Transplacental Transport and Fetal Localization of Bisphenol A, Tetrabromobisphenol A and 2,4,6-Tribromophenol in Mice

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Introduction

Bisphenol A (BPA) is an intermediate in the production of epoxy resins, while its brominated derivative tetrabromobishenol A (TBBPA) and its photolysis degradation product 2,4,6-tribromophenol (TBP) are widely used flame retardants (1). These brominated compounds have been identified in human blood. TBBPA, TBP and a number of 4-hydroxy-PCBs (e.g. 4-OH-CB107) are high affinity ligands for the thyroxin (T4) transporter transthyretin (TTR) in rodents and other species (2). Displacement of T4 from the TTR binding site has been proposed as an important mechanism of endocrine disruption by certain halogenated phenolic environmental pollutants (3-4).

BPA is a fairly potent environmental estrogen receptor agonist that can induce an array of estrogenic effects in several species including mammals, birds and fish. Although the estrogenic activity of TBBPA is less obvious, this brominated BPA analog has been reported to interact with the estrogen receptor and induce estrogenic effects in some in vitro test systems.

While the reproductive and developmental toxicity of BPA is well documented, there is evidence that also halogenated phenolic compounds can pass the placental barrier and induce such toxicity (3-6). Within the objectives of the COMPARE EU project we study the fetal and maternal kinetics and transplacental transport of phenolic environmental pollutants in pregnant mice. To explore the role of TTR in the placental and blood-brain barrier transport, we employ TTR-deficient mice. For comparative reasons, we also explore the transfer to bird embryos following

injection into the yolk or administration to the egg-laying bird (7). In the present communication, we report on the disposition of BPA, TBBPA and TBP in the feto-placental unit in pregnant wild-type mice.

Material and methods

Chemicals

Bis [^{14}C]-phenol A (^{14}C-BPA; spec. act. 48.8 mCi/mmol) and tetrabromo-bis[^{14}C]-phenol A (^{14}C-TBBPA; spec. act. 48.8 mCi/mmol) were prepared from [^{14}C]-phenol and acetone as described by Susan et al. (8). 2,4,6-Tribromo-[^{14}C]-phenol (^{14}C-TBP; spec. act. 48.8 mCi/mmol) was prepared by bromination of [^{14}C]-phenol and isolated after chromatographic purification of the synthesised product.

Animals and treatments

C57 Bl mice were obtained from B&K Universal (Sollentuna, Sweden). The animals were housed in a controlled environment (12 hour light/dark cycle and 23 ºC, 50% humidity) and fed a standard pellet diet and tap water ad libitum. A male mouse was combined with three females in each cage, and mating was confirmed the following morning by the presence of a vaginal plug (day 0 of pregnancy). Animals dosed iv with the labelled test compounds (10 µCi/animal, dissolved in 20 µl DMSO) were killed either on gestation day 14 or 17, 1.5 -72 h following administration. The studies were approved by the Local Ethics Committee for Research on Animals.

Tape-section autoradiography

The mice were killed by carbon dioxide, frozen in an aqueous gel of carboxymethyl cellulose (-70ºC), and subjected to tape-section autoradiography according to Ullberg (9). Sagittal sections (20 µm, 12 sections per mouse) were collected onto tape using a cryostat microtome, freeze-dried and apposed to roentgen film. Autoradiograms were developed after 1-12 months of exposure at -20 ºC.

Results

General observations: Following iv injection, all compounds showed a pronounced elimination via the bile to the intestinal contents and via the urine. A marked excretion of radioactivity in the intestinal contents was evident also in late gestational fetuses, supporting an ability of the fetus to form presumably conjugated metabolites. As demonstrated by the retention of radioactivity in the maternal and fetal compartments at the different post-injection times, ^{14}C-TBP had a shorter half-life than the bisphenol A derivatives. The rate of elimination of ^{14}C-TBBPA was more rapid than that of ^{14}C-BPA, which was strongly retained in all tissues at 72 h.

BPA. The autoradiograms were characterized by a strong uptake in the maternal liver at all post-injection times, a slight uptake in brown fat, and a marked and fairly persistent labelling of the blood. With exception of a marked uptake of radioactivity in the choroid plexus, the penetration to the brain was hindered. Also notable was a marked and persistent accumulation of ^{14}C-BPA in the corpora lutea in the ovary, leaving other ovarial structures such as the follicles and stroma devoid of accumulated radioactivity (Figure 1). A corresponding uptake occurred also in the adrenal cortex. A high and persistent enrichment of ^{14}C-BPA-derived radioactivity was observed in the uterine luminal fluid and the yolk sac epithelium (Figure 1). The transfer to the fetuses was rapid, and the retention in the fetuses was pronounced, giving rise to higher relative concentrations in the fetuses than in most maternal tissues at the longer post-injection times. As was the case in the dam, the penetration of ^{14}C-BPA to the fetal brains was low, confirming the presence of a fetal blood-brain barrier.

TBBPA With some exceptions, ^{14}C-TBBPA showed a similar tissue distribution pattern as ^{14}C-BPA. Notably, however, ^{14}C-TBBPA was not accumulated in the corpora lutea in the ovaries. The uptake of the liver was fairly strong and persistent. A marked radioactivity was present in the blood at all post-injection times, while the uptake in the brain was consistently low. The enrichment of ^{14}C-TBBPA-derived radioactivity in the uterine luminal fluid and yolk sac epithelium was strong at all post-injection times (Figure 2). As with ^{14}C-BPA, also ^{14}C-TBBPA gave rise to a pronounced relative retention in the fetuses, most pronounced at gestation day 17, 24-48 h after treatment. As in the dam, the penetration to the fetal brains was hindered.

TBP As compared to the labelled BPA derivatives, ^{14}C-TBP was more rapidly eliminated by biliary and urinary excretion. At 24-72 h, comparatively low levels of radioactivity remained in the bile-intestines. The distribution pattern was initially similar to that of ^{14}C-BPA and ^{14}C-TBBPA, with a high uptake and retention in the liver, a marked labelling of the blood and a hindered penetration to the maternal brain. Marked enrichment of radioactivity occurred in the uterine luminal fluid and yolk sac epithelium up to 6 h after injection. The transfer to the fetuses was rapid although the overall retention was less pronounced than for the BPA derivatives. An effective fetal blood-brain barrier was present. 24-72 h after injection, the retention in the fetuses was distinct but faint (Figure 3).

Discussion

The most conspicuous finding was that ^{14}C-BPA , ^{14}C-TBBPA and ^{14}C-TBP all gave rise to strong enrichment of radioactivity in the utero-luminal fluid and in the yolk sac epithelium at gestation day 14 and 17. Also noteworthy was the pronounced retention observed in the fetuses, which at the longer post-injection times reached a higher general level of radioactivity than the injected dams. As evidenced by a very low uptake both in the fetal and maternal brains, there seemed to be an efficient blood-brain barrier for all compounds. This finding conforms with the previous observation that injection of PCB 77 in pregnant mice results in a strong retention of a phenolic metabolite in full-term fetuses despite a very low uptake in the fetal brain (10). It has previously been shown that ^{14}C-TBBPA, ^{14}C-TBP and ^{14}C-BPA, as well as several OH-PCBs including 4-OH-CB-107 bind with high affinity to the plasma thyroid transport proteinTTR. This high affinity binding competes with and displaces the natural ligand, T_4. (3-4). TTR is also reported to be important for the transplacental transport of thyroid hormones in vertebrates, as well as for delivery of T4 across the blood-brain barrier. Since our findings show that neither of the labelled test substances could cross the blood-brain barrier, the role of TTR binding is presently being explored using TTR knock-out mice.

Figure 1; ^{14}C-BPA

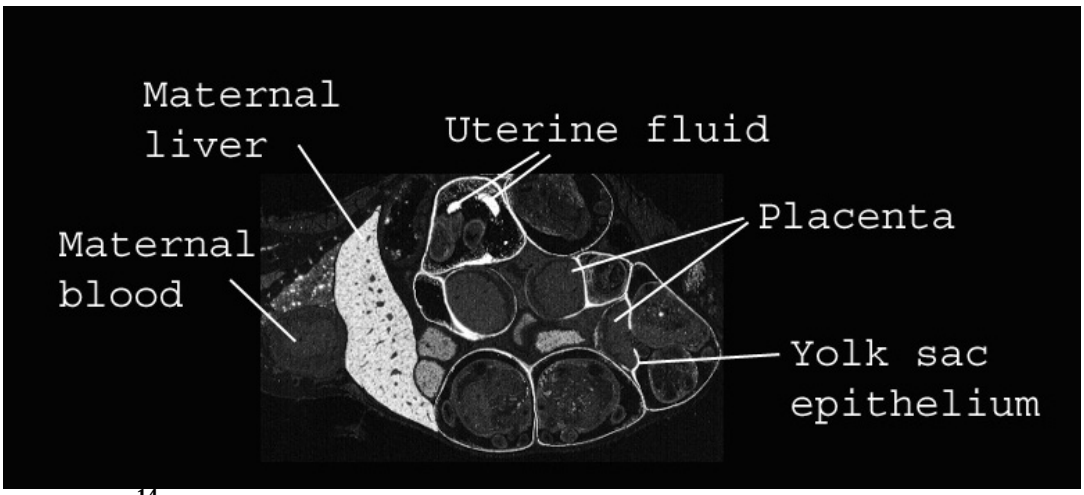

Figure 2; ^{14}C-TBBPA

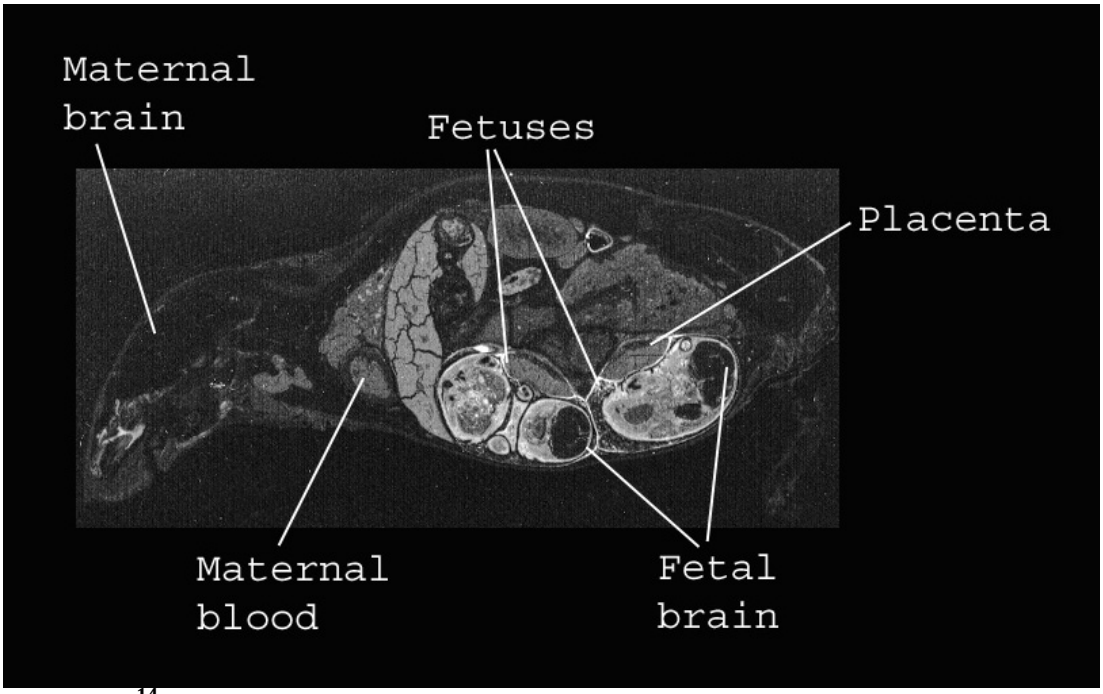

Figure 3; ^{14}C-TBP

The observed selective uptake of ^{14}C-BPA in the corpora lutea (a tissue producing progesterone) is of particular interest and deserves further consideration. Notably, no similar uptake of ^{14}C-TBBPA and ^{14}C-TBP in the corpora lutea was observed.

Since the selected route of administration was i.v. injection, it is obvious that the labelled test substances were eliminated by biliary excretion into the intestine. All three substances were readily excreted, presumably following conjugation of the hydroxyl group. The rate of elimination was different, however, in that ^{14}C-TBP apparently had a considerably shorter half-life than ^{14}C-BPA and ^{14}C-TBBPA. For comparative reasons we also examined the disposition of ^{14}C-BPA , ^{14}C-TBBPA (7) in quail embryos following administration into the yolk. As demonstrated in these studies, the transfer of the labelled BPA derivatives to the fetuses was slow, and embryonic retention similar to that observed in mice fetuses was not observed. A marked concentration of radioactivity was however found in the intestinal contents in the quail embryos, conforming with the view that bird embryos express xenobiotic-metabolising enzymes at an early stage of development compared to mouse fetuses.

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