Metabolism of Bisphenol A in the Rat Syncytiotrophoblast Cell Line, TR-TBT 18d-1

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TR-TBT 18d-1 is a syncytiotrophoblast cell line established from placenta of the transgenic rat harboring temperature-sensitive simian virus 40 large T-antigen (Tg-rat). Previously we have reported high level expressions of drug metabolizing enzymes in the TR-TBT cells. To elucidate the ability of metabolizing xenobiotics in the TR-TBT cells, we measured the metabolism of bisphenol A (BPA) as a model compound in the TR-TBT 18d-1 cells. It was found that BPA was metabolized and secreted as a monoglucuronide conjugate in a manner proportional to the incubation time and the BPA concentration. To elucidate the possible effect of green tea consumption on the placental metabolism, we investigated the effect of green tea on the metabolism of BPA in the TR-TBT cells. Green tea inhibited the conjugation reaction of BPA at the low concentration (IC_{50}=5%), however, epigallocathechin gallate (EGCG) showed only weak inhibition, suggesting another active component existence. These results suggest that TR-TBT cells are useful for the model system of rat syncytiotrophoblasts which act as a barrier to protect fetus from harmful xenobiotics.

Key words —— bisphenol A, glucuronidation, metabolism, placenta

INTRODUCTION

The placenta separates the blood supply of two human beings, mother and fetus, while being simultaneously perfused by both their circulations.[1] The major function of the placenta is to transfer nutrients and oxygen from the mother to the fetus and to assist in the removal of waste products from the fetus to the mother. In addition, it acts as a barrier to protect the fetus from xenobiotics in the maternal blood.[2,3] Drug administration to pregnant women must always be cognizant of potential for fetal exposure. However, the impact of placental metabolism and uptake on fetal exposure of xenobiotics remains difficult to quantify.

Syncytiotraphoblasts, which form a continuous barrier between the maternal and fetal circulation, play an essential role in restriction of xenobiotics including drugs through blood-placental barrier (BPB). It is of importance to clarify the metabolism of xenobiotics in the rat placenta, because rats are frequently and widely used in the embryo- and feto-toxicity studies on foreign chemical compounds. To investigate the functional roles of syncytioblasts in the BPB, Kitano *et al.* have established new syncytiotrophoblast cell lines (TR-TBTs) from the transgenic rat harboring temperature-sensitive simian virus 40 large T-antigen (Tg-rat).[4,5] The conditionally immortalized TR-TBT cells display syncytium-like morphology and express several syncytioblast specific markers and polarized glucose transporters.[4,6] To further the investigation of syncytiotrophoblast cells as BPB, we characterized the metabolism of bisphenol A, a well-known endocrine disruptor, in the TR-TBT cells.

MATERIALS AND METHODS

Materials —— All chemicals were obtained from Wako chemicals (Tokyo, Japan). BPA sulfate was gifted of Dr. S. Ozawa of National Institute of Health in Tokyo, Japan. Green tea was prepared simulating the usual way of brewing tea as follows. Green tea leaves (2 g) were extracted with 100 ml of hot water (75°C) for 2 min. The extract was then filtered and divided into small aliquots, after which it was stored at −80°C until use. Undiluted extract was assigned as 100% (v/v).

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Cell Culture —— TR-TBT 18d-1 cells were plated in 6-well tissue culture plates and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 10 U/ml penicillin and 10 U/ml streptomycin at 33°C in a humidified atmosphere containing 5% CO_2.

HPLC Analysis of BPA Metabolites in TR-TBT Cells —— For studies of BPA metabolism in the TR-TBT cells, BPA was added to the medium at a concentration of 10–50 μM and then the cells were incubated at 37°C. An aliquot (100 μl) was removed at various times, and then 30 μl of the mixture was filtered and injected onto the HPLC. The analysis was performed on an ODS column (Chromolith Performance RP-18e, 100 × 4.6 mmϕ, Merck, Darmstadt, Germany).[7] The mobile phase was composed of 10 mM tetrabutylammonium hydrogen sulfate in water and acetonitrile (79.5:20.5). The flow rate was 1 ml/min and the temperature for the column was 40°C. Fluorescence intensity was monitored at 313 nm with excitation of 273 nm. Almost signals due to the medium constituents were eluted before 2 min.

Identification of BPA Metabolite —— To identify the BPA metabolite, the culture medium (supplemented with 50 μM BPA and incubated for 72 hr) was treated with 10 units of *Escherichia coli* β-glucuronidase for 1 hr at 37°C and the mixture was then analyzed by HPLC as described above.

Determination of BPA Metabolite by Liquid Chromatography-Mass Spectrometry (LC-MS) —— HPLC fractions containing the BPA metabolite were collected, concentrated by evaporation and dissolved in water and acetnitrile (70:30). Then the sample was analyzed using a model M-8000 ion trap LC-MS system (LC/3DQMS, Hitachi, Tokyo, Japan) with sonic spray ionization interface.[8] The analytical column was a Chromolith Performance PR-18e (100 mm × 4.6 mmϕ, Merck) operated at 40°C. The flow rate was 0.3 ml/min. The drift voltage was 130 V. The sampling aperture was heated at 150°C and the shield temperature was 230°C.

RESULTS

Metabolism of BPA in the TR-TBT 18d-1 Cells

In a separate study, we analyzed the expression of drug metabolism enzymes in the TR-TBT cells and found significant expressions of cytochrome P450s (CYPs) and phase II conjugation enzymes.[9]

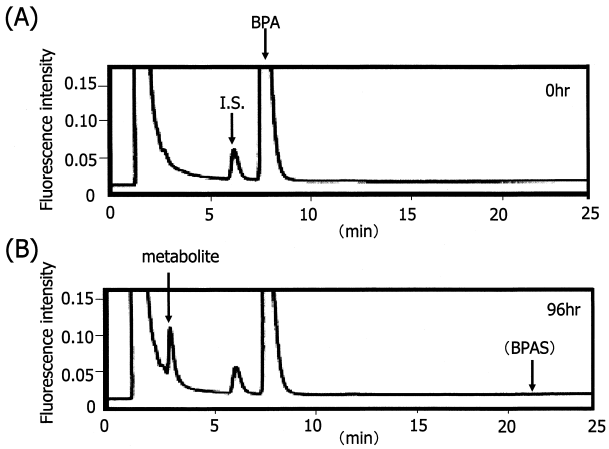

Fig. 1. HPLC Analysis of BPA Metabolite in the TR-TBT Culture Medium

Chromatograms were generated from HPLC analysis of the medium in which the TR-TBT 18d-1 cells were cultured with 50 μM BPA. An aliquot (100 μl) was removed at time=0 (A) and 96 hr (B), and applied to the HPLC. BPA was eluted at 8.4 min (A), and the peak at 3.4 min appeared after 96 hr culture (B). I.S; internal standard (1-naphthol). The position of BPA sulfate (BPAS) was indicated by an arrow in the chart (B).

To evaluate the ability of the cells to metabolize xenobiotics, we measured the metabolism of bisphenol A (BPA) as a model compound in the TR-TBT 18d-1 cells. After supplementing TR-TBT cells cultures with BPA (0–50 μM), the accumulation of BPA metabolites in the growth medium was monitored by the analytical HPLC over a 96 hr period. As shown in Fig. 1, one peak eluted at 3.4 min was accumulating in the culture medium followed by BPA decreasing. The accumulation was in a manner that is almost directly proportional to the incubation time and the BPA concentration (Fig. 2). After 96 hr incubation, more than 50% of BPA was metabolized (data not shown).

To identify the metabolite eluted at 3.4 min on the HPLC, we hydrolyzed it by β-glucuronidase. As shown in Fig. 3, the peak disappeared after the treatment, suggesting the presence of β-glucuronic acid moiety. To further confirm the result, we analyzed the metabolite by LC-MS. As shown in Fig. 4, the chromatographic peak was detected with the retention time corresponding to that of the collected peak and the mass spectrum of the peak showed a major signal at m/z 403, the mass of the deprotonated molecular ion, $[M\text{-}H]^-$ of BPA monoglucuronide. Mass chromatogram at m/z 403 also gave a major peak at the retention time identical to that of the chromatographic peak. All these data indicate that the major metabolite of BPA in the TR-TBT cells is

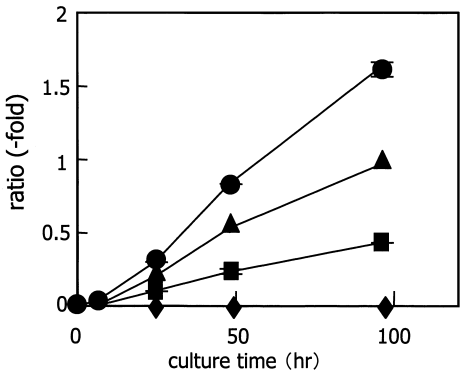

Fig. 2. Accumulation of BPA Metabolite in the Culture Medium of TR-TBT 18d-1 Cells

BPA was supplemented to the medium at the concentration ranging 0 to 50 μM and aliquots were removed at the indicated times, and then analyzed by HPLC (diamond, 0 μM; square, 12.5 μM; triangle, 25 μM; circle, 50 μM). The amount of the metabolite was expressed as the ratio to the amount of internal standard (1-naphthol). Each point represents the average of two independent measurements with an error.

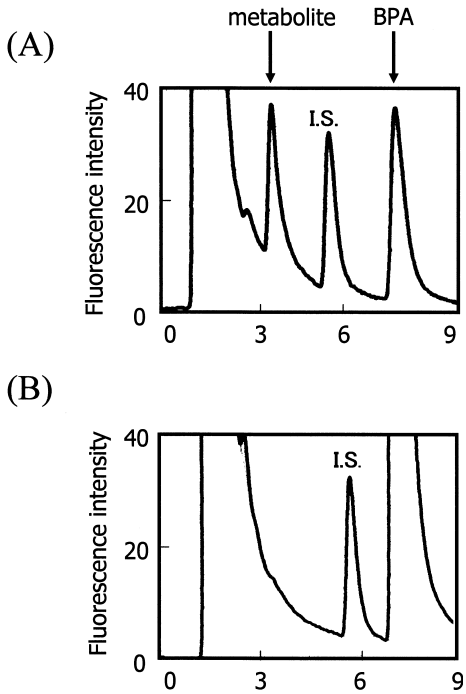

Fig. 3. Hydrolysis of BPA Metabolite by β-glucuronidase

TR-TBT cells were supplemented with 50 μM BPA and incubated for 72 hr, and the aliquot (25 μl) of the medium was treated with (A) or without (B) 10 units of *E. coli* β-glucuronidase for 1 hr at 37°C, and the mixture was then analyzed by HPLC as described above.

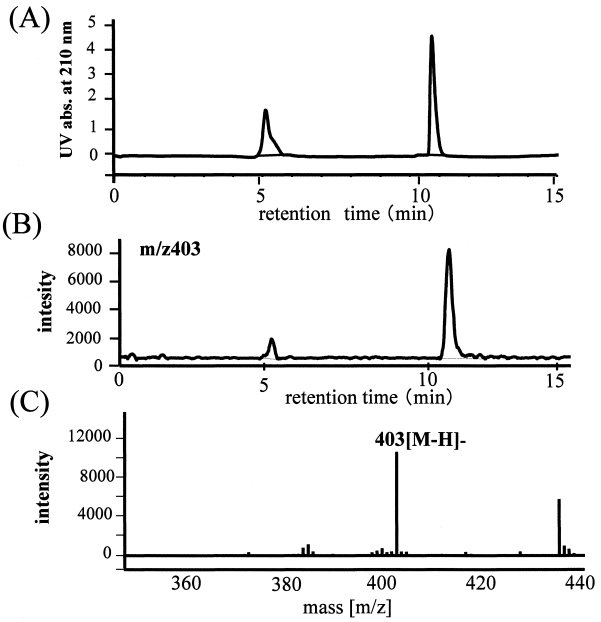

Fig. 4. Analysis of BPA Metabolite by LC-MS

HPLC fractions containing the BPA metabolite were collected, and the mixture was concentrated and dissolved in a mixture of water and acetonitrile (70:30). Then the sample was analyzed by the ion trap LC-MS system with sonic spray ionization interface. (A) Chromatogram of analytical HPLC. The major peak was eluted at 11 min, corresponding to the BPA metabolite because of the slow flow rate (0.3 min/ml). (B) Mass chromatogram at m/z 403. (C) Mass spectrum of the peak fraction eluted at 11 min in (A).

BPA monoglucuronide.

Effects of Green Tea on the Metabolism of BPA in the TR-TBT Cells

Recent studies have shown the induction or inhibition of drug metabolizing enzyme activities by food constituents.[10–12] If these events occurred in the placenta, this might interfere with the normal fetal growth and development. Since the TR-TBT cells might be useful to analyze these effects on drug metabolizing enzymes in the placenta, we examined the effects of green tea on the metabolism of BPA in the TR-TBT cells. As shown in Fig. 5, addition of green tea into the culture medium inhibited the accumulation of BPA glucuronide at low concentrations of green tea (IC_{50}=5%). To identify the component responsible for the inhibition, we analyzed the effects of epigallocathechin gallate (EGCG), a major component of catechins, and caffeine, however, these two compounds did not show any significant inhibition at the concentration equivalent to 10% green tea (data not shown).

DISCUSSION

Here we reported analyses of metabolism of BPA in the syncytiotrophoblastic cell lines, TR-TBT 18d-1, established from the Tg-rat placenta. BPA was metabolized into a monoglu-

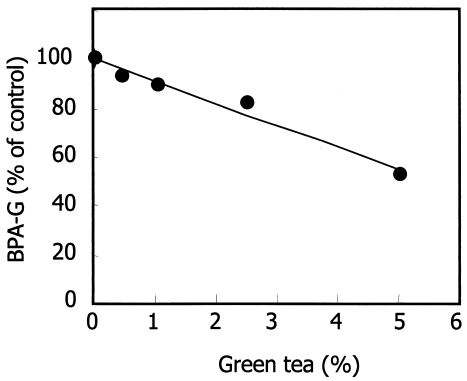

Fig. 5. Effect of Green Tea on the Metabolism of BPA in the TR-TBT Cells

Green tea extract (0–5%(v/v)) was added to the culture medium with 50 μM BPA, and the BPA glucuronide in the medium was measured by analytical HPLC after 48 hr. The amount of BPA glucuronide without green tea extract was assigned as 100%. Each point was the average of two experiments. Undiluted green tea extract was assigned as 100% (v/v).

curonide conjugate in the cells. Several pharmacokinetic studies of the BPA metabolism have demonstrated the extensive metabolism of BPA to BPA-glucuronide,[13–16] and the glucuronidation is mediated by UGT2B1, an isoform of UDP-glucuronosyltransferase (UGT).[17] Since we observed high expression of the UGT2B1 gene in the TR-TBT 18d-1 cells,[9] this UGT isozyme might mediate the BPA glucuronidation in the cells.

We detected a major signal at m/z 403, the mass of the deprotonated molecular ion, $[M\text{-}H]^-$ of BPA monoglucuronide, by LC-MS, however, the possibility can not be excluded that the ion is produced by fragmentation of BPA diglucuronide. More precise analysis of the product should be carried out in the next experiment.

We detected BPA-glucuronide in the culture medium whereas BPA sulfate was not observed (Fig. 1). Since significant expression of phenol sulfating sulfotransferase family genes (SULT1) was detected in the TR-TBT 18d-1,[9] lack of transporter for BPA sulfate might be attributed to the absence of BPA sulfate. This point should be clarified in the next experiments.

Glucuronidation is generally considered to be a detoxification reaction producing metabolites from both exogenous and endogenous substrates. These metabolites are generally more polar and more readily eliminated. Since UGT activity is not observed in the fetal liver as a major path of xenobiotic and endobiotic detoxification,[18] UGT activity in the syncytiotrophoblasts may play a protective role during gestation through metabolism and clearance of toxic compounds such as BPA. In addition, UGTs are also related to the inactivation of some hormones such as testosterone, estrone and thyroid hormones.[19]

Many studies have indicated that food and beverage constituents, such flavonoids and cathecins, affect UGT activities in the hepatic and intestinal cells.[11,20,21] In this report, we found inhibition of BPA glucuronidation in the TR-TBT cells by green tea at the low concentrations (IC_{50}=5%). Although the active component is not clear since the two major physiological active components (EGCG and caffeine) are not responsible, this kind of inhibition of UGT activity by green tea or other beverages and food constituents may influence the normal fetal growth and development. The TR-TBT cells may become a useful tool to investigate the effects of food constituents, environmental chemicals, and drugs on the metabolism of syncytiotrophoblasts in the placenta.

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