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Bisphenol A (BPA) pharmacokinetics with daily oral bolus or continuous exposure via silastic capsules in pregnant rhesus monkeys: Relevance for human exposures

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ABSTRACT

We measured serum dBPA in non-pregnant and pregnant female rhesus monkeys, fetuses and amniotic fluid. dBPA was administered by a daily oral bolus or sc implantation of Silastic capsules; both resulted in daily average serum unconjugated dBPA concentrations of <1 ng/ml. We observed lower serum concentrations of unconjugated dBPA in pregnant females relative to pre-pregnancy values, and generally lower concentrations in fetal serum than in maternal serum. Differences in pharmacokinetics of dBPA were evident between pre-pregnancy, early and late pregnancy, likely reflecting changes in maternal, fetal and placental physiology. The serum ratio of conjugated to unconjugated dBPA after continuous sc release of dBPA was similar to values reported in human biomonitoring studies and markedly lower than with oral administration, suggesting oral bolus exposure is not an appropriate human exposure model. We report elsewhere that there were numerous adverse effects on fetuses exposed to very low serum dBPA in these studies.

1. Introduction

Bisphenol A (BPA) is a high volume production chemical that is used in a wide variety of consumer products, including polycarbonate and other forms of plastics, resins used to line food and beverage containers, thermal printed papers, and composites used in dentistry. As a result of its widespread use, humans are exposed to BPA on a virtually constant basis [1]. Although estimates of daily exposure differ markedly [2–4], BPA contaminates our air, water, and soil [5], and the pervasiveness of human exposure is not disputed [3,6]. Relevant to our research, there is extensive evidence that BPA crosses the placenta in humans and animals, resulting in measurable concentrations of unconjugated (bioactive) BPA in placenta, fetal tissues and blood [3,7–9].

BPA is an endocrine disrupting chemical (EDC) that has been demonstrated to affect signaling mechanisms involving estrogen, androgen, aryl hydrocarbon and thyroid hormone receptors [10,11]. Animal studies have demonstrated that maternal exposure can significantly alter fetal development, resulting in a variety of adverse outcomes in the adult [12–15]. In addition, numerous epidemiological studies have reported associations between BPA and adverse health effects [16], including when exposure occurs during fetal life [17], which has been a main focus of research with laboratory animals [18]. In response, regulatory agencies in some countries have begun to restrict the uses of BPA. For example, Canada has declared BPA a "toxic chemical", the US-FDA banned BPA for use in baby bottles (although this was requested by the baby bottle industry), and the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) has called for the elimination of BPA in food packaging in 2014 [19].

Despite the evidence that BPA induces a wide range of adverse effects whether exposure occurs during development or in adulthood, debate about the level of concern appropriate for BPA continues, with discussion centering on two issues that are addressed in our current study: (1) the routes by which humans are exposed and thus how estimates of the current total daily exposure

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levels relate to the amount of BPA in blood that is unconjugated vs. conjugated [20], and (2) the relevance of animal models for predicting human pharmacokinetics and pharmacodynamics [2,21].

The limited information about BPA metabolism during pregnancy in primates and its importance in assessing developmental exposure, together with the controversy regarding potential routes of exposure to BPA, prompted us to undertake the present set of studies in pregnant female rhesus monkeys. We first conducted pharmacokinetic studies of pregnant females. We used in the present study the same oral dose of deuterated BPA (dBPA) on a subset of the rhesus monkey females from our initial study of non-pregnant females [2] that became pregnant and carried a female fetus during the following breeding season. This allowed us to compare dBPA metabolism in the same females in a non-pregnant and pregnant state; we also examined dBPA at multiple times in pregnancy. We then initiated a second study with a separate group of pregnant monkeys using a different exposure paradigm of continuous exposure via subcutaneously (s.c.) implanted Silastic capsules containing dBPA (Fig. 1). Our hypothesis was that the continuous exposure paradigm would more accurately mimic some of the potential sources of human exposure (transdermal, sublingual/buccal, inhalation) than the single daily oral bolus gavage administration commonly used in toxicological research [1,22–24]. Specifically, there is evidence that human exposure to BPA is likely from multiple sources and multiple routes [1] including dermal exposures from BPA-containing receipt paper [25,26], inhalation exposure to BPA on dust [27–29], iatrogenic exposures from medical devices [30], and also sublingual absorption from food while in the mouth [20]. Thus, subcutaneously implanted Silastic capsules may provide a better model for the exposure of humans that is not accounted for by a single gavage administration, which results in a very low percent of the administered dose being bioavailable relative to other routes of exposure [20].

The pharmacokinetic results of our study, together with a series of publications showing significant adverse effects on the ovaries, mammary glands, brain and lungs of fetuses carried by the same dBPA-treated monkey females [31–34], indicate that there is no mechanism to protect the developing fetus from maternal exposure to BPA during pregnancy. Our data also suggest that continuous exposure to BPA via Silastic capsules produces a profile of conjugated vs. unconjugated BPA in serum similar to that observed in cross-sectional studies in people. In contrast, the corresponding profile of conjugated vs. unconjugated BPA in serum observed following a single daily oral bolus administration in monkeys (both prior to and during pregnancy) is markedly different from what is observed in humans [35,36].

2. Methods

2.1. Animals

Adult female rhesus macaques (*Macaca mulatta*) were housed at the California National Primate Research Center. Animal protocols were reviewed and approved in advance by the Animal Care and Use Committee of the University of California, Davis; all studies were conducted in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animals were caged individually with a 06:00 to 18:00 light cycle and at a temperature maintained at 25–27 °C. Animals were fed a diet of Purina Monkey Chow (Purina Mills, St. Louis, MO, USA) and provided with water ad libitum. Seasonal produce, seeds, and cereal were offered as supplements for environmental enrichment. Cages were made of stainless steel, and water was delivered to each cage by rigid polyvinyl chloride pipes and a water nipple.

Only females with a history of normal menstrual cycles were selected for this study. Females ranged in age from 6 to 13 years, and body weights ranged from 6.25 to 11.25 kg throughout pregnancy (mean, 8.75 kg). All females were naturally mated according to standard California National Primate Research Center procedures. Pregnancy was detected by ultrasound examination, and an estimated day of conception (gestation day—GD 0) was assigned. At approximately GD 40, the sex of all fetuses was determined, and those with female fetuses were continued on the current study and also to determine the effects of dBPA on ovarian, mammary gland, lung and brain development. Fetal growth rate was also monitored by ultrasound every 2–3 weeks during treatment [37]. Cephalic vein blood samples were collected from unanesthetized, cage-restrained animals that were trained to present an arm for the procedure.

2.2. Rationale for deuterated BPA (dBPA) administration

Deuterated (d6) BPA (dBPA, CDN Isotopes, Quebec, Canada) was used in these studies because it can be clearly distinguished from BPA by liquid chromatography mass spectrometry (LC/MS), thus eliminating concern about potential BPA contamination from materials used in the preparation, handling or shipment of samples, although in practice, by using appropriate field and assay blanks, we have been able to rule out contamination with background BPA in our studies, as have others; for example refs. [8,38].

2.3. Treatment groups

Two routes of BPA exposure were used for these experiments (Fig. 1). The first cohort of animals was given small pieces of fruit containing 400 μg/kg body weight of dBPA once per day, modeling an acute oral exposure. Our second route of exposure was via Silastic implants, which models exposure routes that bypass first-pass metabolism in the liver [1,20]. These implants were demonstrated in a preliminary study to release dBPA at a fairly constant rate for 30 days, after which release rate begins to drop; the capsules produced serum levels of about 3.5 ng/ml unconjugated dBPA in non-pregnant females, close to the median reported in pregnant women [8,39]. Both the oral and continuous dose (Silastic implant) cohorts were further subdivided into early and late pregnancy treatment groups as shown in Fig. 1. Early pregnancy animals were dosed from GD 50 to GD 100 (Early Pregnancy, Oral Dose: $N = 5$; Early Pregnancy, Silastic implant: $N = 6$). Late pregnancy animals were dosed from GD 100 to GD 155 (Silastic implant; $N = 6$) or from GD 100 to natural birth on about GD 165 (oral dose; $N = 6$). Female fetuses carried by the early and late pregnancy oral dose females and by additional control females were examined for effects on various tissues that are reported elsewhere [31–34].

2.4. Comparison of the metabolism of single daily oral BPA doses in non-pregnant monkeys and pregnant monkeys on GD 50 and GD 95

To directly compare dBPA metabolism in the non-pregnant and pregnant females, the eleven rhesus monkey females used in our recent pharmacokinetic study [2] were mated during the next breeding season. Four females that became pregnant were determined to be carrying female fetuses, which were the focus of analysis of fetal tissues. The dose of dBPA administered to these four females in our previous study, prior to pregnancy, was the same 400 μg/kg/day dose used here [2]. One additional adult female that had not been examined prior to pregnancy was added to the BPA-exposed group to achieve a sample size of 5 for the pharmacokinetic analysis of dBPA during pregnancy. On GD 50 and then again on GD 95, the concentrations of unconjugated and conjugated dBPA