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Bidirectional placental transfer of Bisphenol A and its main metabolite, Bisphenol A-Glucuronide, in the isolated perfused human placenta

T. Corbel[a,b], V. Gayrard[a,b], S. Puel[a,b], M.Z. Lacroix[a,b], A. Berrebi[c], S. Gil[d], C. Viguié[a,b], P.-L. Toutain[a,b], N. Picard-Hagen[a,b,*]

[a] INRA, UMR 1331 Toxalim, Research in Food Toxicology, F-31027 Toulouse, France
[b] Université de Toulouse, INPT, ENVT, EIP, UPS, F-31076 Toulouse, France
[c] Hôpital Paule de Viguier, Service de Gynécologie Obstétrique, CHU Toulouse, F-31059 Toulouse, France
[d] INSERM, UMR-S 767 "Grossesse normale et pathologique", Faculté de Pharmacie, Université Paris Descartes, F-75006 Paris, France

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ABSTRACT

The widespread human exposure to Bisphenol A (BPA), an endocrine disruptor interfering with developmental processes, raises the question of the risk for human health of BPA fetal exposure. In humans, highly variable BPA concentrations have been reported in the feto-placental compartment. However the human fetal exposure to BPA still remains unclear. The aim of the study was to characterize placental exchanges of BPA and its main metabolite, Bisphenol A-Glucuronide (BPA-G) using the non-recirculating dual human placental perfusion. This high placental bidirectional permeability to the lipid soluble BPA strongly suggests a transport by passive diffusion in both materno-to-fetal and feto-to-maternal direction, leading to a calculated ratio between fetal and maternal free BPA concentrations of about 1. In contrast, BPA-G has limited placental permeability, particularly in the materno-to-fetal direction. Thus the fetal exposure to BPA conjugates could be explained mainly by its limited capacity to extrude BPA-G.

1. Introduction

Bisphenol A (BPA) is a xenoestrogen widely used as a monomer in the manufacture of epoxy resins and polycarbonate plastics that can contribute to the almost ubiquitous human exposure to BPA across a lifespan [1]. Much of the concern regarding BPA safety has arisen from reported adverse effects of BPA that can interfere with the normal development of reproductive organs and mammary glands and also with energy metabolism and cognitive performance when tested, even at low doses, in experimental species during the perinatal period [1].

In humans, highly variable BPA concentrations have been reported in the feto-placental compartment, in umbilical cord blood, in placental tissue and in amniotic fluid [1], indicating a transfer of BPA from mother to the fetus. However, these limited biomonitoring data need to be considered with caution. Indeed high BPA levels at single time points could be due to contamination from the sampling devices or to a recent maternal exposure to BPA at delivery in particular with medical devices used in cesarean section [2]. Thus, the human fetal exposure to BPA still remains unclear and the biomonitoring data do not enable a relationship between maternal and fetal exposure to be established.

BPA toxicokinetic investigations using the pregnant sheep physiological model [3] have shown that the fetus received about 4.5% of the BPA dose administered to the mother and at a late stage of pregnancy the ovine feto-placental unit was very efficient in metabolizing BPA into conjugated compounds that remained trapped within the fetal compartment, leading to a high fetal exposure to BPA conjugates [3,4]. Thus, BPA placental transfer from fetus to mother appears as one of the key parameters determining the fetal exposure to the unconjugated (active) form of BPA and to its conjugates.

Abbreviations: BPA, Bisphenol A; BPA-G, Bisphenol A-Glucuronide; BSA, Bovine serum albumin; Cl, clearance; ER, extraction rate; TR, transfer rate.

* Corresponding author at: Ecole Nationale Vétérinaire de Toulouse 23, chemin des capelles, 31076 Toulouse cedex 3, France. Tel.: +33 561193861; fax: +33 561 193 917.
E-mail addresses: t.corbel@envt.fr (T. Corbel), v.gayrard@envt.fr (V. Gayrard), s.puel@envt.fr (S. Puel), m.lacroix@envt.fr (M.Z. Lacroix), berrebi.a@chu-toulouse.fr (A. Berrebi), sophie.gil@parisdescartes.fr (S. Gil), c.viguie@envt.fr (C. Viguié), pltoutain@wanadoo.fr (P.-L. Toutain), n.hagen-picard@envt.fr (N. Picard-Hagen).

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This highlights the urgent need to develop relevant models to predict human fetal exposure that are not biased by extrapolation from species with different feto-placental physiology. From this standpoint, the perfusion of an isolated human cotyledon appears as a method of choice for studying the placental transport of BPA. Materno-to-fetal placental transfer of BPA of 19–27% was already reported in a placenta model in closed (recirculating) configuration [5,6]. However, this closed system did not allow the fetal and maternal placental clearances to be evaluated contrary to the open (non-recirculating) system. Nonetheless, the single pass system is a metabolically static system that does not enable placental metabolism to be evaluated. Moreover, the preponderance of the glucuronoconjugated form of BPA in the plasma of fetal monkeys [7] and ovine fetus [3] after maternal administration raises the question of its maternal or fetal origin. The goal of our study was to evaluate in an open (non-recirculating) human cotyledon, the placental transfer of BPA and BPA-G in both the materno-to-fetal and feto-to-maternal directions in order to estimate the fetal exposure from a known maternal internal exposure.

2. Materials and methods

2.1. Chemicals

BPA (purity ≥ 99%) and antipyrine were purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France) and were dissolved in ethanol at a concentration of 1 mg/mL and water at a concentration of 100 mg/mL respectively. BPA-G was purified in our laboratory as previously described [8] and was dissolved in ethanol at a concentration of 10 mg/mL. All solutions were stocked at −20 °C until use. Albumin from bovine serum (BSA Fraction V) was purchased from PAA Laboratories (Vélizy-Villacoublay, France) and was added extemporaneously to the perfusion media.

All the materials for the placenta perfusion including the materials used for the preparation of solutions, sampling, processing and analysis were glass or BPA-free plastic containers and the absence of leaching of BPA from the tubing was verified.

2.2. Placentae

The study received institutional approval and each patient gave informed consent to participate in the study. 22 term placentae (582.2 ± 96.5 g) from uneventful pregnancies were obtained from HIV-seronegative women delivered in the CHU Paule de Viguier, Toulouse, France after cesarian sections ($n = 5$) or normal vaginal delivery ($n = 17$).

2.3. Perfusion technique

Collected placentae were perfused within 30 min of the delivery in an open double circuit system according to the method initially described by Schneider et al. [9] and modified as follows [10,11]. Briefly, after a visual examination to check placenta integrity, a truncal branch of the chorionic artery and its associated vein supplying a peripheral cotyledon were catheterized (Microtube Tygon S54HL, 1.02 mm i.d.; 1.78 mm o.d. Saint Gobain, Courbevoie, France).

The fetal circulation was established at a flow rate of 6 mL/min to ensure a balance between arterial and venous fetal flows. The absence of vascular leakage was checked. On the maternal side, the perfused area progressively whitened, which allowed visualization of the selected cotyledon. The irrigated cotyledon was isolated by cutting it out and it was placed into a thermostated glass receptacle maintained at 37 °C with the maternal villi facing upwards. The maternal perfusion was established at a flow rate of 12 mL/min through 2 hypodermic cannulas inserted into the intervillous space.

The maternal and fetal media were Earle's medium (Euromedex, Souffel Weyersheim, France) supplemented with BSA fraction V. The pH were adjusted continuously throughout the experiment to 7.42 ± 0.01 for the maternal and 7.22 ± 0.01 for the fetal perfusion media.

2.4. Experimental design

The study was designed to evaluate the placental transfer of BPA and BPA-G, both in materno-to-fetal and in the feto-to-maternal directions.

Antipyrine (20 μg/mL), a reference control substance for passive diffusion and integrity of the barrier, and the test substances, BPA or its main metabolite BPA-G, were added to the maternal or fetal reservoirs to study maternal-to-fetal or fetal-to-maternal transfer, respectively. The nominal BPA concentrations infused into the fetal or the maternal compartments were 45 ng/mL (0.2 μM) and 250 ng/mL (1 μM); these concentrations were selected to test the proportionality of BPA transfer rate. As BPA binds to albumin [12], this experiment used perfusion medium containing 30 g/L of BSA to reflect the physiological protein plasma concentration at late pregnancy [13]. Bovine serum albumin was used instead of human albumin because the amino acid sequences of bovine and human albumin are 76.5% homologous [14] and the binding capacities toward BPA of ruminant and human serum albumin are similar [3,12]. BPA-G was added at a concentration of 1000 ng/mL (2.5 μM) in the fetal or maternal media supplemented with 2 g/L of BSA. Because the permeability across membrane of hydrophilic substances is generally low, this high concentration of BPA-G was chosen for effective detection in the opposite compartment after its placental transfer.

Each perfusion experiment was performed for 90 min. Maternal and fetal exudates were collected every 5 min after addition of test substances and the volume was measured.

For determining maternal-to-fetal transfer, the two analytes (antipyrine and BPA or BPA-G) were added simultaneously in the maternal medium. Samples (1 mL) were collected from the maternal inflow reservoir before (time 0) and after addition of the test molecules and at the end of the perfusion, from the maternal outflow perfusate at 0, 30, 60 and 90 min, and from the fetal outflow perfusate at time 0 and every 5 min up to 90 min.

Fetal-to-maternal transfer was assessed after addition of the two analytes (antipyrine and BPA or BPA-G) to the fetal reservoir at the same concentrations as for the materno-to-fetal transfer while the maternal inflow perfusate was drug free. Samples (1 mL) were collected from the fetal inflow reservoir before (time 0) and after addition of the test molecules and at the end of the perfusion, from the fetal outflow perfusate at 0, 30, 60 and 90 min, and from the maternal outflow perfusate at time 0 and every 5 min up to 90 min.

At the end of the perfusion, the isolated cotyledon was perfused with phosphate-buffered saline at 4 °C for 10 min and this washing solution was collected.

All samples were immediately chilled in iced and centrifuged for 10 min at 3000 × *g* and 4 °C and the supernatant was collected and stored at −20 °C until assayed.

Tissue samples (about 1 g) were taken from the placenta before the perfusion and after the washing perfusion from an area within the cotyledon (clearly perfused white area) to determine tissue concentrations of BPA and BPA-G.

2.5. Placental tissue homogenization

The homogenization method was validated in order to check that the sample preparation did not lead to hydrolysis of BPA-G. Briefly, standards solutions of *d16*-BPA and *d6*-BPA-G were prepared by dissolving each compound in acetonitrile/H_20 (50/50,

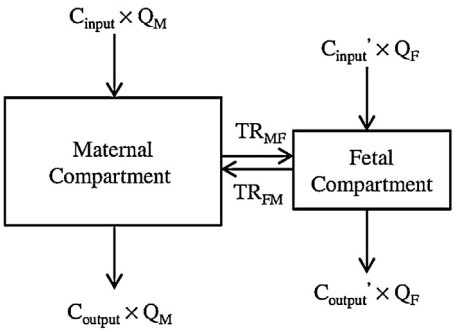

Fig. 1. Two-compartment model of the open-configuration double circuit placental perfusion. TR_{MF} and TR_{FM} were the materno-to-fetal and the fetal-to-maternal transfer rates, respectively. C_{input} and $C_{input'}$ were the maternal and fetal inflow concentrations, respectively. C_{output} and $C_{output'}$ were the maternal and fetal outflow concentrations, respectively. Q_M and Q_F were maternal and fetal system flow rates, respectively.
$C_{input'} \times Q_F = 0$ in materno-to-fetal experiment and $C_{input} \times Q_M = 0$ in feto-to-maternal experiment.

vol/vol). These two internal standards were added to placenta tissue (placental samples) or water (control samples) at the final concentration of 200 ng/mL for *d16*-BPA and 400 ng/mL for *d6*-BPA-G in glass tubes at 0 °C. Placental and control samples were homogenized in 1 mL methanol/g tissue (or 1 mL methanol/mL water) for 1 min on ice using an Ultra-Turrax®. The efficiency of extraction was monitored by calculating the ratio between areas of internal standard in control samples and areas of internal standard in placenta samples. The absence of any hydrolysis that might have occurred during homogenization and extraction was verified by monitoring the absence of *d6*-BPA in the samples.

2.6. Analytical procedure

Antipyrine concentrations in the perfusion medium were determined by HPLC coupled with UV detection from an adapted method previously described [15]. The calibration curve ranged from 0.5 to 20 μg/mL. The mean intra- and inter-day coefficients of variation for three concentration levels were lower than 15% and the limit of quantification (LOQ) was validated at 0.5 μg/mL.

BPA and metabolite concentrations in perfusion media and in placental tissue were determined by an UPLC/MS/MS after acetonitrile extraction as previously described [8]. The calibration curves ranged from 1-1000 ng/mL and 10–5000 ng/mL for BPA and BPA-G, respectively. The mean intra- and inter-day coefficients of variation for three concentration levels and for BPA and BPA-G were lower than 15% and the limits of quantification (LOQ) were validated at 1 ng/mL and 10 ng/mL, respectively.

2.7. Calculation methods

Only concentrations at steady state, reached after 15 min both for antipyrine and BPA or BPA-G, were used for calculations of standard parameters: transfer rate, extraction rate and transplacental clearance. These standard parameters were calculated according to the Challier formulas [16,17]. The open-configuration double circuit placental perfusion could be represented as a two-compartment model (Fig. 1).

In this configuration, the transfer rate (TR) was calculated for each steady state time point during perfusion as the ratio between the concentrations in the receiving compartment to the nominal concentration in the entrance compartment according to the following equations:

$$TR_{MF} = \frac{C_{output}'}{C_{input}} \times 100 \quad (1)$$

$$TR_{FM} = \frac{C_{output}}{C_{input}'} \times 100 \quad (2)$$

where TR_{MF} and TR_{FM} were the materno-to-fetal and the feto-to-maternal transfer rates, respectively; C_{input} and $C_{input'}$ were the maternal and the fetal inflow concentrations, respectively; $C_{output'}$ and C_{output} were the fetal and maternal outflow concentrations, respectively.

In the materno-to-fetal direction, the perfusions were validated if the transfer rate of antipyrine was above the generally accepted threshold of 20% [11], whereas a threshold of antipyrine transfer rate above the mean value of 15% previously reported by Challier et al. and Sudhakaran et al. [17,18] was arbitrarily chosen to validate the feto-to-maternal perfusions.

The clearance index in the materno-to-fetal direction and vice versa was the mean ratio of the transfer rate of BPA or BPA-G to that of antipyrine calculated every 5 min throughout the perfusion.

To determine the extraction rate (ER), corresponding to the fraction of the dose transferred from entrance compartment to receiving one, it is necessary to take into account the mean flow rate in maternal (Q_M) and fetal (Q_F) circulations determined every 5 min throughout perfusion. The extraction rate (ER) was calculated according to the following equations:

$$ER_{MF} = \frac{Q_F \times C_{output}'}{Q_M \times C_{input}} \times 100 \quad (3)$$

$$ER_{FM} = \frac{Q_M \times C_{output}}{Q_F \times C_{input}'} \times 100 \quad (4)$$

The materno-to-fetal transplacental clearance (Cl_{MF}) and the feto-to-maternal transplacental clearance (Cl_{FM}), corresponding to the volume of perfusate cleared of the substance per unit time, were calculated with the following equations taking into account the principle of mass conservation:

$$Cl_{MF} = \frac{Q_M \times (C_{input} - C_{output})}{C_{input}} = \frac{Q_F \times C_{output}'}{C_{input}} \quad (5)$$

$$Cl_{FM} = \frac{Q_F \times (C_{input}' - C_{output}')}{C_{input}'} = \frac{Q_M \times C_{output}}{C_{input}'} \quad (6)$$

The mass balance was calculated as the ratio of the sum of the quantities of substrate in the perfusion media, tissue and PBS washings to the measured amount of the substrate in the maternal or fetal reservoir using the following equation:

$$\%_{Recovery} = \frac{[V_F \times \text{Pool}\, C_F + (V_F \times C_F)_{Wash}] + [V_M \times \text{Pool}\, C_M + (V_M \times C_M)_{Wash}] + [C_{tissue} \times \text{Weight}_{cot}]}{V \times C} \quad (7)$$

where V_F and V_M were the fetal and maternal perfused volumes, respectively; Pool C_F and Pool C_M were the BPA or BPA-G concentrations in the fetal and maternal pool (total volume collected throughout the perfusion), respectively; $(V_F \times C_F)_{Wash}$ and $(V_M \times C_M)_{Wash}$ were the fetal PBS washings volume and the matching concentration and the maternal PBS washings volume and the matching concentration, respectively; C_{tissue} and $Weight_{cot}$ were the BPA or BPA-G concentration in the cotyledon collected after perfusion and the weight of the perfused cotyledon, respectively; and V and C were the total perfused volume and the BPA or BPA-G concentration in the loaded reservoir, respectively.

The amount of substrate in the tissue was corrected by the corresponding quantity determined in the tissue before perfusion.

The modified equation of Henderson–Hasselbach described below [19,20] was used to predict the ratio between fetal and maternal steady-state concentrations (F:M) of free BPA in vivo:

$$F:M = \frac{\%\text{unbound}_M}{\%\text{unbound}_F} \times \frac{1+10^{pK_a-pH_F}}{1+10^{pK_a-pH_M}} \times \frac{Cl_{MF}}{Cl_{FM}+Cl_F} \quad (8)$$

where % unbound$_M$ and % unbound$_F$ were the fractions of free BPA (not bound to plasma proteins) equal to 0.07 in maternal plasma [12] and estimated from maternal values to be 0.06 in fetal plasma [21], pK_a was the pK_a of BPA of 10.2 [22]; pH$_F$ and pH$_M$ were the pH of fetal and maternal blood respectively; Cl$_{MF}$, Cl$_{FM}$ and Cl$_F$ were the materno-to-fetal clearance, the feto-to-maternal clearance and the fetal non-placental clearance, respectively. The mean materno-to-fetal and feto-to-maternal BPA clearances were determined in the perfusion model. The fetal BPA non-placental clearance (Cl$_F$) was considered negligible compared to Cl$_{FM}$, in accordance with previous data obtained in the ovine model [3].

2.8. Statistical analysis

Descriptive statistics were used and data are expressed as means (±SD). All statistical analyses were done using general linear models on Systat 12.0 (SPSS, Inc.).

For both the feto-to-maternal and materno-to-fetal BPA transfer, the mean of the placental transfer of BPA and of antipyrine during time points along the experiment were compared by a three-way ANOVA with substrate, concentration and perfusion time and double and triple interactions between substrate, concentration, perfusion time as fixed-effect and placenta nested within the BPA concentration as a random-effect factor.

The time course transfer rates of BPA-G and antipyrine were compared by a two-way ANOVA with substrate and perfusion time and their interaction as a fixed factor.

The time course of the BPA clearance index was analyzed by a three-way ANOVA with direction (maternal-to-fetal and fetal-to-maternal), concentration and perfusion time and their interactions as fixed-effect factors and placenta nested within the interaction between BPA concentration and direction as a random-effect factor.

The time course of the clearance index of BPA-G was analyzed by a two-way ANOVA with direction and perfusion time, their interaction as fixed effect factors and placenta nested within the direction as a random effect factor.

The materno-to-fetal and feto-to-maternal extraction rates were compared using a one-way ANOVA with direction as fixed effect.

3. Results

For all validated perfusions, the overall mean clearance of antipyrine was 1.66 ± 0.63 mL/min and 2.44 ± 0.34 mL/min in the materno-to-fetal and feto-to-maternal directions, respectively. The wet weight of the perfused cotyledons was 36.6 ± 13.6 g. The average flow rate was 5.27 ± 0.36 mL/min in the fetal circulation and 12.6 ± 0.67 mL/min in the maternal circulation and remained stable throughout the perfusion.

Before perfusion, BPA was detected in 19 placentae (86%) at a mean concentration close to the BPA LOQ (1.63 ± 0.49 ng/g), whereas no BPA-G was detected in any placenta.

For the 22 placentae, at the end of the perfusion, the overall mean recovery (±SD) of infused molecules in fetal and maternal perfusates and in the cotyledon were 105 ± 17% (range 80–131%) and 83 ± 11% (range 69–99%) for BPA perfusions and 105 ± 18% (range 81–126%) and 92 ± 5% (range 86–97%) for BPA-G perfusions in the feto-to-maternal and in the materno-to-fetal directions, respectively.

BPA-G was never detected in any perfusates or cotyledons following BPA placental perfusions no matter the direction of the transfer. Similarly, BPA was never detected in perfusates or cotyledons following BPA-G perfusion in both feto-to-maternal and materno-to-fetal direction.

3.1. Placental transfer of BPA

Fig. 2A and B depicts the mean (±SD) time courses of materno-to-fetal placental transfer of BPA at the concentration of 45 ng/mL (n = 3) and 250 ng/mL (n = 4) and of antipyrine at 20 μg/mL.

The placental transfer rate of antipyrine and BPA increased rapidly during perfusion and the plateau was reached from 15 min to the end of the perfusion. The materno-to-fetal transfer rates (mean ± SD; range) of BPA at concentrations of 45 ng/ml (36.0 ± 8.34%; 26.8–43.0%, n = 3) and 250 ng/mL (24.2 ± 8.74%; 15.3–34.3%, n = 4) were similar but systematically slightly lower than that of antipyrine (34.6 ± 10.6%, 20.9–48.7%, n = 7, p < 0.0001) (Table 1).

The materno-to-fetal transplacental clearances of BPA were 1.94 ± 0.55 mL/min and 1.22 ± 0.43 mL/min for the BPA perfusates concentrations of 45 and 250 ng/mL, respectively (Table 1). In materno-to-fetal experiments, 11.2 ± 5.92% and 10.4 ± 2.18% of the BPA added in the maternal perfusate at the concentration of 45 ng/mL and 250 ng/mL respectively were transferred across the barrier during the perfusion. Only 0.45 ± 0.60% and 0.34 ± 0.21% of the BPA dose perfused at the concentration of 45 or 250 ng/mL remained in the perfused cotyledon.

Fig. 2C and D shows the time courses (mean ± SD) of feto-to-maternal placental transfer of BPA at the concentration of 45 ng/mL (n = 3) and 250 ng/mL (n = 4) and of antipyrine. The feto-to-maternal placental transfer rate of antipyrine and BPA increased rapidly during perfusion and reached a plateau from 15 min until the end of the perfusion. The feto-to-maternal transfer rate (mean ± SD; range) of BPA at the fetal concentration of 45 ng/mL (16.1 ± 2.28%; 13.5–17.8%, n = 3) and of 250 ng/mL (14.2 ± 1.52%; 12.4–15.9%, n = 4) was systematically slightly lower than that of antipyrine (19.3 ± 2.30%; 16.3–23.1%, n = 7, p < 0.0001) (Table 1).

The feto-to-maternal transplacental clearances of BPA were 1.95 ± 0.25 mL/min and 1.87 ± 0.24 mL/min for the BPA perfusate concentrations of 45 and 250 ng/mL, respectively (Table 1). 33.1 ± 4.40% and 36.2 ± 5.69% of BPA added in the fetal perfusate at the concentration of 45 ng/mL and 250 ng/mL, respectively were transferred across the placenta barrier. After 90 min of placenta perfusion, 3.43 ± 4.07% and 2.50 ± 1.76% of the BPA perfused at the concentrations of 45 or 250 ng/mL remained in the cotyledon.

The overall mean clearances index of BPA (and range) were 0.84 (0.66–0.93) in the materno-to-fetal direction and 0.81 (0.61–1.13) in the feto-to-maternal direction and were not affected neither by the placental direction nor by the BPA dose (p > 0.05) (Table 1). However, the effect of interaction between dose and direction tended to be significant (p = 0.06) suggesting that the BPA placental transfer could be lower for a BPA concentration of 250 ng/mL compared to 45 ng/mL.

By adjusting these materno-to-fetal and feto-to-maternal transplacental clearances using Eq. (8), the free BPA feto-to-maternal ratio at term could be estimated to 1.06.

3.2. Placental transfer of BPA-G

The time courses of the mean (±SD) materno-to-fetal (n = 4) and feto-to-maternal placental transfers (n = 4) of BPA-G (1000 ng/ml) and antipyrine throughout the perfusion are shown in Fig. 3. The materno-to-fetal and feto-to-maternal placental transfer rates of

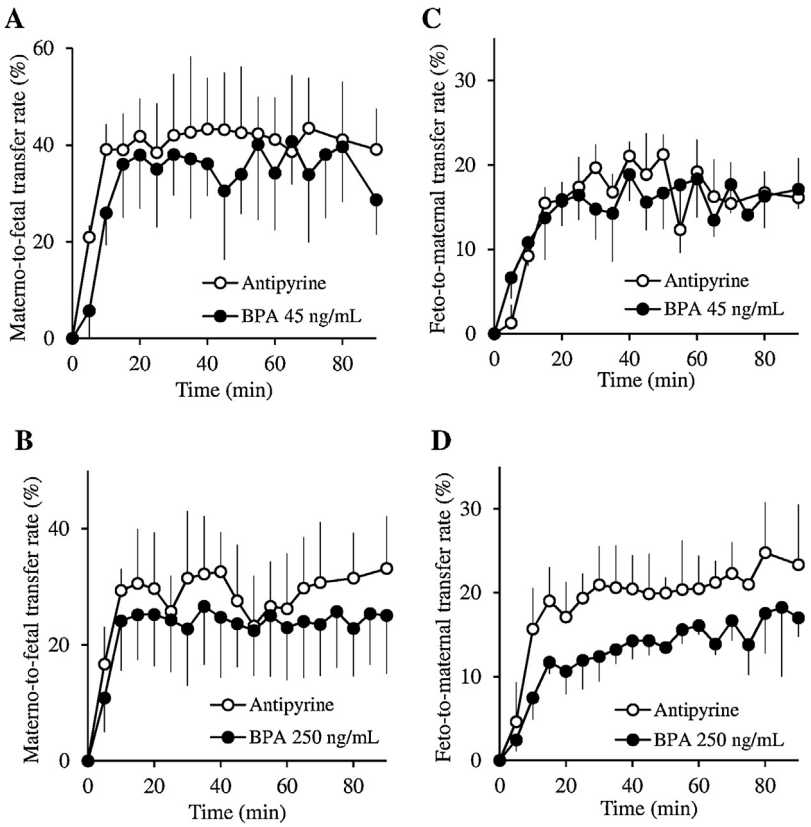

Fig. 2. Time courses of the mean (±SD) materno-to-fetal (A and B) and feto-to-maternal (B and C) transfer rates of antipyrine and BPA at the concentration of 45 ng/mL (A and C, $n = 3$) and of 250 ng/ml (B and D, $n = 4$) during the perfusion.

BPA-G and of antipyrine increased rapidly during perfusion and reached a plateau from 15 min until the end of perfusion.

The materno-to-fetal transfer rate (mean ± SD; range) of BPA-G (3.81 ± 1.41%; 2.02–5.28%, $n = 4$) was significantly lower than that of antipyrine (25.8 ± 1.36%; 24.0–27.1%, $p < 0.0001$) (Table 2). The feto-to-maternal transfer rate (mean ± SD; range) of BPA-G (7.77 ± 2.39%; 4.80–9.86%, $n = 4$) was two-times lower than that of antipyrine (18.2 ± 2.21%; 16.1–21.3%, $p < 0.0001$) (Table 2).

The clearance index of BPA-G (mean ± SD; range) in the materno-to-fetal direction (0.15 ± 0.05%; 0.09–0.21%) was significantly lower than in the reverse direction (0.44 ± 0.17%; 0.27–0.61%). The extraction rate of BPA-G in the materno-to-fetal direction (1.71 ± 0.73%) was much lower than in the feto-to-maternal direction (20.5 ± 8.72%, $p < 0.05$). The percentage of the BPA-G dose recovered in the perfused cotyledon was 0.32 ± 0.17% in the materno-to-fetal direction and 0.44 ± 0.27% in the reverse direction.

The transplacental clearance of BPA-G in materno-to-fetal direction was 0.21 ± 0.08 mL/min whereas it was 1.04 ± 0.37 mL/min in the reverse direction (Table 2).

4. Discussion

Pregnancy represents a critical window of exposure during which developmental processes are extremely sensitive to estrogenic interferences. BPA is one of the xenobiotics of major concern for fetal development. The degree of human exposure to BPA, particularly during fetal development is not clear. This highlights the urgent need for a better understanding and characterization of the dynamics of maternal–fetal–placental exchanges of BPA. The

Table 1
Materno-to-fetal and feto-to-maternal BPA perfusion parameters.

Direction	Dose	Placenta	TR Ant (%)	TR BPA (%)	CI	Cl Ant (mL/min)	Cl BPA (mL/min)
Materno-to-fetal	45 ng/mL	Mean	41.55	35.97	0.86	2.24	1.94
		SD	10.27	8.34	0.04	0.68	0.55
	250 ng/mL	Mean	29.36	24.23	0.83	1.47	1.22
		SD	8.26	8.74	0.13	0.38	0.43
	Overall mean		34.59	29.26	0.84	1.80	1.53
	SD		10.57	10.04	0.09	0.63	0.59
Feto-to-maternal	45 ng/mL	Mean	17.58	16.05	0.95	2.14	1.95
		SD	1.13	2.28	0.18	0.16	0.25
	250 ng/mL	Mean	20.62	14.21	0.71	2.70	1.87
		SD	2.11	1.52	0.09	0.37	0.24
	Overall mean		19.31	15.00	0.81	2.46	1.90
	SD		2.30	1.97	0.18	0.41	0.23

Ant: antipyrine; TR: transfer rate; CI: clearance index; Cl: placental clearance.

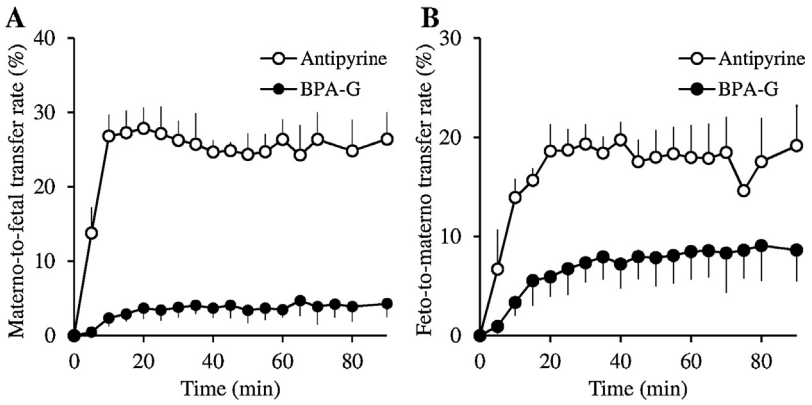

Fig. 3. Time courses of the mean (±SD) transfer rates of antipyrine and BPA-G during the materno-to-fetal perfusion (A, $n = 4$) and during the feto-to-maternal perfusion (B, $n = 4$).

present study uses the isolated perfused human placenta to examine the placental transport of BPA and its main metabolite, BPA-G in humans. Antipyrine, a reference marker for transcellular passive diffusion, was used as a useful reference substance by which the transfer rate of lipid-soluble xenobiotics may be characterized [23,24]. The antipyrine clearance observed in this study (1.14–3.18) conforms closely to values reported previously [17,24,25].

The main result obtained in this human perfused placental cotyledon model is that BPA can cross the placenta rapidly in both directions. By reporting the clearances of BPA and BPAG as a fraction of antipyrine (clearance index), several variable hemodynamic factors in the perfusion system are corrected thus allowing comparisons between different conditions. The materno-to-fetal clearance index of BPA of 0.86 determined in this ex vivo perfusion of a human placental cotyledon in an open system is in agreement with the transfer index reported in a closed perfusion system of 1.1 [6]. Moreover, the clearance index of BPA was similar in the materno-to-fetal and feto-to-maternal directions and was unaffected by the BPA concentration. However, for a high BPA concentration of 250 ng/mL, although not relevant to human exposure, there was a trend toward saturation of the transfer in the feto-to-maternal direction. All together, these observations suggest that placental exchange of BPA through the term placenta involves mainly passive diffusion.

On the other hand, BPA has been shown to be a substrate of the P-glycoprotein efflux pump [26] and in a BeWo cell-line, a part of BPA is actively effluxed by the P-gp protein [5], suggesting a role for P-gp in the protection of the fetus against toxic xenobiotics [27]. Further investigation is required to determine the role of membrane transporters in the maternal and fetal BPA placental transfer.

From the extraction ratio determined in our model (Eqs. (3) and (4)) and taking into account the placental blood flow described in the literature [20,28], maternal and fetal BPA placental clearances were estimated to 74 mL/min and 113 mL/min, respectively. The fetal placental clearance of BPA thus represents approximately 36% of the umbilical blood flow estimated at term [28]. Taking into account a BPA maternal total clearance of 2400 mL/min in humans as estimated by an allometric approach (Collet et al. personal communication), it means that only 3.1% of the BPA was eliminated via the maternal placental route. This result is consistent with previous data obtained in the pregnant ovine model showing that 2% of the BPA dose infused to the mother was eliminated by the placental route [3].

In this present study, using the modified equation of Henderson–Hasselbach (Eq. (8)) [19], we estimated a ratio between maternal and fetal concentrations of free (unbound to plasma proteins) BPA of 1.06, corresponding to a total (unbound + bound) BPA ratio of 0.9, suggesting that the internal exposure of the fetus at term is similar to that of its mother. This F:M ratio is rather high compared to the ratio between umbilical cord and maternal plasma concentrations reported in biomonitoring studies at birth (0.66 and 0.125 [29,30]). Our F:M ratio may be overestimated because we do not take into consideration the role of fetal drug clearance in determining the extent of fetal exposure. However, phase II metabolism activities develop in the human fetal placental unit, at least toward the end of pregnancy [31], and further investigation of the contribution of fetal metabolism to BPA clearance is required to predict fetal BPA concentrations.

Moreover, this F:M ratio determined at term could be considered as the maximal ratio for further reasons. Firstly, there is a gradual rise in the feto-maternal serum albumin concentration ratio from about 30% at 12 weeks to about 120% at term, leading to an increase in the ratio of the free BPA between the maternal and fetal circulation [32,33]. So the determination of BPA protein binding in maternal and fetal plasma in early and late stage pregnancy is necessary to evaluate the change in free maternal to fetal BPA plasma concentration ratios throughout pregnancy. Secondly, for a lipid-soluble molecule such as BPA, the limiting factors in the rate of placental clearance are the blood flow perfusing the placenta, the surface area available for exchange (from 3.4 to 12.6 m^2 between the seventh month of pregnancy and the

Table 2
Materno-to-fetal and feto-to-maternal BPA-G perfusion parameters.

Direction	Placenta	TR Ant (%)	TR BPA-G (%)	CI	Cl Ant (mL/min)	Cl BPA-G (mL/min)
Materno-to-fetal	Mean	25.79	3.81	0.15	1.42	0.21
	SD	1.36	1.41	0.05	0.09	0.08
Feto-to-maternal	Mean	18.16	7.77	0.44	2.39	1.04
	SD	2.21	2.39	0.17	0.23	0.37

Ant: antipyrine; TR: transfer rate; CI: clearance index; Cl: placental clearance.

term) and membrane thickness (from 50–100 μm at the second month to 4–5 μm at term) [20]. The uterine flow increases from 50 mL/min at 10 weeks of gestation to 600 mL/min at term, whereas the umbilical blood flow showed a reduction with gestational age, from 130 mL/(min.kg) at 20 weeks to 104 mL/(min.kg) at 38 weeks [28]. All together these physiological changes throughout pregnancy could contribute to a maximal fetal internal exposure related to that of its mother in late pregnancy. Thus, the data lead to the hypothesis that the fetus is not likely to be overexposed to free BPA in comparison to its mother and that the maximal internal fetal exposure observed at term corresponds to that of its mother.

Contrary to BPA, the BPA-G maternal-to-fetal clearance normalized to the respective antipyrine clearance was approximately 30% of the feto-to-maternal clearance (0.15 vs. 0.44). These low clearances, both materno-to-fetal and feto-to-maternal, compared to those of the highly diffusible reference substance antipyrine could be explained by the hydrophilic properties of BPA-G that prevent its efficient transfer across biological interfaces. The differential transfer of BPA-G across the placenta suggests that a transport system may be involved in restricting the maternal transfer of BPA-G. Several ATP-dependent transporters including multi-drug resistance proteins 1 and 2 (MRP-1, MRP-2) and OATP transporters, predominantly expressed in the syncytiotrophoblast, are known to transport glucuronide conjugates [34–36]. Further investigations using specific inhibitors of placental drug transporters will be necessary to elucidate the process of BPA-G transfer across the placenta.

The low BPA-G materno-fetal extraction rate of 2% in the human placenta system is in accordance with the small amount of the BPA-G maternal dose (0.13%), which is transferred into the fetal compartment after infusion of its mother in a rat model [36]. All together these results suggest that BPA-G passage across the placenta is extremely limited in the materno-to-fetal direction and that the high fetal exposure to BPA conjugates observed in primates after BPA maternal exposure [7] could be of feto-placental origin.

The data provided by this human placental cotyledon model should be evaluated with further limitations in mind. Firstly, this single pass system involves a metabolically static system in contrast to the dynamic state of pregnancy, which is why we have been unable to find evidence for BPA metabolism by our placental lobules at term. However, even in a closed perfusion system, only 3.2% of BPA was detected in conjugated form after the fetal placental transfer [6]. Moreover, it seems that for most drugs placental metabolism is relatively minor compared to that of the maternal or fetal liver and is not a significant factor in limiting the extent of their passage across the placenta [37]. Secondly, the results from the perfused cotyledon model at term cannot be extrapolated to earlier periods during pregnancy for the reasons explained above (physiological changes throughout pregnancy).

5. Conclusion

In conclusion, we showed that BPA crosses the placenta bidirectionally at a level similar to that of antipyrine suggesting a passive diffusion of this xenobiotic. It would appear therefore, that the human placenta has little capacity to restrict fetal exposure to BPA associated to environmental exposure of its mother. Nevertheless, it is unlikely that the fetus is overexposed to BPA compared to its mother. In contrast, BPA-G has extremely limited maternal placental permeability suggesting that from total circulating BPA (aglycone plus conjugates), mainly the aglycone fraction of BPA could be transferred to the fetal compartment. Thus, the fetal exposure to BPA conjugates could be explained mainly by the contribution of the fetal metabolism at least at the end of pregnancy. Once in the fetal compartment, it is likely that the feto-placental compartment is able, even slowly, to extrude BPA-G, since the BPA-G feto-to-maternal clearance index considerably exceeded the materno-to-fetal one. However, the limited feto-to-maternal BPA-G clearance could lead to a accumulation of glucuronoconjugates in the fetal compartment, particularly in amniotic fluid. Although BPA-G is not estrogenic [38], interference of this metabolite with other developmental processes could be not ruled-out.

Thus, the major factors determining the relationships between fetal and maternal internal exposures are the relative maternal and fetal BPA protein binding, the placental blood flows and the ontogeny of fetal metabolic process converting BPA into BPA-G. These physiological processes determining fetal exposure will have to be further investigated to evaluate the risk of fetal exposure to BPA in humans.

Conflict of interest

The authors have no conflict of interest to declare.

Transparency document

The Transparency document associated with this article can be found in the online version.

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