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Mammary Excretion and Placental Transfer of Bisphenol A in Rats

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Abstract

This study examined the extent of mammary excretion and placental transfer of bisphenol A in rats. Bisphenol A was given by simultaneous i.v. bolus injection plus infusion to steady-state at low, medium and high doses. The steady-state serum levels of bisphenol A were linearly increased with increasing the dosing rate. The systemic clearance (mean range, 119.2-154.1 ml/min/kg) remained unaltered over the dosing rate studied. The levels of bisphenol A in milk exceeded those in serum, with the steady-state milk to serum concentration ratio being 2.4-2.7. The steady-state milk levels of bisphenol A were also increased linearly with increasing the infusion rate. In a separate study, the kinetic disposition of bisphenol A in the rat maternal-fetal unit was studied in pregnant rats. After i.v. injection, bisphenol A concentration in the maternal serum declined biexponentially. Bisphenol A was rapidly distributed into placenta, fetus and amniotic fluid, with maximum concentrations in these tissues achieved within 1 hr of injection. The decline of bisphenol A in placenta, fetus and amniotic fluid paralleled that of maternal serum. A simultaneous computer simulation showed that the observed concentrations were well represented by a 5-compartmental model consisting of the maternal central, placenta, fetus, amniotic fluid, and maternal tissue compartments.

Introduction

Bisphenol A [2,2-bis(p-hydroxyphenyl)propane] is a monomer intermediate used primarily in the manufacture of epoxy resins and polycarbonate plastics (Keith 1997). Because these resins and plastics are widely used in food, drink and medical packaging industry (Olea et al., 1996; Krishinan et al., 1993), there is a potential of oral exposure in humans to bisphenol A in trace amounts. Bisphenol A is listed by EPA as an endocrine modifying chemical (U.S. EPA 2000). This chemical has been shown to increase cell proliferation in various human breast cancer cell lines (Schafer et al., 1999; Krishnan et al., 1993), in the mammary gland and the reproductive tract in rats (Steinmetz et al., 1998; Colerangle and Roy 1997). Bisphenol A has also been extensively examined for its reproductive, fetal, perinatal and postnatal toxicities at relatively high doses or physiologically relevant doses (Fisher et al., 1999; Nagao et al, 1999; vom Saal et al, 1998; Morrissey et al., 1987).

Bisphenol A is known to be rapidly metabolized and excreted in urine in small quantities, with the major urinary metabolite being the monoglucuronide conjugate in rats (Pottenger et al., 1997). The pharmacokinetics of bisphenol A has been reported to be linear over an i.v. dose range from 0.2-2.0 mg/kg (Yoo et al., 2000). When given by nonparenteral routes (oral, s.c. and i.p.), a majority of the administered dose is excreted into feces (52.28-83.17%) (Pottenger et al., 2000). C-bisphenol A appears to be absorbed to a greater extent after s.c. and i.p injections compared to oral administration (10 and 100 mg/kg doses) in rats (Pottenger et al., 2000).To our knowledge, no literature information is available on the mammary excretion and kinetics of bisphenol A during pregnancy. This study was conducted to determine the extent of mammary excretion of bisphenol A during lactation and its kinetic disposition in the pregnant rat.

Methods

Pregnant (16-18 days of gestational age) and lactating (4-6 days post partum age) female Sprague Dawley rats were used in the study. The rats were kept in plastic rat cages housed in an animal facility (temperature 23 ± 2°C) with light/dark cycle of 12/12 hr and relative humidity of 50 ± 10%. The animals were fed standard rat diet and had free access to water throughout the study. Three groups of lactating rats received three separate 4 hr infusion at low, medium and high rates of 0.13, 0.27, 0.54 mg/hr, respectively. Intravenous bolus doses of 0.47, 0.94 and 1.88

mg/kg were given for the low, medium and high dosing regimens, respectively, immediately prior to starting each infusion. The three infusion regimens were designed to achieve the steady-state serum concentrations of approximately 75, 150, 300 ng/ml. Blood samples (0.2-0.3 ml each) were collected at 2, 3, and 4 hr after initiation of the infusion. Due to limited sample volumes, milk samples were obtained at 4 hr only. Thirty minutes prior to milk sampling, oxytocin was given to the rat by s.c. injection (5 i.u.) to stimulate milk production. Obtained serum and milk samples were kept at -20°C until analysis.

Analysis of Bisphenol A

Bisphenol A concentrations in serum and milk were determined by a validated HPLC with fluorescence detection method developed in our laboratory (Shin et al., 2000). Briefly, bisphenol A was extracted by a single liquid-liquid extraction with 2 ml of methylene chloride under acidic conditions. A Shimadzu HPLC component system consisting of a model SCL-10A system controller, RF-10AXL fluorescence detector, LC-10AT pump, SIL-10A autosampler, and CTO-10A column oven was used in the assay. Chromatographic separations were achieved using a Spherisorb S5 ODS2 analytical column (Waters, 4.6 mm x 250 mm, 5 mm) (Milford, MA, USA). The mobile phase consisted of acetonitrile:deionized water (54:46, v/v) and the flow rate was maintained at 0.5 ml/min at 40°C. The excitation and emission wavelengths were set at 278 nm and 315 nm, respectively. The standard curve was linear over the concentration range of 1-1,000 ng/ml, with a typical correlation coefficient (r) >0.999. The extraction recovery of bisphenol A was >91.3% determined at 5, 10, 20, 50, 100, 200, 500 and 1,000 ng/ml (n=5 each). The intra- and inter-day assay accuracy was >91.5% and >90.8%, respectively, with the assay coefficients of variation being <5.7% and <10.5%, respectively. The limit of quantification of this assay method was 1 ng/ml using a 100 ml of biological sample volume.

Data Analysis

The systemic clearance after infusion was calculated as Cls = infusion rate/Css,serum. Parameter values were expressed as the mean S.D. A one-way analysis of variance (ANOVA) was used to test the difference in the mean systemic clearance obtained after infusion at three dosing regimens. The statistical significance was set at $p<.05$.

Results

The designated infusion regimens resulted in the achievement of steady-state serum concentrations of bisphenol A by 2 hr (Figure 1). The average steady-state serum concentration (Css,serum) was calculated as the mean of concentrations determined at 2, 3 and 4 hr after initiation of infusion. Average steady-state concentrations of

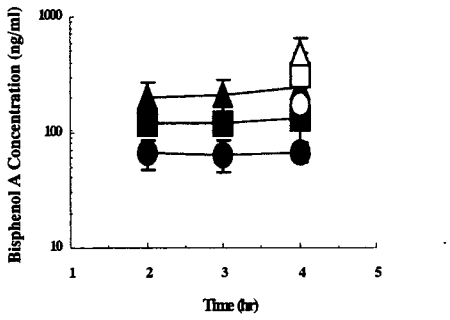

Figure 1. Average steady-state concentrations of bisphenol A in milk (open symbols) and serum (closed symbols) following i.v. injection and infusion to steady-state in lactating rats at low (circles), medium (squares), and high (triangles) doses (n=4-6 each).

Table 1. The systemic clearance (Cls) and the steady-state concentrations of bisphenol A in serum (Css,serum) and milk (Css,milk) in lactating female rats after simultaneous i.v. bolus injection followed by infusion

Parameter	Infusion Regimen		
	Low (n=4) (0.13 mg/hr)	Medium (n=5) (0.27 mg/hr)	High (n=6) (0.54 mg/hr)
Body W (g)	295± 5	282± 28	289 ± 27
Cl5 (ml/min/kg)	119.2 ± 23.8	142.4 ± 45.3	154.1 ± 44.6
Css,serum (ng/ml)	66.1± 15.5	120.0 ±34.7	217.1 ± 65.0
Css,milk (ng/ml)	173.1 ± 43.3	317.4 ± 154.4	493.9 ± 142.2
M/S Ratio	2.7 ± 0.9	2.6 ± 1.2	2.4 ± 0.6

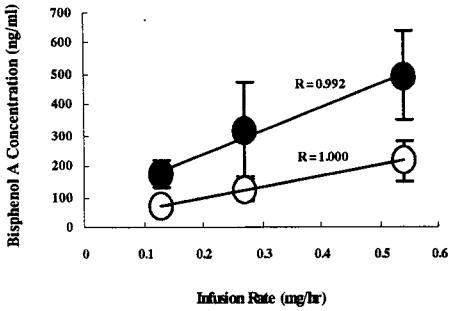

Figure 2. Relationship between the infusion rate and the steady-state serum (?) or milk () levels of bisphenol A in lactating rats. Each point is the mean S.D. of 4-6 rats.

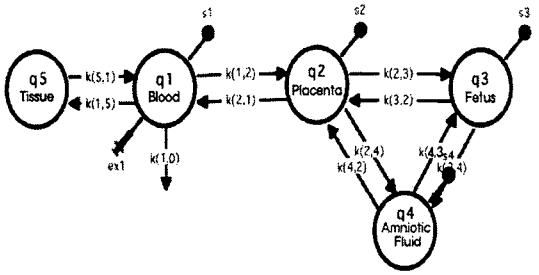

Figure 3. Schematic representation of the pharmacokinetic model consisting of the maternal central, placental, fetal, amniotic fluid and maternal tissue compartments.

bisphenol A in serum (Css,serum) and milk (Css,milk) are summarized in Table 1. Bisphenol A was excreted into milk extensively, with the milk levels exceeding the serum levels (M/S ratio 2.4-2.7). The Css,serum and Css,milk were linearly increased as the infusion rate was increased (Figure 2). The mean systemic clearance of bisphenol A ranged from 119.2 to 154.1 ml/min/kg at three infusion rates. There was no significant difference among these values.

A 5-compartmental model was used to describe the bisphenol concentrations in maternal central (q1), placenta (q2), fetus (q3), amniotic fluid (q4), and maternal tissue compartment (q5) (Figure 3). In this model, k10 is the elimination rate constant from the maternal central compartment, k12 and k21 are the intercompartmental transfer rate constants between maternal blood and placenta, k23 and k32 are the transfer rate constants between placenta and fetus, k34 and k43 are the transfer rate constants between fetus and amniotic fluid, and k24 and k42 are the transfer rate constants between placenta and amniotic fluid.

Table 2. Intercompartmental transfer rate constants and volume of distribution of bisphenol A in the materal-fetal unit

Parameter	Value	Parameter	Value
k10 (hr-1)	1.20 ± 0.51	Cl10 (ml/min)	12.6 ± 1.4
k12 (hr-1)	0.26 ± 0.26	Cl12 (ml/min)	3.2 ± 3.2
k21 (hr-1)	7.33 ± 7.76	Cl21 (ml/min)	0.7 ± 0.7
k15 (hr-1)	3.50 ± 1.97	Cl15 (ml/min)	33.2 ± 18.8
k23 (hr-1)	60.10 ± 15.07	Cl23 (ml/min)	5.7 ± 1.3
k32 (hr-1)	34.85 ± 0.29	Cl32 (ml/min)	12.5 ± 0.1
k24 (hr-1)	1.32 ± 1.26	Cl24 (ml/min)	0.1 ± 0.1
k42 (hr-1)	7.58 ± 1.54	Cl42 (ml/min)	1.9 ± 0.4
k34 (hr-1)	0.17 ± 0.17	Cl34 (ml/min)	0.1 ± 0.1
k43 (hr-1)	8.95 ± 1.57	Cl43 (ml/min)	2.3 ± 0.4

Maternal serum bisphenol A concentration-time data were initially described by a two-compartmental open model by WinNonlin weighted (1/c) least-squares regression. Pharmacokinetic parameters such as volume of the maternal central (Vc) and peripheral (Vt) compartments were determined. The volumes of the placenta and fetus were determined from measured weight assuming 1 g of tissue is equivalent to 1 ml of volume. The volume of amniotic fluid (Va) was estimated by collecting amniotic fluid into a syringe. Model differential equations were fitted simultaneously to all concentration-time data by Scientist weighted (1/c) least-squares regression analysis.

Intercompartmental distribution clearances (Clij) were calculated from the product of transfer rate constants and distribution volume. Computer simulated bisphenol A concentration-time curves as a function of time are shown in Figure 4.

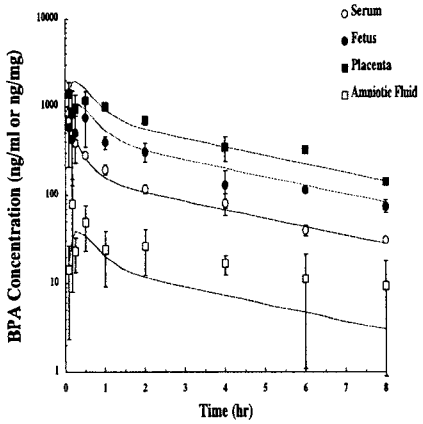

Figure 4. Average concentration-time curves of bisphenol A in the maternal serum, fetus, placenta and amniotic fluid obtained after i.v. injection of 2 mg/kg.

Discussion

The extent of mammary excretion of xenobiotics under non-steady-state conditions requires collection of serial milk and serum samples and comparison of the AUC values. We used a single point determination at steady-state, based on the theory that the M/S ratio at steady-state (M/Ssteady-state = Css,milk/Css,serum) is equivalent to the milk to serum AUC ratio after a single dose (M/Ssingle dose = AUCmilk/AUCserum) (McNamara et al., 1991). Consistent with high lipophilicity (o/w partition coefficient 2.2) (Staples et al., 1998), bisphenol was excreted into rat milk extensively, with the milk levels exceeding the serum levels (M/S ratio 2.4-2.7). A dose-proportional increase in milk levels indicates that the mammary excretion of bisphenol A is mediated by passive diffusion. The systemic clearance determined at three infusion rates was unaltered, confirming the linear pharmacokinetics over the concentrations examined (Yoo et al., 2000).

Average bisphenol A concentrations in serum, placenta, fetus and amniotic fluid as a function of time after i.v. injection of 2 mg/kg are shown in Figure 3. Bisphenol A concentration in the serum declined biexponentially. Bisphenol A was rapidly distributed into placenta, fetus and amniotic fluid, with maximum concentrations in these tissues achieved within 1 hr of injection. The decline of bisphenol A in placenta, fetus and amniotic fluid paralleled that of maternal serum. Estimates of transfer rate constants and clearances are shown in Table 2. The transfer of bisphenol A from maternal plasma to the placenta was rapid. The transfer rate constants between placenta and fetus were greater than those between placenta and amniotic fluid. As shown in Figure 3, computer simulated bisphenol A concentration-time curves were well fitted to the observed concentrations in each compartment.

In summary, bisphenol A was highly excreted into milk, with the M/S ratio being 2.4-2.7. Milk levels of bisphenol A were increased linearly with increasing the infusion rate, indicating that the excretion into milk was mediated by passive diffusion. The extent of mammary excretion and placental transfer should be considered in studying the prenatal and postnatal toxicokinetics of bisphenol A after exposure during pregnancy and lactation.

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