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Development of a physiologically based pharmacokinetic model for bisphenol A in pregnant mice

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Abstract

Bisphenol A (BPA) is a weakly estrogenic monomer used to produce polymers for food contact and other applications, so there is potential for oral exposure of humans to trace amounts via ingestion. To date, no physiologically based pharmacokinetic (PBPK) model has been located for BPA in pregnant mice with or without fetuses. An estimate by a mathematical model is essential since information on humans is difficult to obtain experimentally. The PBPK model was constructed based on the pharmacokinetic data of our experiment following single oral administration of BPA to pregnant mice. The risk assessment of bisphenol A (BPA) on the development of human offspring is an important issue. There have been limited data on the exposure level of human fetuses to BPA (e.g. BPA concentration in cord blood) and no information is available on the pharmacokinetics of BPA in humans with or without fetuses. In the present study, we developed a physiologically based pharmacokinetic (PBPK) model describing the pharmacokinetics of BPA in a pregnant mouse with the prospect of future extrapolation to humans. The PBPK model was constructed based on the pharmacokinetic data of an experiment we executed on pregnant mice following single oral administration of BPA. The model could describe the rapid transfer of BPA through the placenta to the fetus and the slow disappearance from fetuses. The simulated time courses after three-time repeated oral administrations of BPA by the constructed model fitted well with the experimental data, and the simulation for the 10 times lower dose was also consistent with the experiment. This suggested that the PBPK model for BPA in pregnant mice was successfully verified and is highly promising for extrapolation to humans who are expected to be exposed more chronically to lower doses.

Keywords: Risk assessment; Bisphenol A; PBPK model; Offspring

Introduction

Bisphenol A (4,4′-isopropylidene-2-diphenol, BPA) is used as a monomer for the production of polycarbonate and epoxy resins, and also as a stabilizer or antioxidant for many types of plastics in many consumer products, including food-ware, food-can linings, baby bottles and dental composite fillings and sealants. The use of these products raises the potential of oral exposure of humans directly to trace amounts of BPA, released from these products or via water contaminated with BPA that has leached out of plastic wastes in landfill (Yamamoto et al., 2001).

There has been concern about the estrogenic potential of BPA. Gaido et al. (1997) confirmed the weak estrogenicity of BPA in a yeast-based steroid hormone receptor gene transcription assay, showing BPA to be approximately 15,000 times less potent than 17β-estradiol, and Kuiper et al. demonstrated that BPA binds to human estrogen receptors and stimulates the transcriptional activity of both estrogen receptor subtypes (Kuiper et al., 1997, 1998). The estrogenicity of BPA is attributed to its weak *in vitro* agonist activity, in the order of 1/10 000 of that of estrogen, and differences in the binding to ERa and ERb (Matthews et al., 2001). BPA also exerts an estrogen-like effect *in vivo* on the estrus cycle of rats (Laws et al., 2000), on the development of the reproductive tract and mammary gland in female mice offspring (Nikaido et al., 2004), and on the size of reproductive organs, as well as sperm production, of male mice offspring by prenatal exposure to BPA (Nagel et al.,

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1997; vom Saal et al., 1998), although these observations are still controversial (Cagen et al., 1999; Yoshida et al., 2004).

Attention has been renewed on the effect of prenatal or neonatal exposure to BPA on the development of the nervous system (Kabuto et al., 2004; Shikimi et al., 2004) and behavior of offspring (Carr et al., 2003; Negishi et al., 2004). The effect of prenatal exposure to BPA on the immune system (Yoshino et al., 2004) and the disruption of thyroid hormone functions (Moriyama et al., 2002; Seiwa et al., 2004) in mice have also been reported.

Based on such an accumulated information in test animals on the reproductive and developmental effect of BPA, the endocrine-like effect on humans due to BPA has been under discussion.

In this study, we developed a physiologically based pharmacokinetic (PBPK) model describing the pharmacokinetics of BPA in a pregnant mouse with the prospect of future extrapolation to humans. Clarifying the controversial issues on the low-dose effects of BPA is vital (Gray and Cohen, 2004; Gray et al., 2004). The PBPK model was constructed based on the pharmacokinetic data of the experiment we executed on the pregnant mice following single oral administration of BPA (Kawamoto et al., 2005). The model was verified and given a trial for the extrapolation to the lower and prolonged exposure that is expected in humans.

Recently, other investigators have reported on developing a PBPK model for BPA in non-pregnant rats and humans (Shin et al., 2004). Their model was basically constructed to simulate pharmacokinetics of BPA in steady state after multiple i.v. injections. In contrast, the PBPK model we developed is in pregnant animals with oral administration, which is the most likely exposure route for humans. Our model can simulate the characteristic pharmacokinetics of orally administered BPA in a non-steady state and estimate the prenatal exposure level to BPA.

The present study was designed to develop a physiologically based pharmacokinetic (PBPK) model for BPA in pregnant

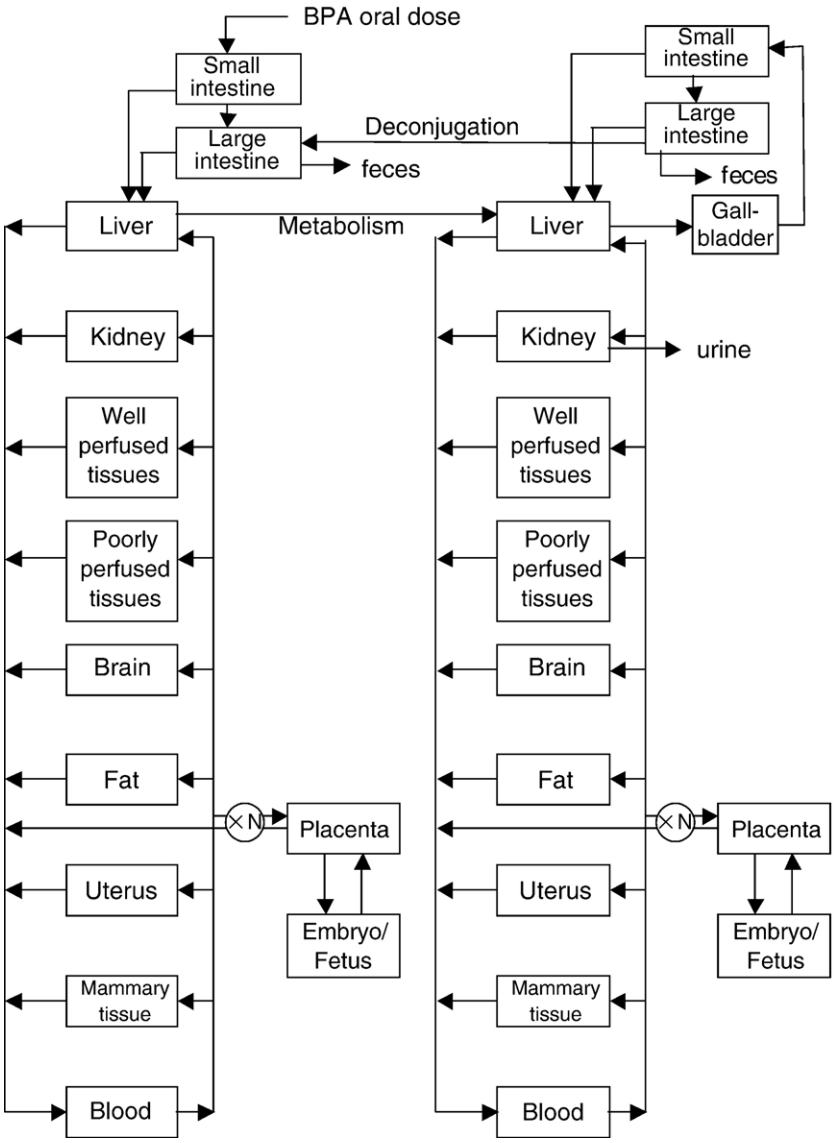

Fig. 1. The schematic diagram of the PBPK model for BPA in pregnant mice.

mice that could reasonably predict tissues dosimetries after single oral administration of BPA. The model was verified and given a trial by simulating independent data on BPA disposition after multiple oral administrations. The model was further verified by using experimental data retrieved from the literature.

Methods

Experimental data

The experimental data used for the calibration of the PBPK model were obtained from our previous pharmacokinetic study on pregnant mice following a single oral administration of BPA (Kawamoto et al., 2005). Ten mg/kg of ^{14}C-BPA was orally administered to pregnant mice on gestation day 15. Maternal blood and tissues, as well as fetuses, were sampled at 20 min, 1, 3, 6 and 24 h after administration. BPA and its metabolites were quantified in the maternal serum, liver and fetuses. Radioactivity i.e. total amount of BPA and its metabolites was counted in other tissues. Radioactivity was represented as the weight equivalent to BPA (e.g. mg BPA eq.) based on the specific activity of ^{14}C-BPA administered to the mice. We also studied the elimination of these compounds into the urine and feces.

The time courses of total concentrations (BPA plus its metabolites) following three repeated 10 mg/kg or lower doses (1 mg/kg) of oral administration of BPA to pregnant mice was also examined (Kawamoto et al., 2005).

PBPK model description

The schematic diagram of the PBPK model for BPA on pregnant mice is presented in Fig. 1. The basic structure follows the physiologically based kinetic model of rat and mouse gestation developed by O'Flaherty et al. (1992). The brain compartment was added to the model since it is considered to be one of the target organs of BPA. The yolk sac and chorioallantoic placentas were modeled as a single placenta for simplification. Two gastrointestinal compartments, small and large intestines, and the gallbladder compartment were introduced to model the enterohepatic circulation observed in our previous experimental study (Kawamoto et al., 2005).

The constructed PBPK model consists of two analogous kinetic models for BPA and its metabolites, respectively, which are connected to each other at the liver compartment in the metabolism phase. Several metabolites of BPA distinguished in our experimental study (Kawamoto et al., 2005), in which BPA glucuronide was a main metabolite, were lumped into one (represented as BPA-gluc) in the description of the model, as the toxicity of BPA is considered to be substantially weakened through metabolism (Elsby et al., 2001).

The model equations for BPA are described as follows:

$$V_{KI}\frac{dC_{KI}}{dt} = Q_{KI}\left(C_{BL} - \frac{C_{KI}}{P_{KI}}\right) - KE_{KI}C_{KI}V_{KI} \quad \text{Kidney}$$

$$V_{WP}\frac{dC_{WP}}{dt} = Q_{WP}\left(C_{BL} - \frac{C_{WP}}{P_{WP}}\right) \quad \text{Well perfused tissues}$$

$$V_{PP}\frac{dC_{PP}}{dt} = Q_{PP}\left(C_{BL} - \frac{C_{PP}}{P_{PP}}\right) \quad \text{Poorly perfused tissues}$$

$$V_{LI}\frac{dC_{LI}}{dt} = Q_{LI}\left(C_{BL} - \frac{C_{LI}}{P_{LI}}\right) - \frac{dMET}{dt}V_{LI} + K_aA_{SG} + 0.1K_aA_{LG} \quad \text{Liver}$$

$$V_{BR}\frac{dC_{BR}}{dt} = Q_{BR}\left(C_{BL} - \frac{C_{BR}}{P_{BR}}\right) \quad \text{Brain}$$

$$V_{F}\frac{dC_{F}}{dt} = Q_{F}\left(C_{BL} - \frac{C_{F}}{P_{F}}\right) \quad \text{Fat}$$

$$\frac{dA_{SG}}{dt} = DOSE - K_aA_{SG} - KE_{SG}A_{SG} \quad \text{Small intestine}$$

$$\frac{dA_{LG}}{dt} = KE_{SG}A_{SG} - 0.1K_aA_{LG} - KE_{LG}A_{LG} + K_dA^g_{LG} \quad \text{Large intestine}$$

$$V_{U}\frac{dC_{U}}{dt} = Q_{U}\left(C_{BL} - \frac{C_{U}}{P_{U}}\right) \quad \text{Uterus}$$

$$V_{MT}\frac{dC_{MT}}{dt} = Q_{MT}\left(C_{BL} - \frac{C_{MT}}{P_{MT}}\right) \quad \text{Mammary tissue}$$

$$V_{PL}\frac{dC_{PL}}{dt} = Q_{PL}\left(C_{BL} - \frac{C_{PL}}{P_{PL}}\right) + PA1\frac{C_{EF}}{P_{EF}} - PA2\frac{C_{PL}}{P_{PL}} \quad \text{Placenta}$$

$$V_{EF}\frac{dC_{EF}}{dt} = PA2\frac{C_{PL}}{P_{PL}} - PA1\frac{C_{EF}}{P_{EF}} \quad \text{Embryo/fetus}$$

$$V_{BL}\frac{dC_{BL}}{dt} = \sum_{ti} Q_{ti}\left(\frac{C_{ti}}{P_{ti}} - C_{BL}\right) \quad \text{Blood}$$

where V_{ti} is the volume of a tissue (L), C_{ti} is the BPA concentration in a tissue (mg/L), Q_{ti} is the blood flow rate to a tissue (L/h), P_{ti} is the tissue/blood partition coefficient of BPA, A_{ti} is the BPA amount in an organ, and PA1 and PA2 are the diffusion coefficients (L/h) of BPA from the fetus to the placenta and from the placenta to the fetus respectively. The abbreviations of other parameters in the equations are defined in Tables 1 and 2.

The model equations for BPA-gluc are presented below (the same equations as for BPA are not shown):

$$V_{LI}\frac{dC^g_{LI}}{dt} = Q_{LI}\left(C^g_{BL} - \frac{C^g_{LI}}{PG_{LI}}\right) + \frac{dMET}{dt}V_{LI} + K_{aG}A^g_{SG} + 0.1K_{aG}A^g_{LG} - K_{bG}C^g_{LI}V_{LI} \quad \text{Liver}$$

$$\frac{dA^g_{BI}}{dt} = K_{bG}C^g_{LI}V_{LI} - K_{bG2}A^g_{BI} \quad \text{Gallbladder}$$

$$\frac{dA^g_{SG}}{dt} = -K_{aG}A^g_{SG} + K_{bG2}A^g_{BI} - KE_{SG}A^g_{SG} \quad \text{Small intestine}$$

$$\frac{dA^g_{LG}}{dt} = KE_{SG}A^g_{SG} - 0.1K_{aG}A^g_{LG} - KE_{LG}A^g_{LG} - K_dA^g_{LG} \quad \text{Large intestine}$$

where C^g_{ti} is the BPA-gluc concentration in a tissue (mg/L), PG_{ti} is the tissue/blood partition coefficient of BPA-gluc, and A^g_{ti} is the BPA-gluc amount in an organ. The abbreviations of other parameters in the equations are defined in Table 2.

The metabolism of BPA into its metabolites is given by:

$$\frac{dMET}{dt} = \frac{V_{max}\cdot f_s\cdot SB\cdot\alpha\cdot C_{LI}/P_{LI}}{K_m + f_s\cdot SB\cdot\alpha\cdot C_{LI}/P_{LI}}$$

where the abbreviations of parameters in the above equation are defined in Table 2.

Parameterization

Physiological parameters. Most physiological parameters for the PBPK model were set based on the data presented by Brown et al. (1997). The changes

Table 1
Physiological parameters of pregnant mice on gestation day 15

Body weight (g)	56	
Cardiac output (L/h)	1.958	
	Blood flow (L/h)	Organ volume (mL)
Kidney	0.154	0.733
Well perfused tissues	0.120	0.518
Poorly perfused tissues	0.948	33.1
Liver	0.334	2.92
Brain	0.056	0.733
Fat	0.147	3.77
Uterus	0.017	0.440
Mammary tissue	0.085	2.18
Placenta	0.097	1.73
Blood	–	2.11
Fetuses	–	7.74
Gastric emptying rates (1/h)		
Small intestine (KE_{SG})	0.400	
Large intestine (KE_{LG})	0.080	

Assumed tissue density is 1 g/mL.

in organs volumes and blood flow from the basic data with regards to gestation days were evaluated using the equations given in the gestation model by O'Flaherty et al. (1992). The body weight of a pregnant mouse with fetuses was set as 56 g based on the actual body weight of pregnant mice used for our previous pharmacokinetic experiment.

The gastric emptying rates were determined based on the values of transit time in gastric intestines (Davies and Morris, 1993). The values reported in the literature are mostly from dogs and humans. We applied the value of dogs to the mouse (rodents) since the transit time in the small intestine of the rat (rodents), which is the only value reported on the transit time of rat, is quite similar to that of dogs.

The gallbladder was assumed to be emptied at the administration time and every 3 h after the administration. Mice are known to eat food irregularly, so it is hard to estimate when the gallbladder is empty. The assumption on the timing of emptying the gallbladder was made from our experimental result that the increase in BPA concentration in the blood was observed almost every 3 h after the administration of BPA (data not shown), which was thought to be caused by the enterohepatic circulation.

The values of physiological parameters are summarized in Table 1.

Table 3
Tissue/blood partition coefficients in pregnant mice

	BPA	BPA-gluc
Kidney	0.858	3.18
Well-perfused tissues	1.43	0.271
Poorly perfused tissues	0.682	0.387
Liver	384	6.76
Brain	1.34	0.125
Fat	1.16	0.220
Uterus	0.693	0.581
Mammary tissue	0.957	0.270
Placenta	0.880	0.680
Fetuses	0.308	0.0580

Biochemical parameters. The serum protein binding of BPA was measured by ourselves *in vitro* with an ultrafiltration method using mouse serum spiked with BPA (Kawamoto and Morisawa, in preparation). The blood/serum concentration ratio of BPA was determined based on our pharmacokinetic experiment on mice (Kawamoto et al., 2005).

The metabolic kinetic parameters for BPA were measured *in vitro* using mouse S9 (Kawamoto and Morisawa, in preparation). Factor α scaling from the metabolic activity obtained *in vitro* to *in vivo* needed to be introduced and the factor was determined by fitting. The deconjugate rate for BPA metabolites was determined by fitting.

The absorption rate in the small intestine and the urine and bile elimination rate were assumed to occur in a first-order fashion and obtained by fitting. The absorption rate at large intestine was assumed to be 10% of that in the small intestine. The bile elimination rate was set only for BPA-gluc, because Inoue et al. (2001) reported that most BPA was metabolized to glucuronides and that the metabolites were mainly excreted into the bile on perfusion of BPA solution into the liver of SD rats, and that BPA was excreted only into the veins, but not detected in the bile.

The values of biochemical parameters are summarized in Table 2.

Physicochemical parameters. The tissue/blood partition coefficients of BPA in equilibrium were determined by our experiment *in vitro* according to the method reported by Jepson et al. (1994). For the liver, the partition coefficient needed to be raised 312 times from the experimental value (1.23) in equilibrium to reach 384, so that the model simulation could fit the experimental BPA concentration in the liver, assuming the partition of BPA to the liver *in vivo* is caused by the active transport of BPA into hepatic cells, as well as by

Table 2
Biochemical parameters of the PBPK model for BPA in pregnant mice

Parameter	BPA		BPA-gluc	
	Abbreviation	Value	Abbreviation	Value
Absorption rate (1/h)	K_a	10.0	K_{aG}	0.10
Urinary excretion rate (1/h)	KE_{KI}	0.0	KE_{KIG}	7.50
Biliary excretion rate (1/h)				
Liver to gallbladder	–		K_{bG}	8.59
Gallbladder to intestine	–		K_{bG2}	10000 (every 3 h)
Metabolic constant in liver				
Maximum reaction rate (mg/h)	V_{max}	1970	–	
Michaelis constant (mg/L)	K_m	3.78	–	
Unbound fraction in serum (–)	f_s	0.0317	–	
Concentration ratio of serum to blood	SB	1.6	–	
Scaling factor	α	60	–	
Deconjugate rate in large intestine (1/h)	–		K_d	2.4
Diffusion coefficient (L/h)				
Fetus to placenta	PA1	2.0E-05	PAG1	6.6E-14
Placenta to fetus	PA2	5.2E-05	PAG2	3.3E-06

physicochemical affinity. In the experimental method we used, hepatic cells are not thought to be alive and the measured partition coefficient reflects only the physicochemical affinity between liver tissue and BPA. Values over 300 for the liver partition coefficient have also been reported by others (Bikhazi et al., 1983). Those of BPA-gluc (representation of all BPA metabolites in the description of the model) were based on the estimate by the algorithm of Poulin and Krishnan

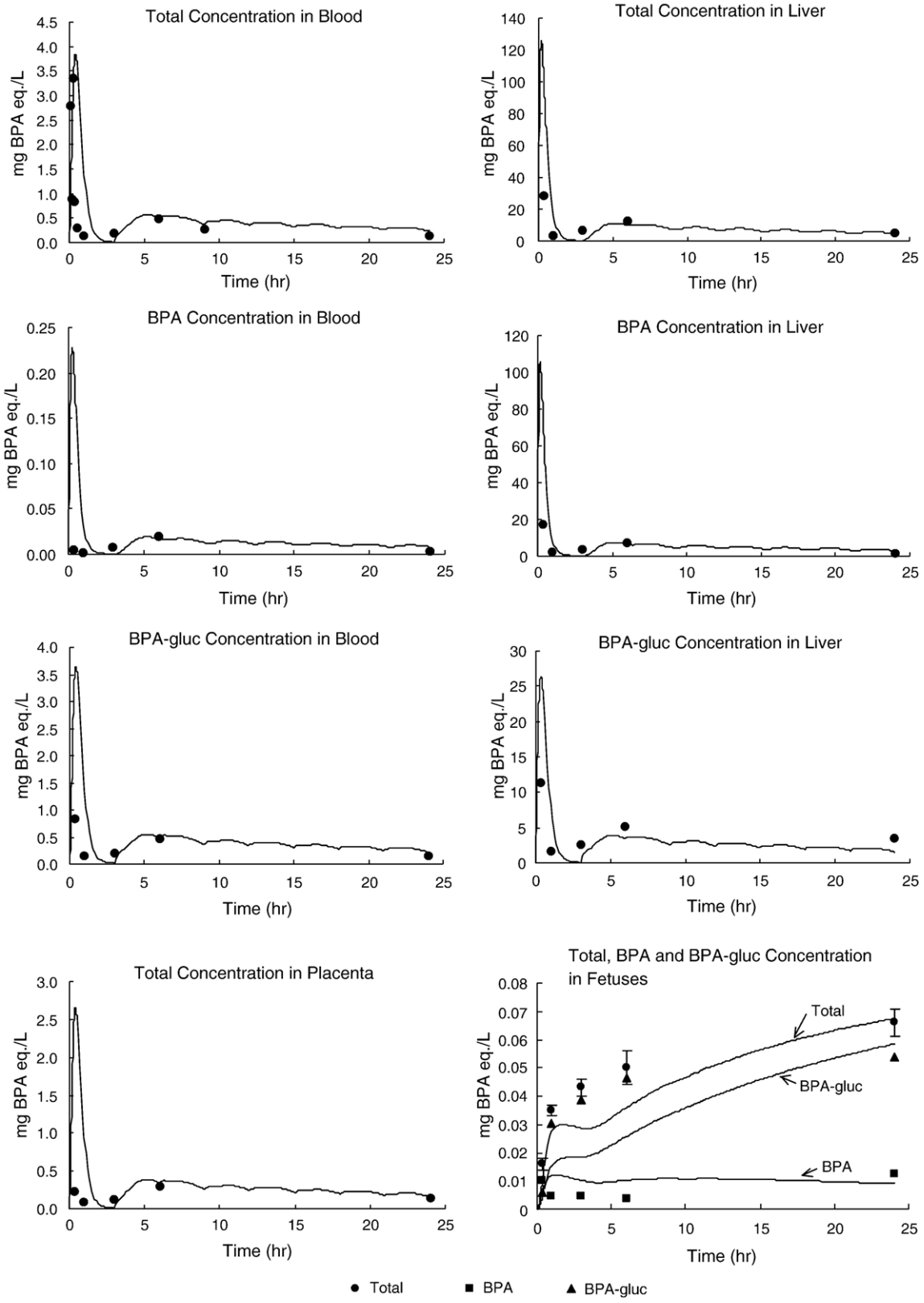

Fig. 2. A comparison of the parameterized PBPK model simulation (line) with the experimental data (points) in pregnant mice following a single oral administration of BPA (10 mg/kg).

(1995) from the n-octanol/water partition coefficient of the genuine glucuronide of BPA, with some adjustment by fitting. The fitting was through visual inspection of model simulations compared with our experimental data (10 mg/kg) (Kawamoto et al., 2005). The values of physicochemical parameters are summarized in Table 3.

Model calibration

The PBPK model was numerically solved using the Euler Method. The simulation of the parameterized model was compared with the experimental pharmacokinetic data on pregnant mice with a single oral administration (10 mg/kg) (Kawamoto et al., 2005).

Model verification

The calibrated PBPK model was applied to the repeated dosing condition (three doses of 10 mg/kg). The changes in organ volumes and blood flow during the repeated dosing period (3 days) with regards to gestation days were described in the model with the equations given by O'Flaherty et al. (1992). The model simulation was compared with our experimental data on time courses of total concentrations following three-time repeated oral administration of BPA to pregnant mice (Kawamoto et al., 2005).

Extrapolation to a lower dose

The verified PBPK model was applied to the lower dosing condition (1 mg/kg). The model simulation was compared with our experimental data on time courses of total concentrations following the lower dose of oral administration of BPA to pregnant mice (Kawamoto et al., 2005).

Results

Parameterization and calibration

The simulation of the parameterized model was compared with the experimental pharmacokinetic data on pregnant mice with a single oral administration (10 mg/kg), resulting in good consistency over the whole period after the administration in the concentrations for all tissues. The results for some of the tissues are shown in Fig. 2. Good consistency was observed in BPA and its metabolite concentrations, as well as total concentrations in maternal blood and the liver. This indicated that the metabolic kinetic parameters were successfully set, whereas the factor scaling from the metabolic activity obtained from our experiment *in vitro* to *in vivo* needed to be introduced (Tsukamoto et al., 2001) to reproduce the experimental data in maternal blood and the liver. The factor was determined by fitting. We assumed the factor represented in large part the active transport of BPA into hepatic cells *in vivo*.

In fetuses, the concentration of BPA metabolites was underestimated by the model during the short period after administration.

The simulation reproduced the second peaks of concentrations in maternal blood and tissues and the following slow decline in the concentrations, which were thought to be caused

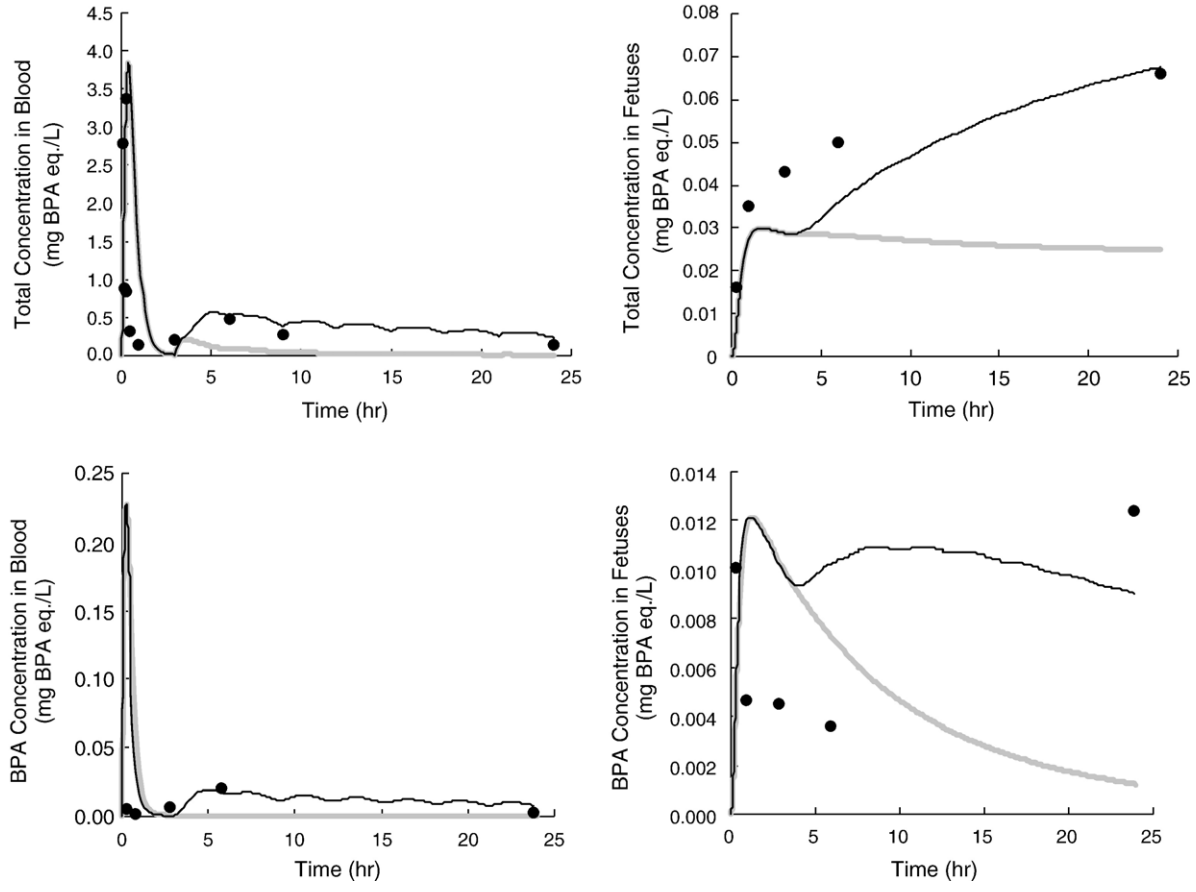

Fig. 3. A comparison among the PBPK model simulation with (solid line) and without (gray line) the enterohepatic circulation mechanism incorporated in the model, and the experimental data (points) in pregnant mice following a single oral administration of BPA (10 mg/kg).

by the enterohepatic circulation. The enterohepatic circulation mechanism described in the model, and the assumption that the gallbladder is emptied at the administration time and every 3 h after the administration, was successfully performed. Without the enterohepatic circulation mechanism, setting the deconjugate kinetic parameter zero when running the model, the simulation failed to reproduce the second peaks and the subsequent slow decline in concentrations (Fig. 3). This indicated that the enterohepatic circulation was practically responsible for the time courses in dams. The enterohepatic circulation described in the model also worked to make BPA and its metabolites stay longer in fetuses.

Model verification

The calibrated PBPK model was applied to the repeated dosing condition (three dose of repeated 10 mg/kg). The model simulation resulted in good consistency with our experimental data (Kawamoto et al., 2005) on time courses of total concentrations for all the tissues following administration of three oral dose of BPA to pregnant mice. Fig. 4 shows the results for some of the tissues. The experiment showed a higher tendency toward accumulation of BPA and its metabolites in fetuses than in dam (Kawamoto et al., 2005), which was described well by the model. This indicated that the PBPK model we developed could be used to predict the pharmacokinetic BPA behavior in both pregnant dams and fetuses.

Extrapolation to a lower dose

The verified PBPK model was applied to the lower dosing condition (1 mg/kg). The model simulation resulted in fair consistency with our experimental data (Kawamoto et al., 2005) on the total concentration level after a short interval following the lower dose of oral administration of BPA to pregnant mice (Fig. 5). Although during the short period after the administration the model predicted a higher peak concentration at an earlier time in dams than the experimental data (Fig. 5). The model predicted a sharper decline for the total concentration after the peak in dams. In fetuses, the model simulation fairly reproduced the experimental concentration after a short interval following administration, although over a short period the model underestimated the concentration (Fig. 5).

Discussion

Parameterization and calibration

Although we simply raised the liver/blood partition coefficient rather than redefined liver partitioning by encoding for the

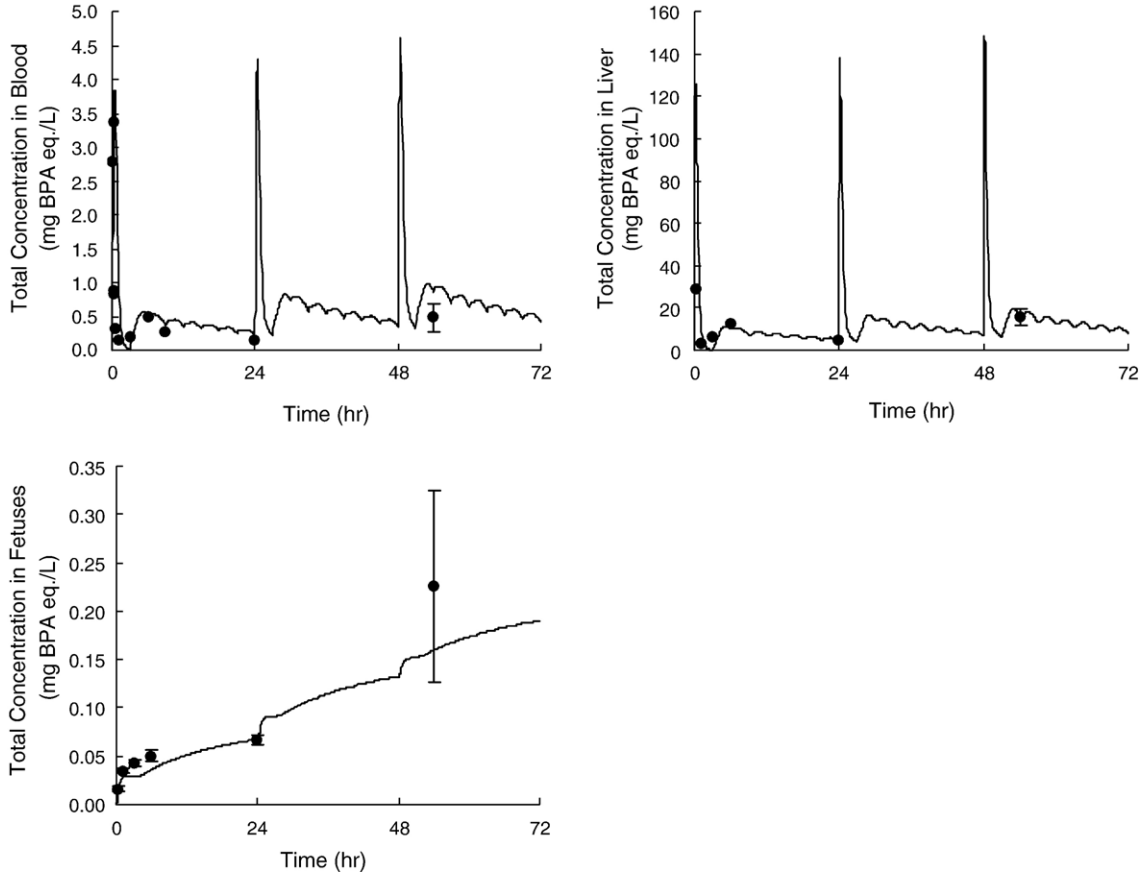

Fig. 4. A comparison of the PBPK model simulation (line) with the experimental data (points) on pregnant mice following administration of three oral doses (once a day) BPA (10 mg/kg at each time).

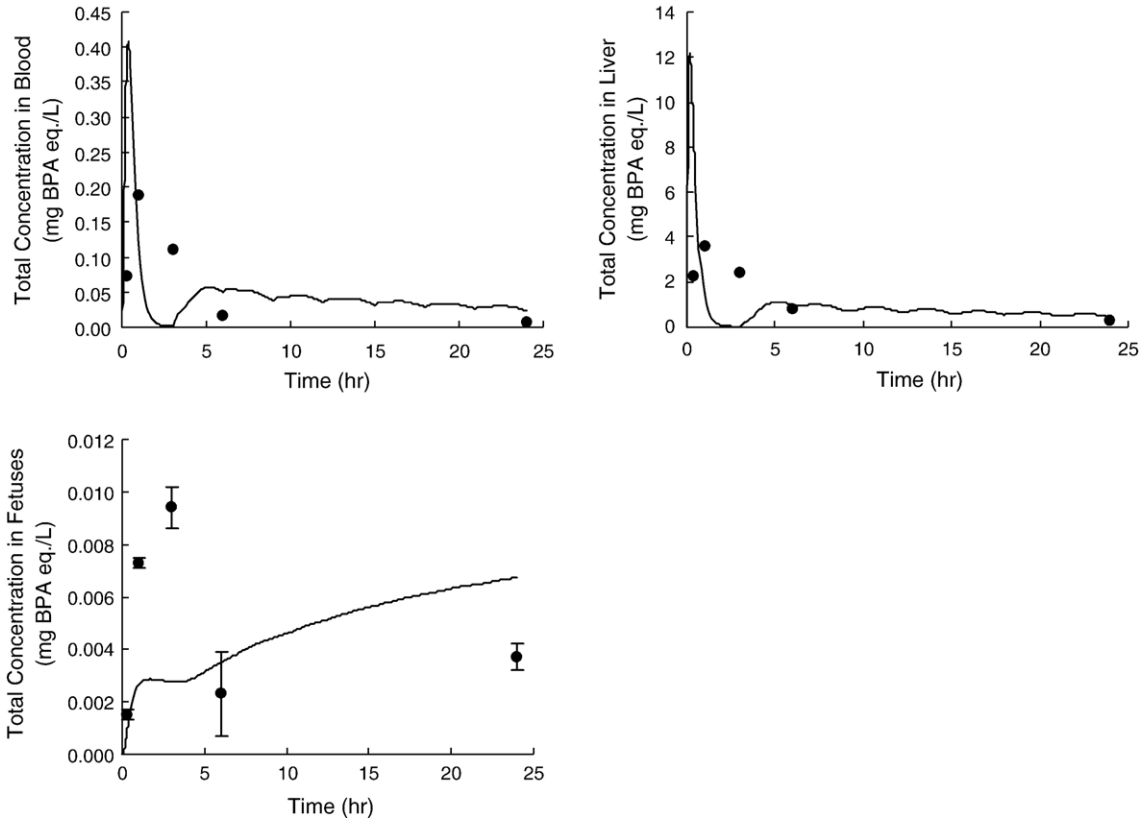

Fig. 5. A comparison of the PBPK model simulation (line) with the experimental data (points) on pregnant mice following a lower dose single oral administration of BPA (1 mg/kg).

active uptake mechanism, good consistency between the model simulations and the experimental data were observed in BPA and its metabolite concentrations, as well as total concentrations in maternal blood and the liver. This showed that the metabolic parameters, including the factor scaling from the metabolic activity obtained in vitro to in vivo, were successfully determined. However, the liver/blood ratio of the BPA concentration may not change with increasing dose and might be saturated at a higher dose. Further experiments are required to build the active uptake mechanism into the model.

In fetuses, the simulation of BPA metabolite concentrations was underestimated during a short period after administration. A more detailed model constructed from analogous models for more species of metabolites might be able to reproduce experimental data more precisely, without lumping several BPA metabolites into one. However, the simple diffusion-limited transfer of BPA and its metabolites between the placenta and the fetus described in the model could evaluate fairly well the exposure level of fetuses, not considering the amniotic fluid compartment as in Shin et al. (2002).

The model fits appear to have quite a bit of instability. This is because we introduced enterohepatic circulation into the model. The PBPK model suggested that the enterohepatic circulation was practically responsible for the delayed clearance of BPA and its metabolites in mouse dams and fetuses. In this mouse model, the gallbladder was assumed to be emptied at the administration time and every 3 h after the administration. In the human PBPK model extrapolated from this mouse model, the gallbladder would be modeled to be emptied at each meal.

Model verification

The PBPK model for pregnant mice could be applied to evaluate the pharmacokinetics of BPA following repeated administrations, which is a more chronic condition. The model well described the higher tendency of accumulation of BPA and its metabolites in fetuses than in dams that was shown in our experiment (Kawamoto et al., 2005). This indicated that the PBPK model we developed could be used to predict the pharmacokinetic BPA behavior in both pregnant mouse dams and fetuses in various conditions.

Extrapolation to a lower dose

The simulation by the PBPK model showed a somewhat more radical transition of concentration than the experiment soon after the administration in dams, while later the prediction was fairly consistent with the experiment. In fetuses, the model was not successful to predict the time course of concentration only in the short period after administration. During the short period, the experimental time course for the low dose (1 mg/kg) curved differently from the one for the standard dose (10 mg/kg), whereas the predicted time courses was a similar shape between the two doses and the concentrations were shifted approximately 10 times. This indicated that the chemicals were transferred

through the placenta by a rather more complicated mechanism than the simple diffusion-limited transfer modeled in this PBPK model. The mechanism is not thought to depend linearly on the concentrations of the transferred chemicals in practice. Another hypothesis is that various metabolites, represented as one symbolized metabolite (BPA-gluc) in the model, are transferred through the placenta in various dose-dependent manners. The transfer mechanism of BPA and its metabolites through the placenta should be confirmed experimentally and be mathematically modeled to improve the PBPK model.

Although the doses used in model specification and validation are quite high compared with probable human exposure, the lower dose was not appropriate due to the radioactivity of ^{14}C-BPA used in our previous study. Surely, high-to-low dose extrapolation is an important and difficult issue. However, once the PBPK model has been developed for a high dose, it will be possible to validate and/or modify it for lower doses by performing low-dose experiments and monitoring the BPA concentration in certain organs, e.g., blood and fetus.

Extrapolation to human

At the moment, the PBPK model that we have developed for pregnant mice can roughly predict the exposure level of fetuses to BPA successfully over a range of doses and in the range of gestations, not the precise kinetic behavior of BPA in detail. This prediction can be the basis for the estimates in humans, excluding the problem on the extrapolating between species, since the chronic toxicity caused by BPA is the concern. The PBPK model for human could be developed based on that for mouse. Certainly, it is likely that there is species specificity in BPA pharmacokinetics. However, the model could be validated with epidemiological data on the BPA concentration in the blood and umbilical cord of pregnant women (Ikezuki et al., 2002; Schonfelder et al., 2002).

The model simulation suggested a new experimental subject, which is important from the viewpoint of the human reproductive risk. The manifested subject for further experimental study is the dose-dependency of the fraction of metabolites in fetuses. In a previous experimental pharmacokinetic study on pregnant mice, we fractionated metabolites in tissues for one standard dose only. Coupling this new information into the PBPK model, the model will be able to predict more precisely the pharmacokinetics of BPA in pregnant mammals.

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