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Transport of Benzo[α]pyrene in the Dually Perfused Human Placenta Perfusion Model: Effect of Albumin in the Perfusion Medium

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Abstract: Transport of benzo[α]pyrene (BaP) across the placenta was examined because it is a ubiquitous and highly carcinogenic substance found in tobacco smoke, polluted air and certain foods. Foetal exposure to this substance is highly relevant but is difficult to estimate. The human placenta is unique compared to other species; since it is available without major ethical obstacles, we have used the human placenta perfusion model to study transport from mother to foetus. Placentas were donated after births at Rigshospitalet in Copenhagen from pregnant mothers who signed an informed consent. BaP is lipophilic and studies using cell culture medium in 6-hr placenta perfusions showed minimal transport through the placenta. To increase the solubility of BaP in perfusion medium and to increase physiological relevance, perfusions were also performed with albumin added to the perfusion medium [2 and 30 mg/ml bovine serum albumin (BSA) and 30 mg/ml human serum albumin (HSA)]. The addition of albumin resulted in increased transfer of BaP from maternal to foetal reservoirs. The transfer was even higher in the presence of an HSA formulation containing acetyltryptophanate and caprylate, resulting in a foetal–maternal concentration (FM) ratio of 0.71 ± 0.10 after 3 hr and 0.78 ± 0.11 after 6 hr, whereas the FM ratio in perfusions without albumin was only 0.05 ± 0.03 after 6 hr of perfusion. Less BaP accumulated in placental tissue in perfusions with added albumin. This shows that transplacental transport of the pro-carcinogenic substance BaP occurs, and emphasizes the importance of adding physiological concentrations of albumin when studying the transport of lipophilic substances.

Benzo[α]pyrene (BaP) is a five-ring polycyclic aromatic hydrocarbon that is mutagenic and highly carcinogenic. A large number of experiments have demonstrated that BaP causes tumours at several sites by several routes of administration, in both sexes, and in several animal species. Based on the sufficient evidence in experimental studies and strong evidence that the mechanisms of carcinogenesis in animals also operate in human beings, International Agency for Research on Cancer (IARC) has classified BaP as carcinogenic to humans (IARC, group 1) [1]. BaP is the most studied carcinogenic polycyclic aromatic hydrocarbon and one of the most potent. It is often used as a toxicological prototype or surrogate for all carcinogenic polycyclic aromatic hydrocarbons [2]. BaP itself is a pro-carcinogen; it is the metabolism of BaP to reactive intermediates capable of forming DNA adducts that can lead to cancer or inflammatory responses [3].

Human exposure to BaP occurs primarily by smoking tobacco, inhalation of polluted air, and by ingestion of food or water contaminated by combustion effluents [2]. BaP is present as a component of the total content of polycyclic aromatic hydrocarbons in the environment, and is found in coal tar, in automobile exhaust fumes, tobacco smoke and in charbroiled food. A recent Swedish study revealed that indoor BaP levels are four times higher in homes where wood burning is used for heating (n = 13), compared to reference homes with electrical heating or a heat pump (n = 10) [4]. Upon evaluation of the content of BaP and other polycyclic aromatic hydrocarbons in food, the European Food Safety Authority (EFSA) recently reported that BaP was detected in 50% of different food commodity samples; median dietary exposure in Europe was estimated to be between 3.9 and 6.5 ng/kg body weight/day, with seafood products accounting for the highest relative amounts [5]. A recent Korean assessment of dietary exposure to BaP showed higher levels of BaP in fried chicken, smoked dried beef and certain types of potato chips; accordingly, Lee and Shim recommend changes in food processing to reduce cancer risks [6]. BaP content was measured to be between 3.4 and 28.4 ng per cigarette [7], and for non-smokers, personal exposure to BaP due to second-hand smoke is estimated to be around 8 ng per hour [5].

Human exposure to BaP is almost ubiquitous; a study performed in the Ukraine investigating the effect of pollution on 200 normal pregnancies detected BaP in all but one placenta, in amounts up to 6.15 ng/g dry weight [8]. BaP is distributed to distal tissues in experimental animals, and the transport of BaP in the bloodstream can be explained by its partitioning into plasma proteins and lipoproteins [9]. During pregnancy, BaP metabolites (mBaP) are capable of forming DNA adducts, causing oxidative damage, or disrupting normal blastocyst development [10]. BaP and mBaP in the maternal bloodstream may pass to the foetal circulation and expose the developing foetus to the aforementioned

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hazards. Increased risks to foetal development would be expected not only from the transplacental transfer of maternal mBaP, but also from placental and foetal metabolism of BaP. Autrup *et al.* observed BaP-DNA adducts in the umbilical cord blood of 21 smoking and 30 non-smoking women, and they reported a positive association between the adduct level in maternal and umbilical cord blood [11].

During pregnancy, the placenta mediates the transfer of oxygen, nutrients and waste products between the maternal and foetal circulations. In addition to these vital functions, the placenta also represents the portal of foetal exposure to xenobiotics, including drugs and environmental toxins. *Ex vivo* human placental perfusion experiments can provide valuable information concerning the transport of potentially harmful environmental substances across the placental barrier, expected foetal exposure and placental metabolism [12]. These non-invasive studies are representative of placental function at term but it is not without some limitations. Maternal and foetal blood flows, for example, are most often represented by a buffer solution.

Physiological concentrations of maternal albumin during the second and third trimesters are around 30 mg/ml, which is about 40% lower than albumin levels before and after pregnancy [13]. In order to investigate the importance of these albumin concentrations on the transplacental transport of BaP, this work uses perfusion medium containing concentrations of albumin corresponding to the levels seen during pregnancy. The effect of albumin on the transport of BaP is compared using perfusion medium with no albumin, bovine serum albumin at two concentrations (2 mg/ml and 30 mg/ml), and human serum albumin (30 mg/ml). Since foetal albumin levels are higher than maternal levels [14], and to represent the higher viscosity of foetal blood, 40 mg/ml albumin was used in the foetal perfusion medium during these experiments.

It has been previously demonstrated that albumin affects both the transplacental transfer of drug substances and the binding of drugs to placental tissue. For example, Nanovskaya *et al.* recently reported that the presence of albumin in placental perfusion experiments caused an increase in the foetal–maternal ratio of rosiglitazone from 0.17 to 0.33; this was accompanied by a decrease in the amount of drug bound to placental tissue [15].

BaP has been shown to bind to albumin in a saturable manner [16]; the reported value of 230 μg BaP/ml in human serum includes binding not only to albumin but also to serum lipoproteins, which account for a greater percentage of the total protein-bound BaP [17]. The amino acid sequences of human serum albumin (HSA) and bovine serum albumin (BSA) are 76.5% identical. Steinhardt *et al.* reported the only major differences to be the number of tryptophans (BSA has one more) and that HSA has more valines [18]. The binding capacities of BSA and HSA are also similar. Franke and Büchner reported a binding affinity of 0.063 mol BaP per mol of HSA [19]; Bothorel and Desmazes reported an almost identical binding of BaP to BSA (0.064 mol/mol) [20].

This work describes the placental perfusion of the pro-carcinogen BaP and the effect of albumin on its transplacental transfer. These experiments demonstrate the anticipated risks of foetal exposure to this pro-carcinogen and also provide details concerning the use of albumin in placental perfusion medium as a relevant physiological model of the distribution of substances between the maternal and foetal circulations.

Materials and Methods

Placentas. Placentas from uncomplicated pregnancies and births were obtained immediately after vaginal birth or elective caesarean section at Copenhagen University Hospital, Rigshospitalet. Informed written consent was provided by the mothers before donation. The project was approved by the Ethical Committees in the Communities of Copenhagen and Frederiksberg (KF 01-145/03 + KF(11) 260,063) and the Danish Data Protection Agency.

Dually perfused placenta method, perfusion medium and albumin. The perfusion model has been described in detail previously [12,21], and is adapted from the models described by Schneider & Huch [22] and by Myllynen *et al.* [23]. Briefly, one vascular unit of the placenta was perfused by cannulation (Flocare Pur Sondes-MP, Ch 5/50 + Ch 6/60) of the foetal circulation in one villous tree with simultaneous supply of maternal perfusion medium to the intervillous space with the system maintained at 37°. The foetal and maternal perfusion media (each 100 ml) consisted of RPMI 1640 medium (Panum Institute, University of Copenhagen, Denmark) supplemented with L-glutamine, 1% penicillin and streptomycin. We have observed that if antibiotics are not added to the perfusion medium, bacteria can appear after 150 min., which may cause a decrease in the pH and pO_2 and lead to an unsuccessful experiment (leakage from the foetal reservoir). The perfusion medium was bubbled with 95% O_2/5% CO_2 for about 15 min. until the pH = 7.4, after which heparin (5000 IU/ml, 5 ml/l medium) was added. Albumin was added to the media in some of the perfusions as 2 mg/ml BSA (96% lyophilized powder, Sigma-Aldrich) (n = 2), 30 mg/ml BSA (n = 2), or 30 mg/ml HSA (20% solution, product # 109,697, CSL Behring Gmbh) (n = 3). Dextran (Sigma-Aldrich) (2 mg/ml) was added to increase the viscosity of the media in experiments without 30 mg/ml albumin. Foetal solution was gassed with 95% N_2/5% CO_2 and maternal solution was gassed with 95% O_2/5% CO_2 throughout the perfusion. Once the pO_2 had reached the desired levels during pre-perfusion (10–15 kPa in foetal and 25–35 kPa in maternal solution), a minimum of gassing was required to maintain the desired levels, and foaming was not an issue. Before starting perfusion with a substance, a system adherence test is performed using the maternal circulation through an empty perfusion chamber. The test aims to study the dissolution of the compound, transmission from surroundings, and binding to the perfusion equipment. As success criteria for the perfusions, the leakage from the foetal reservoir must be less than 3 ml/hr, and an FM-ratio greater than 0.75 for the control substance antipyrine should be realized during the perfusion.

C14-BaP and antipyrine. Radio-labelled [7,10-C^{14}]-BaP (50 μCi/ml) in a toluene solution (GE Healthcare) was added to the maternal perfusion medium for a final concentration of 1.0 μM. Antipyrine (Aldrich-Chemie, Steinheim, Germany) was included as a positive control at a final concentration of 100 μg/ml. In the perfusions containing physiological albumin concentrations, about 30 ml was taken from the maternal medium during the pre-perfusion and the added substances were herein mixed for at least 20 min. This was then returned into the remaining maternal media and an initial time-point sample was taken before the onset of the perfusion. Details regarding the metabolism of BaP are not reported in this

current work, but it was verified that some of the radioactivity detected in maternal and foetal perfusates represents BaP metabolites.

Sample analysis and control. Samples were collected before adding test substances, and at 0, 2, 30, 60, 90, 120, 150, 180, 240, 300 and 360 min. from both the maternal and foetal reservoirs after addition of BaP and the positive control substance (antipyrine). The samples were centrifuged for 5 min. at 4000 *g* and the supernatant was transferred to vials. Two millilitres of Ecoscint (XR LS-372) scintillation liquid (BN instruments A/S) was added to 200 μl of supernatant, and another 200 μl of supernatant was stored at −20° until antipyrine detection by high performance liquid chromatography (HPLC). Pellets from maternal and foetal samples were collected in separate vials after centrifugation; the pellets were then centrifuged again and the supernatant was replaced with 200 μl fresh media in which the pellet was resuspended and added to 2 ml scintillation liquid. Tissue samples (1 g) were taken from the placenta before the perfusion and after the end of perfusion from an area within the cotyledon (clearly perfused white area), and from an unperfused area beside the cotyledon (still red), taking care to avoid tissue exposed during the perfusion. These samples were cut into small pieces and extracted in 3 ml of acetonitrile (J.T. Baker) for at least 72 hr. After this extraction, the tissue was removed and 4 ml of scintillation liquid was added before scintillation counting. The pH and O_2 tension in the maternal perfusion medium, in the foetal perfusion medium, and in the foetal venous outflow were measured every 30 min. using an ABL5 blood gas analyser (Radiometer, Denmark). Adjustments were made using 1 M HCl or 1 M NaOH to adjust pH, and increasing or decreasing the rate of 95% N_2/5% CO_2 gassing in the foetal perfusate or 95% O_2/5% CO_2 gassing in the maternal perfusate to adjust pO_2 after each measurement. An extraction procedure was employed in order to identify the presence of water-soluble metabolites of BaP; 300 μl of centrifuged samples from foetal and maternal perfusates was mixed with 300 μl of chloroform for 3 min, followed by centrifugation for 5 min. at 4000 *g*. Two hundred microlitres from the upper aqueous phase (containing BaP metabolites) and 200 μl from the lower organic phase (containing unmetabolized BaP) were analysed separately by scintillation counting and corrected for partitioning of unmetabolized BaP by comparison to extracted standards. All data are presented as the mean ± SD unless otherwise stated.

Analysis. Radioactivity was detected by liquid scintillation (Liquid Scintillation Analyser, TRI-CARB 2300TR, Packard Instruments Company, Inc., Downers Grove, IL, USA) and a calibration curve was included in each test round. The scintillation counter was programmed to count each sample twice for a maximum of 10 min. The second counting was used for analysis. Antipyrine was detected on a LaChrom HPLC system equipped with a C-18 column and a SecurityGuard precolumn as described elsewhere [24,25].

Foetal-maternal concentration ratio and permeability rate. The foetal–maternal concentration (FM) ratio is the concentration in foetal perfusion medium divided by the concentration in maternal perfusion medium. The FM ratio enables comparison between perfusions and between studies using different placentas and concentrations. An indicative qualitative measure of the permeability coefficient is estimated from the initial linear part of the relationship between perfusion time and the foetal–maternal concentration ratio (FM ratio). The indicative permeability rate illustrates the initial rate of transfer from maternal to foetal circulation, whereas the FM ratio rather illustrates the final level of exchange at the end of perfusion. The FM ratios resulting from the perfusions with and without HSA were compared at each time point by Student's t-test, and differences were deemed statistically significant if $P < 0.05$.

Mass balance. The mass balance calculation after perfusion is adapted from an equation from Mose *et al.* [24]. It is an expression of the counts per minute in perfusion media, tissue and samples relative to the initial counts in the maternal circulation at the beginning of perfusion, as determined from the standard curve or an independent sample. In the recovery equation below, C_M means counts in the maternal compartment at the end of perfusion, V_M is the volume of the maternal compartment at the end of perfusion, V_A is the volume analysed in the scintillation counter, C_F is the count in the foetal compartment at the end of perfusion, V_F is the volume of the foetal compartment at the end of perfusion, C_j is the count in each sample removed during the perfusion from both maternal and foetal compartments for samples 1 through n, V_j is the volume of each sample removed during the perfusion, C_C is the count from the cotyledon sample, M_{CT} is the total mass of the perfused cotyledon, M_{CA} is the mass of the cotyledon sample analysed in the scintillation counter, C_T is the counts in the surrounding tissue sample, M_{TT} is the total mass of the surrounding tissue, M_{TA} is the mass of the sample of the surrounding tissue analysed in the scintillation counter, C_I is the initial counts in the maternal compartment after adding the test substance, and V_I is the initial volume of the maternal compartment.

$$\text{Recovery} = \frac{\left(\frac{C_M \cdot V_M}{V_A} + \frac{C_F \cdot V_F}{V_A} + \sum_{j=1}^{n} \frac{C_j \cdot V_j}{V_A} + \frac{C_C \cdot M_{CT}}{M_{CA}} + \frac{C_T \cdot M_{TT}}{M_{TA}}\right)}{\frac{C_I \cdot V_I}{V_A}} \cdot 100\%$$

Histology. Immediately after each study, representative samples of placental tissue from the perfused areas were fixed in 10% formaldehyde and paraffin blocks were prepared following standard procedures of the Department of Pathology at Rigshospitalet. The paraffin embedded tissue was sectioned by use of a microtome to 7 μm slices. These were placed on routine glass slides, coloured with eosine and hematoxylin, and then the placental tissue morphology was evaluated.

Results

Twelve placentas from uncomplicated deliveries resulted in successful perfusions with the test substance BaP (out of a total of 34 perfusions with BaP including unsuccessful and terminated perfusions). The average maternal age was 35 ± 4 years, two mothers were smokers, and one smoker had taken prescription medication, but this had no effect on the transport of BaP. One placenta was delivered by normal birth, the rest were from elective caesarean section, and the mean placental weight was 768.5 ± 82.3 g. A requirement for a successful perfusion is a volume loss below 3 ml/h from the foetal circulation; in these perfusions, the mean volume loss for all 12 placental perfusions was 1.59 ± 1.26 ml/h. Table 1 presents more details of the mean perfusion variables, including the pH and pO_2 levels of the maternal reservoir, foetal reservoir, and the foetal outflow during the 6-hr perfusions. The pO_2 levels in the foetal outflow are higher than the pO_2 levels in the foetal reservoir, indicating successful transfer of oxygen across the placenta during the perfusions.

The positive control substance antipyrine passed rapidly from the maternal to the foetal compartment. Figure 1 shows that the mean FM ratio for all 12 perfusions was 0.82 ± 0.19 after 150 min. of perfusion, and 0.95 ± 0.23 after 300 min. of perfusion. The indicative permeability rate for antipyrine was 0.74/h, but the rapid transfer of antipyrine necessitates more samples within the first hour to more

Table 1.

Perfusion variables in all 6-hr BaP perfusion.

Perfusion variables: Mean (range)		Albumin content in perfusion medium			
		No albumin (n = 5)	2 mg/ml BSA (n = 2)	30 mg/ml BSA (n = 2)	30 mg/ml HSA (n = 3)
Volume loss (ml)*	Maternal reservoir	8.1 (−9.7–15.8)	0.8 (−0.8–2.3)	−10.4 (−10.4– −10.3)	−12.0 (−35–2.6)
	Foetal reservoir	9.0 (−2.9–29.4)	11.8 (8.8–14.7)	6.6 (6.1–7.0)	16.6 (11.6–20.9)
	Foetal reservoir (ml/h)	1.3 (−0.4–4.2)	1.7 (1.3–2.2)	0.97 (0.9–1.04)	2.4 (1.7–3.1)
pO_2 (kPa)†	Maternal reservoir	63.6 (53–69)	74.1 (71–77)	46.2 (38–54)	45.7 (45.3–46.3)
	Foetal reservoir	7.4 (6.6–9.8)	8.5 (7.6–9.3)	6.9 (6.7–7.1)	13.9 (12.4–14.6)
	Foetal outflow	10.3 (8.5–11.6)	9.43 (8.5–10.4)	10.9 (9.2–12.7)	17.7 (13.7–20.1)
pH†	Maternal reservoir	7.15 (7.11–7.19)	7.24 (7.18–7.29)	7.26 (7.25–7.26)	7.15 (7.05–7.21)
	Foetal reservoir	7.26 (7.2–7.3)	7.28 (7.26–7.29)	7.31 (7.27–7.35)	7.28 (7.22–7.33)
	Foetal outflow	7.18 (7.15–7.21)	7.18 (7.13–7.22)	7.28 (7.2–7.35)	7.26 (7.17–7.32)
Foetal flow rate (ml/min)‡		3.1 (2.9–3.5)	3.0 (2.9–3.0)	3.0 (3)	3.0 (2.8–3.3)
Time (min), birth to placenta laboratory		29.4 (16–36)	33.5 (29–38)	29.5 (27–32)	27.7 (19–37)
Pre-perfusion time (min)		44.8 (37–59)	54.5 (48–61)	44.5 (42–47)	59.3 (44–85)
Maternal age (years)		34.5 (33–38)	32.5 (32–33)	41 (39–43)	33.7 (28–37)

*The samples removed during the perfusion are taken into account when doing the volume loss calculations. Volume gain in the maternal reservoir is due to the content of blood in the placenta which is washed out during the perfusion. The foetal system is under pressure and will lose a few ml to the maternal system during a 6-hr perfusion.

†These values represent the most extreme, and are not a true average of the perfusion as the pH and pO_2 were adjusted immediately after each measurement.

‡The foetal flow rate is measured with a volumetric container sampling the foetal outflow for 2 min. This is carried out every 20–30 min. to ensure a stable flow. The maternal flow rate is ca. 9 ml/min. as determined by the pump flow.

accurately determine the permeability rate for this substance. The addition of albumin to the perfusion medium had no effect on the transfer of antipyrine through the placenta, in agreement with the observations of Nanovskaya *et al.* [26].

Figure 2 demonstrates a clear difference in the permeability rate of BaP in the presence of physiological concentrations of HSA in the perfusion medium, compared to perfusion medium without albumin. Perfusions with 30 mg/ml HSA (n = 3) resulted in an indicative permeability rate for BaP of 0.31/h, and the mean FM ratio was 0.78 after 6 hr. At all time-points between 30 min. and 360 min., the FM ratios with HSA were significantly higher than the FM ratios with no albumin ($P < 0.05$). With 30 mg/ml BSA, the indicative permeability coefficient was 0.08/h, and the mean FM ratio was 0.38 after 6 hr. When only 2 mg/ml BSA or no albumin was added, the results overlapped; the indicative permeability rate was less than 0.01/h, and the mean FM ratio after 6 hr was 0.05. Extraction of perfusate samples demonstrated the formation of water-soluble BaP metabolites during placental perfusion.

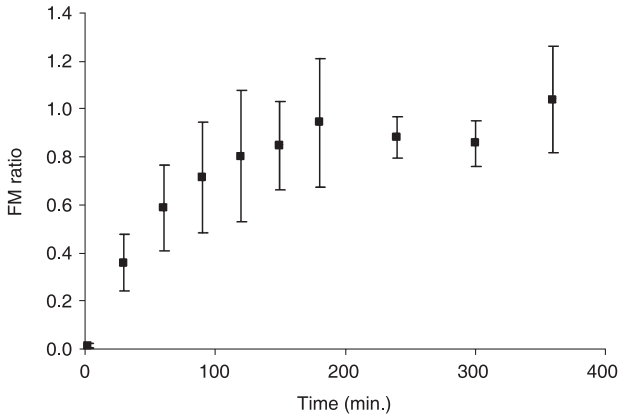

Fig. 1. FM ratio of antipyrine passage in all BaP perfusions (n = 12). The FM ratio is the foetal concentration divided by the maternal concentration.

The recovery was calculated by using the aforementioned equation. The mean recovery was between 78–84%, as shown in table 2. Scintillation counting of the pellets from the centrifuged samples containing red blood cells account for some of the BaP not represented in the mass balance equation. Pellet samples taken at the end of a perfusion demonstrated a loss of 2.8% of the added BaP in the pellets from maternal samples and 0.04% of the added BaP in the pellets from foetal samples. Table 2 shows that after perfusions with little or no albumin, most of the BaP was found in placental tissues. In perfusions with 30 mg/ml albumin, on the other hand, less BaP had accumulated in the tissue – especially in the cotyledon – and more BaP remained in the maternal and foetal reservoirs. For each albumin concentration, system adherence tests with blood added to the perfusion medium were performed to account for binding of BaP to the experimental system. Regardless of the type of perfusion medium used, BaP did not bind to the system tubing. Although albumin increased the solubility of BaP in the perfusion medium, the mean concentration of BaP fell from 0.87 μM to 0.15 μM after a 6-hr tubing test. Most of this difference (mean equivalent of 0.56 μM) was attributed to the red blood cells in the pellets of centrifuged samples from the tubing tests.

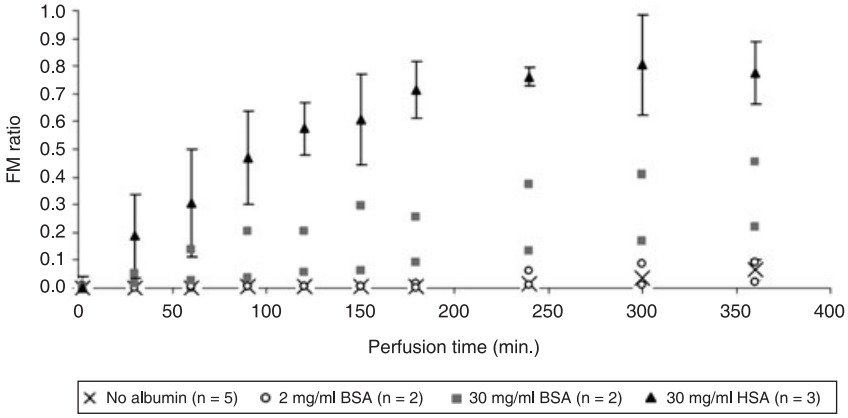

Fig. 2. FM ratio of 1.0 μM benzo[α]pyrene in 6-hr placental perfusions in medium containing different concentrations of BSA or HSA.

As evidence of a successful perfusion, histological examinations of sections of the perfused placenta were performed. No tissue damage was observed in successful perfusions, indicating that BaP is not directly toxic to the placental tissue during the perfusion, regardless of the perfusion medium used. The perfused placental tissue showed normal chorionic villi as appropriate for development at term. The foetal vessels were dilated, indicating a successful perfusion.

Discussion

These experiments show that in the presence of physiological concentrations of albumin, the pro-carcinogen BaP crosses the perfused human placenta to the foetal circulation, and placental metabolism of BaP was observed. BaP is a pro-carcinogen, meaning that possible deleterious effects to the foetus would follow its conversion to harmful metabolites. The metabolism of BaP to a 7,8-epoxide is catalysed predominantly by CYP1A1, although other cytochrome P450 isoenzymes may also participate [3]. Epoxide hydrolase can mediate further transformation to a 7,8-dihydrodiol, and then a second epoxidation – by CYP1A1, peroxidize-catalysed epoxidation, or cooxidation – leads to benzo[α]pyrene-7,8-dihydriodiol-9,10-epoxide (BPDE) [3], which is the reactive metabolite forming DNA-adducts. BPDE-DNA adducts can cause p53 mutations, especially G $\rightarrow$ T transversions [27].

Maternal exposure to BaP can occur by smoking tobacco, inhalation of polluted air, and ingestion of certain foods. This work demonstrates that maternal exposure to BaP results in significant foetal exposure to BaP. This is especially disturbing in cases of smoking during pregnancy because cigarette smoke significantly (14.7-fold) up-regulates the expression of placental CYP1A1 [28], an enzyme which is implicated in the conversion of BaP to the carcinogenic metabolite BPDE. This means that when a mother smokes tobacco, not only is foetal exposure to BaP increased, but the ability of the placenta to convert BaP to BPDE is also increased, thereby multiplying the risks to the developing foetus.

Arnould *et al.* found that urinary cotinine levels (representing exposure to tobacco smoke) correlated well with the amounts of BPDE-DNA adducts in placenta ($r^2 = 0.905$) and umbilical cord blood ($r^2 = 0.684$) [29]. Even some of the non-smoking mothers in their study had low but detectable amounts of cotinine and BPDE-DNA adducts, meaning that second-hand exposure to tobacco smoke increases the risks of foetal exposure to harmful BaP metabolites. Sanyal *et al.* have also observed significantly increased metabolism of BaP in placental samples from smokers [30], as well as detectable amounts of cotinine and polycyclic aromatic hydrocarbon-DNA adducts in some placentas from non-smokers [31], although interindividual variability in placental metabolic activity has been noted [28,30]. Placental metabolism of BaP is not the only concern, however, as the observations of Hansen *et al.* suggest that the foetus is also capable of metabolizing BaP to DNA-binding compounds [32]. Gurtoo *et al.* reported that maternal exposure correlated with the amount of DNA adducts in the placenta and umbilical cord

Table 2.

Mean mass balance of BaP after 6-hr placental perfusion (range).

BaP	% found in maternal perfusate	% found in foetal perfusate	% found in samples	% found in cotyledon	% found in rest tissue	% found in total
Medium containing no albumin	21.7 (12–40)	1.3 (0.3–2.4)	1.6 (0.8–1.8)	42.6 (21–60)	14.3 (3–30)	81.5 (44–129)
Medium containing 2 mg/ml BSA	24.3 (18–30)	1.4 (0.3–2.4)	1.2 (1–1.3)	33.6 (20–46)	20.7 (17–24)	81.2 (72–90)
Medium containing 30 mg/ml BSA	22.7 (18–28)	6.4 (3–10)	2.2 (2–2.5)	23.3 (11–36)	23.1 (11–36)	77.7 (69–86)
Medium containing 30 mg/ml HSA	38.8 (23–65)	21.9 (11–37)	4.2 (3.2–5.6)	14.4 (5–30)	4.7 (1.2–7.5)	84.0 (47–144)

blood, as had Autrup *et al.* [11,33]. It should also be noted that BPDE is not the only metabolite of BaP [34], and that besides its pro-carcinogenicity, BaP can cause aryl hydrocarbon receptor (AhR) pathway-related abnormalities in placental vasculature and intrauterine growth restriction in C57B1/6 mice [35] and neurodevelopmental effects in Long-Evans rats [36].

An interesting observation from this work is that the transplacental transfer of BaP is minimal when the perfusion medium contains little or no albumin. An objective of this work was to compare the absence and presence of physiological concentrations of albumin in the perfusion medium, as well as to compare human versus bovine albumin. The presence of 30 mg/ml BSA caused an increase in the FM ratio compared to the absence of albumin. Although albumin does not cross the placental barrier, it is taken up by trophoblast cells and recycled to the maternal compartment [37]. This means that although albumin binds to BaP to some extent, it would not serve as a carrier for BaP all the way to the foetal compartment, but it may increase the uptake of BaP into the trophoblast layer for subsequent dissociation and transport to the foetal side. Furthermore, the presence of albumin in the perfusion medium reduces the amount of BaP that is bound to placental tissues (as shown in table 2), thus making more BaP available for transplacental transport.

The FM ratio for BaP was higher in the presence of human albumin compared to bovine albumin at the same concentrations (30 mg/ml). This is due to the different formulations used during the experiments. BSA was added to the perfusion medium as a powder to the desired concentration. The HSA, on the other hand, was obtained as a concentrated liquid which was diluted during the preparation of the perfusion medium to a final concentration of 30 mg/ml. This concentrated solution contained not only 200 mg/ml HSA, but also stabilizers: 16 mM sodium acetyltryptophanate, 16 mM sodium caprylate and 140 mM sodium chloride. In human serum, more BaP binds to lipoproteins (containing fatty acid chains) than to albumin [17]. It is therefore presumed that in the experiments containing HSA, BaP was preferentially bound to the caprylic acid (a fatty acid) present in this formulation, which may promote its transplacental transfer in both directions.

In perfusion experiments with guinea pig placentas, the transport rate of BaP was higher when heparinized blood was used as the perfusion medium, compared to Ringer solution containing albumin; when physiological saline containing dextran (without albumin) was used, no BaP could be detected in the foetal compartment [38]. This matches the rank order from the current study, where perfusion medium without albumin resulted in the least BaP transfer, and perfusions with albumin showed more BaP transfer. Although perfusion medium containing HSA, caprylic acid, and acetyltryptophanate is not equivalent to heparinized guinea pig blood, when it is considered that BaP binds preferentially to serum lipoproteins compared to albumin, and considering the lipophilic components of the HSA formulation used in the human placental perfusions, it is not surprising that these experiments resulted in greater BaP transfer compared to those experiments with less lipophilic components in the perfusion medium.

Kihlström also investigated hydrophilic BaP metabolites (mBaP) and found that BaP crossed the guinea pig placenta more rapidly than mBaP; however, the concentration of mBaP in the plasma of foetuses with intact umbilical circulation during the experiments was higher than the concentration of BaP [38]. This significant influence of foetal metabolism of BaP is in agreement with the aforementioned report of Hansen *et al.* from human samples [32] and demonstrates that the risks of foetal exposure to reactive BaP metabolites is not limited to transplacental transfer following maternal metabolism, but is also affected by both placental and foetal metabolic activity.

In conclusion, BaP was shown to translocate from the maternal to foetal compartment in dually perfused human placenta experiments, and placental metabolism of BaP was observed. The transfer rate and FM ratio of BaP increased significantly when physiological concentrations of albumin were added to the perfusion medium, and the transfer was even higher in the presence of a human serum albumin formulation containing acetyltryptophanate and caprylate. Exposure to BaP may pose serious risks to foetal development since the conversion of BaP to carcinogenic metabolites can be mediated by placental and foetal enzymes.

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