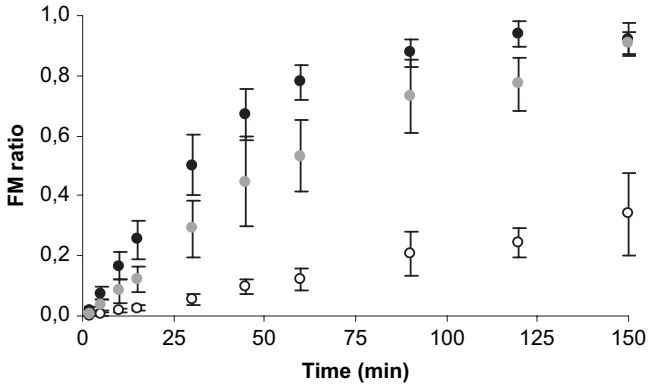


FIG. 4. Placental transfer of caffeine (black), benzoic acid (gray), and glyphosate (white). Data are the means ± SD of seven ex vivo human placenta perfusions with caffeine, seven with glysphosate, and five perfusions with benzoic acid. The time is the number of minutes counting from the addition of caffeine (10 μ*M* or 200 μ*M*), benzoic acid (200 μ*M*), or glyphosate (200 μ*M*) to maternal circulation, and the FM ratio is the concentration in fetal circulation divided by the concentration in maternal circulation.

Caffeine (200 μ*M* and 10 μ*M*) rapidly crossed from maternal to fetal circulation. Four perfusions were preformed with 200 μ*M* and three perfusions using 10 μ*M*. However, the transfer kinetics were the same for both concentrations of caffeine, resulting in an average FM ratio of 0.92 ± 0.05 after 150 min of perfusion (n = 7) and an indicative permeability coefficient of 1.03/h (Table 2). The mass balance of caffeine in perfusion media and tissue after end perfusion was 81.7 ± 7.3% when calculated in relation to added radioactivity. During perfusion, 12.3 ± 3.8% of the added [^{14}C]caffeine accumulated in the tissue and approximately 4% was bound to the perfusion system.

Benzoic acid (200 μ*M*) rapidly crossed from maternal to fetal circulation. However, a more restricted passage was found in the beginning of the perfusion compared to the placental passage of antipyrine and caffeine. The indicative permeability coefficient of benzoic acid was 0.6/h, compared to 1.03/h for caffeine, although benzoic acid and caffeine had more or less the same FM ratio after 150 min perfusion. The FM ratio was 0.91 ± 0.04 after 150 min of perfusion (n = 5). The mass balance of benzoic acid in perfusion media and tissue after end perfusion was 80.4 ± 8.4% when calculated in relation to added radioactivity. During perfusion, 11.4 ± 2.2% of the added [^{14}C]benzoic acid accumulated in the tissue and approximately 3% was bound to the perfusion system.

The quality and tissue integrity were ensured by continuous measurements. Time from birth of child to placenta preparation in the lab was 31 ± 6 min and the preperfusion lasted 39 ± 12 min (n = 19). Loss of fetal perfusion media during preperfusion and perfusion was 6.3 ± 3.4 ml. The average flow rate in fetal circulation was 7.2 ± 0.4 ml/2 min. All perfusions had an uptake of oxygen from fetal artery compared to fetal vein. The total average oxygen tension was 48.4 ± 8.3 kPa in maternal, 16.5 ± 2.9 kPa in fetal outflow, and 12.6 ± 2.1kPa in fetal perfusion media. Figure 5 shows the average oxygen tension in specific time intervals. The overall average pH was 7.11–7.44: 7.25 ± 0.08 in maternal, 7.35± 0.09 in fetal outflow, and 7.13 ± 0.06 in fetal perfusion media (n = 19).

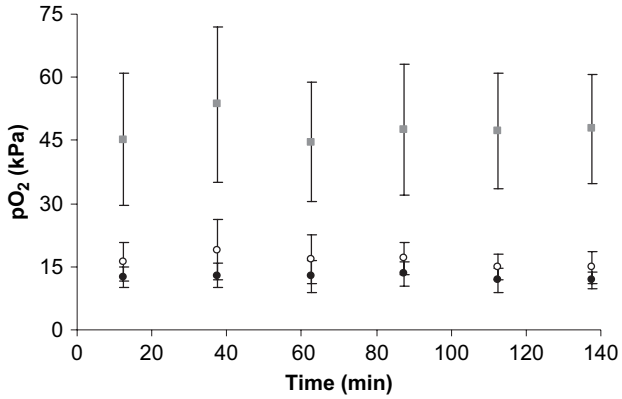


FIG. 5. Oxygen tension (kPa) in maternal compartment (gray), fetal artery (black), and fetal vein (white). Data are shown as the average of all pO_2 measurements in defined time intervals of 25 min in 19 perfusions.

DISCUSSION

Our data on the placental caffeine transfer demonstrated that caffeine rapidly crossed the placenta showing transfer kinetics similar to passive diffusion. Benzoic acid showed a slower transfer rate in the first part of the perfusion but the fetal–maternal concentration ratio reached the level of caffeine and antipyrine within 150 min of perfusion. Glyphosate showed a restricted transfer rate throughout perfusion and the fetal–maternal transfer ratio was 0.34 after 150 min of perfusion.

Transfer across the human placenta depends on the functionality of the placenta, uterine and umbilical blood flow, transporters located in the membrane, chemical gradients across the membrane, and the physiochemical properties of the compound (Audus, 1999; Pacifici & Nottoli, 1995). Physiochemical properties such as molecular weight, lipophilicity [log P_{ow})], degree of ionization (pK_a), and protein binding affect the rate of placental transfer (Audus, 1999). In general, neutral compounds with a molecular weight below 1000 g/mol diffuse freely across the placenta. Benzoic acid, caffeine, and glyphosate are all ionized and charged at physiological pH and have molecular weights between 169 g/mol and 212 g/mol (Table 1). Glyphosate is the most hydrophilic compound, followed by caffeine and benzoic acid. Glyphosate is ionized at three places and has a net negative charge. The transfer across the placenta was restricted and glyphosate did not accumulate in the tissue. The result is in agreement with partial absorption from the gastrointestinal tract (Willams et al., 2000) and the negligible penetration through skin (Wester et al., 1991; Nielsen et al., 2007). The recovery of glyphosate in perfusion media and tissue after end perfusion was low (68%) and varied greatly between perfusions. One plausible explanation is an incomplete extraction with an extraction solvent too lipophilic to extract glyphosate from the tissue. However, glyphosate was not reported as a compound with cumulative tendencies (Williams et al., 2000), and only a minor part of the missing glyphosate (5%) was bound to the perfusion system.

Caffeine is 99.99% positively charged at physiological pH and freely soluble in water. Caffeine freely crossed the placenta as previously reported (Abdi et al., 1993; Grosso et al., 2006). Plasma protein binding affects the level of free caffeine, as 10–46.3% is bound to plasma proteins when diluted 1:1 in plasma (Musteata et al., 2006). When plasma is diluted 1:10 in phosphate-buffered saline (PBS), the plasma protein binding is reduced to 16.5% (Musteata et al., 2006). However, in the present study, plasma proteins were not added to the perfusion media and only blood flushed from the isolated placenta cotyledon was present in the media, making the dilution of plasma proteins much higher than 1:10. Thus, the influence of plasma protein binding is expected to be very limited. Despite a partition coefficient below one, caffeine showed a tendency to accumulate in tissue. This might reflect tissue protein binding rather than tissue fat (membrane) accumulation. The recovery of caffeine in perfusion media and tissue after end perfusion was 82% and uniform between perfusions. Only a minor part of the missing caffeine (4%) was bound to the perfusion system.

Benzoic acid is more lipophilic compared to glyphosate and only slightly soluble in water. At physiological pH, benzoic acid is 99.99% ionized, which increases the water solubility. The transfer across placenta was slower in the beginning but reached the level of caffeine and antipyrine within 150 min. A proton-dependent monocarboxylic transporter system is located in the trophoblasts that transport benzoic acid in both directions (Carstensen et al., 1983; Utoguchi et al., 1999). Our results support the transplacental transfer of benzoic acid, and limited permeability rate during the first part of the perfusion indicates that transporters are involved. The differences seen in the transfer rates of benzoic acid and caffeine might be due to differences in tissue accumulation or binding to the perfusion system, which may reduce the concentration of benzoic acid in the maternal circulation rather quickly and thereby the transfer rate. Benzoic acid did not bind to the perfusion system (3%) but showed a tendency to accumulate in tissue (11%), which was similar to caffeine. Protein binding can also decrease placental transfer because benzoic acid binds to plasma proteins and blood cells, leaving only 14% as free benzoic acid in whole blood (Láznícek & Láznícková, 1995). The recovery of benzoic acid in perfusion media and tissue after end perfusion was 80% and only a minor part of the missing benzoic acid (3%) was bound to the perfusion system.

The tendency to obtain low recoveries of the radiolabeled compounds after perfusion may be associated with differences between the Krebs Ringer buffer used to dilute the standards to the calibration curve and the more blood-filled Krebs Ringer buffer in fetal and especially maternal samples. The biological material present in samples and not standards might affect the counting efficiency resulting in quenching. However, any difference between standards and samples due to quenching is likely to apply to a similar extent for each compound and would therefore not invalidate the conclusion. Alternatively, the low recoveries can be associated with the nonoptimal method to calculate mass balance where a theoretical initial concentration in maternal circulation is used instead of a zero sample. In future studies it is recommended to collect a zero sample from maternal circulation and dilute the calibration curve standards in Krebs ringer buffer containing blood.

The indicative permeability coefficient was included to demonstrate the initial differences in permeability of the model compounds. The indicative measure of permeability rate attempts to rank the compounds according to their initial transfer rates instead of the final concentration ratio at end perfusion (FM ratio). In the present study, the indicative permeability rate is included to illustrate the differences between benzoic acid and caffeine placental passage: information that is hidden using the FM ratio only. The permeability in placenta physiology is traditionally calculated as a product of permeability and membrane surface area equal to the net flux of compound divided by the concentration difference at steady state (Schneider et al.,

1985; Illsley et al., 1985). As the surface area of the perfused membrane is unknown, the wet weight of the perfused tissue may be used as an indicator of surface area, though this measure is also difficult to obtain for individual cotyledons. The present estimate, though simple, seems to illustrate differences between compounds, and seems sufficiently valid to give the expected identical (concentration independent) results for two different concentrations of caffeine.

QSAR modeling with placenta perfusion data like those presented for benzoic acid, caffeine, and glyphosate in relation to their physiochemical properties might result in a model able to predict placental transfer of other compounds with comparable physicochemical properties. However, in order to properly develop QSAR models it would probably be necessary to study a more extensive group of more closely related compounds. The present data set is preliminary as it is limited in size and includes compounds with large heterogeneity. Homogeneous data sets for a structurally defined class of compounds give improved statistics and better QSAR models (Hewitt et al., 2007). How to model placenta transfer as a biological activity in a mother–placenta–fetal compartment system that is metabolically active is a mathematical and statistical challenge. The presence of transporters, pH gradients, and protein binding further complicates the modeling. Nevertheless, QSAR modeling for placental transfer may provide a fast and low-cost screening method without any ethical problems, usable in the classification and characterization of new compounds.

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