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ORIGINAL RESEARCH

Determination of bisphenol A in rat brain by microdialysis and column switching high-performance liquid chromatography with fluorescence detection

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ABSTRACT: A sensitive column switching HPLC-fluorescence detection for determination of bisphenol A (BPA) in rat brain by coupling with microdialysis was developed. A microdialysis probe was inserted into the hypothalamus of rat brain and an artificial cerebrospinal fluid was used for perfusion. BPA in brain dialysate was subjected to a fluorescent derivatization with 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride (DIB-Cl), and the excess reagent was removed by a column-switching technique. Separation was carried out on two ODS semimicro-columns with the mobile phase of acetonitrile–H_2O–methanol–tetrahydrofuran (55:10:35:2.5, v/v) and acetonitrile–0.1 M acetate buffer (pH 3.0)–methanol (35:10:55, v/v) at a flow rate of 0.10 and 0.15 mL/min for a precolumn and a separation column, respectively. Fluorescence intensity was monitored at 475 nm with excitation of 350 nm. BPA could be sensitively detected at 0.3 ppb in 60 μL brain microdialysate at a signal-to-noise ratio of 3. By the proposed method, concentrations of BPA in rat brain and plasma were monitored for 8 h after single i.v. or oral administration. It is proved that BPA is capable of penetrating the blood–brain barrier. The ratio of the area under the concentration–time curve of BPA in rat brain to that in blood was estimated to be about 3.0–3.8%. Copyright © 2002 John Wiley & Sons, Ltd.

INTRODUCTION

In recent years, the global concerns on endocrine disrupting chemicals which mimic endogenous hormone action and thus interfere with normal endocrine function have been increased. Bisphenol A (BPA) has been used not only as a monomer material of polycarbonate plastics (Krishnan *et al.*, 1993), but also in the manufacture of the resin used to line food and beverage cans (Brotons *et al.*, 1995; Hoyle and Budway, 1997), as a component of plastic used in dental fillings (Olea *et al.*, 1996), and as a flame retardant. BPA exhibits weak estrogenic activity *in vitro* and *in vivo* (Steinmetz *et al.*, 1998; Matthews *et al.*, 2001). It binds to human estrogen receptors (ER) and stimulates the transcriptional activity of both ER subtypes (Kuiper *et al.*, 1998; Perez *et al.*, 1998). Miyakoda *et al.* reported that orally administered BPA could easily cross the placental barrier and enter the fetus in animal experiments (Miyakoda *et al.*, 2000). It has also been reported that exposure to BPA in the period of sexual differentiation of the brain can influence adult behavior (Farabollini *et al.*, 1999).

The blood–brain barrier (BBB) is formed by the single layer of endothelial cells that line the inner surface of capillaries in the brain. The tight construction of the vessels in the head guards against brain entry. Excluded molecules include water-soluble compounds, proteins, toxins, most antibiotics and monoamines. It has been reported that endogenous estrogens could definitely pass through the BBB (McCall *et al.*, 1981, Marynick *et al.*, 1976). BPA is a lipophilic compound which has an octanol–water partition coefficient value (log*P*) of around 3–4 (Bayer Leverkusen, 1989; Blanchard, 1984). Hence, we are concerned as to whether BPA could pass through the BBB.

In the present work, we developed a sensitive method for the determination of BPA in rat brain microdialysate by coupling with microdialysis and column switching HPLC-fluorescence detection. Brain microdialysis was used to study the concentration of the target compound in

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Abbreviations used: AUC, area under the concentration–time curve; BPA, bisphenol A; BBB, blood-brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; ER, estrogen receptor; LOD, limit of detection; RSD, relative standard deviation; UGT, UDP-glucuronosyltransferase.

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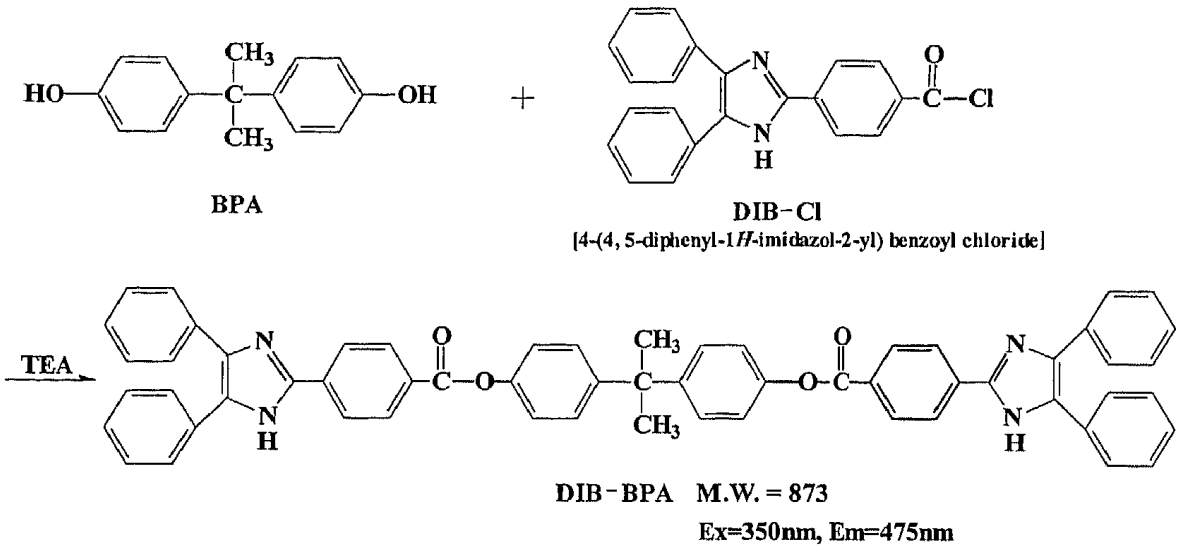

Figure 1. Chemical structure of BPA and its derivatization with DIB-Cl.

extracellular space. By using microdialysis, in which macromolecules are excluded, the sample clean-up step could be simplified. BPA in brain microdialysate was subjected to a fluorescent derivatization with 4-(4,5-diphenyl-1*H*-imidazol-2-yl)benzoyl chloride (DIB-Cl), and the excess reagent was removed by a column-switching technique. The chemical structure of BPA and its derivatization scheme with DIB-Cl are shown in Fig. 1. By the proposed method, BPA in brain microdialysate was monitored until 8 h after a single oral or intravenous (i.v.) administration. Meanwhile, concentrations of BPA in rat plasma were also determined to determine the unconjugated BPA level after BPA administration.

EXPERIMENTAL

Reagents and chemicals

BPA [2,2-bis(4-hydroxyphenyl)propane], propylene glycohol, triethylamine, 25% ammonia solution, hydrochloric acid and sodium acetate were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Acetonitrile and methanol were of HPLC grade (Wako). Tetrahydrofuran and acetic acid were obtained from Kishida Chemicals Co. (Osaka, Japan). Urethane was obtained from ICN Biomedicals Inc. (Aurora, Ohio, USA). DIB-Cl was synthesized in our laboratory as reported previously (Nakashima *et al.*, 1995). Water was passed through a pure line WL21P (Yamato Sciences, Tokyo, Japan).

Animals

Adult male Wistar rats (280–340 g) were obtained from the Otsubo Experimental Animals (Nagasaki, Japan). The rats were kept in plastic cages housed in a controlled room with an ambient temperature (24 ± 1 °C), relative humidity (50 ± 1 °C) and a light/dark cycle of 12/12 h. The animals were fed standard laboratory diet (Oriental Yeast Co. Ltd, Chiba, Japan) and tap water *ad libitum*. In case of oral administration, animals were fasted and provided tap water *ad libitum* 12 h before the experiment.

Drug administration

For oral administration, the proper amount of BPA was dissolved in propylene glycol, and a 200 mg/kg dose of BPA was then administered. For i.v. administration, BPA was dissolved in 45% propylene glycol in saline, and the doses used were 10 or 20 mg/kg.

Apparatus

HPLC system for determination of BPA in rat brain dialysate. The HPLC system consisted of two HPLC pumps (LC-10AD$_{VP}$, Shimadzu, Kyoto, Japan), a Shimadzu RF-10A$_{XL}$ fluorescence detector, a 7125 injector with a 10 μL loop (Rheodyne, Cotati, CA, USA), a HPV-6 column-switching valve with a 20 μL loop (GL Sciences, Tokyo, Japan), and a Rikadenki R-01 recorder (Tokyo, Japan). The mobile phases were acetonitrile–H_2O–methanol–tetrahydrofuran (55:10:35:2.5,v/v) for pump 1 and acetonitrile–0.1 M acetate buffer (pH 3.0)–methanol (35:10:55, v/v) for pump 2, which were delivered at flow rate of 0.10 and 0.15 mL/min, respectively. The temperature for both columns was maintained at 27°C in a column oven (CTO-10AS$_{VP}$, Shimadzu, Kyoto, Japan). Samples were injected on column 1 (precolumn, Wakosil-II 5C$_{18}$, 150 × 1.0 mm i.d., Wako) and purged with mobile phase 1 to pass through the loop of column switching valve. After an injection time of 5.8 min, the valve was switched and then the analyte was loaded onto column 2 (separation column, Develosil ODS-5, 250 × 1.5 mm i.d., Nomura Chemical Co. Ltd, Seto, Japan) and eluted with mobile phase 2 to the fluorescence detector. This valve position was kept until analysis was finished. Fluorescence intensity was monitored at 475 nm with excitation of 350 nm.

HPLC system for determination of BPA in rat plasma. The HPLC system consisted of a HPLC pump (Shimadzu), a Shimadzu RF-10A$_{XL}$ fluorescence detector, a 7125 injector with a 10 μL loop (Rheodyne), and a recorder (Rikadenki R-01). Isocratic separation

was carried out on a Develosil ODS-5 column (Nomura) with a mobile phase of acetonitrile–H_2O–methanol–tetrahydrofuran (55:10:35:2.5, v/v) at a flow rate of 0.15 mL/min. The column was maintained at 27°C in a column oven (Shimadzu). Fluorescence intensity was monitored as stated above.

Rat brain microdialysis of BPA

A brain microdialysis system consisted of a CMA/100 microinjection pump (Carnegie Medicin, Stockholm, Sweden) and a microdialysis probe. The microdialysis probe was made of regenerated cellulose in a concentric design (CUP11, BAS, Tokyo, Japan, 2.0 mm length, 0.24 mm, o.d., with a cut-off at nominal molecular mass of 6000 Da). After anesthetization with intraperitoneal (i.p.) injection of urethane (1.5 g/kg), the rats were fixed on a stereotaxic folding apparatus (Narishige SR-5, Narishige Scientific Instrument Laboratories, Tokyo, Japan) for the microdialysis probe to be implanted in the MPA (medial preoptic area) of the hypothalamus (coordinates AP −0.5, ML −0.5, DV −8.8 mm, relative to bregma and dura surface in the rat brain, provided by the atlas of Paxinos and Watson, 1986). The body temperature was maintained at 37°C with a heating pad. The artificial cerebrospinal fluid (CSF) solution (composition in mM—NaCl 125, NaH_2PO_4 0.5, Na_2HPO_4 2.5, $CaCl_2$ 1.2, KCl 2.5, $MgCl_2$ 1.0, pH 7.4) was used to perfuse the microdialysis probe at a flow rate of 1.0 μL/min delivered by a microinjection pump. After 60 min of perfusion for stabilization, BPA solution was administered orally or intravenously. Brain microdialysate fractions were collected into glass vials at 20, 40, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 min for i.v. administration and every 60 min until 480 min for oral administration. Sample collected before BPA administration was used as a control. The obtained samples were kept in a freezer (−10°C) until analysis.

Derivatization of BPA with DIB-Cl for HPLC analysis

Each brain microdialysate sample was evaporated to dryness under nitrogen gas stream. To the residue, 100 μL of 5 mM DIB-Cl suspension in acetonitrile and 5 μL of 1.5 M triethylamine in acetonitrile were added, and reacted at room temperature for 20 min. Then, 10 μL of 12.5% ammonia solution were added to the reaction mixture to stop the reaction. After standing for 10 min, 10 μL of 5% acetic acid were added for neutralization. A 10 μL portion of the resultant solution was injected onto the HPLC system.

In vitro recovery and delivery of microdialysis probe

A standard solution of BPA in artificial CSF (25 ppb) was placed in the vial. The probe was inserted into the standard solution and perfused with artificial CSF solution. The system was allowed to equilibrate for 60 min. Typically, at least five sequential dialysate samples were collected, at 60 min intervals. Recovery was calculated as the ratio between the concentration in the dialysate (C_d) and the concentration of the standard solution in the vial (C_s) as shown in equation (1).

$$\text{Recovery } (\%) = 100 \times C_d/C_s \quad (1)$$

The standard solution of BPA in artificial CSF (25 ppb) was used as the perfusion fluid through the probe. The probe was inserted into the artificial CSF solution in a vial. Again, the system equilibrated for 60 min and five sequential samples were collected. Delivery was calculated as the ratio of the decrease in the perfusate concentration relative to the initial concentration of the perfusate (C_p), as shown in equation (2).

$$\text{Delivery } (\%) = 100 \times (C_p - C_d)/C_p \quad (2)$$

In vivo delivery of microdialysis probe

For *in vivo* delivery determinations, brain microdialysis probes were inserted into the hypothalamus of the rat brain. Artificial CSF solution containing BPA (25 ppb) was perfused through the probe at a constant flow rate by the microinjection pump. After a 60 min stabilization period, the dialysate (C_d) and perfusate (C_p) concentrations of BPA were determined by HPLC. The *in vivo* delivery of BPA across the microdialysis probe was calculated using equation (2).

Blood sampling and plasma pretreatment for HPLC analysis

In order to monitor the BPA level in rat plasma, a simple HPLC fluorescence detection method was employed. For oral BPA administration, blood was drawn at every 30 min intervals for 4 h and followed by every 60 min intervals for the next 4 h. For i.v. administration, blood was drawn at 5, 10, 15, 30, 50, 75, 105, 135, 165, 195, 225, 270, 330, 390 and 450 min after administration. Blood collected before BPA administration was used as a control in both cases. After mixed with EDTA in glass test tube, blood samples were centrifuged at 1000*g* for 10 min to separate plasma and then the plasma samples were kept frozen (−10°C) before analysis.

To a 50 μL portion of rat plasma 100 μL of 0.2 M HCl were added and mixed well, then the mixture was extracted with 900 μL of chloroform by vortex-mixing for 1 min. After centrifugation at 1000*g* for 15 min, the organic layer was transferred into a screw-capped reaction vial and evaporated to dryness under nitrogen gas stream. To the residue, fluorescent labeling reagent was added and the derivatization reaction was carried out as described above. Then a 10 μL portion of the resultant solution was injected onto the HPLC.

Method validation

The intra-day and inter-day assays of BPA (four replicates) at concentrations of 2.0 and 5.0 ppb in rat microdialysates and 0.5 and 2.0 ppm in rat plasma were performed on the same day and on four sequential days, respectively. The accuracy (percentage recovery) was calculated from the nominal concentration (C_{nom}) and the mean value of the observed concentration (C_{obs}) as follows [equation (3)]:

$$\text{Recovery } (\%) = (C_{obs}/C_{nom}) \times 100 \quad (3)$$

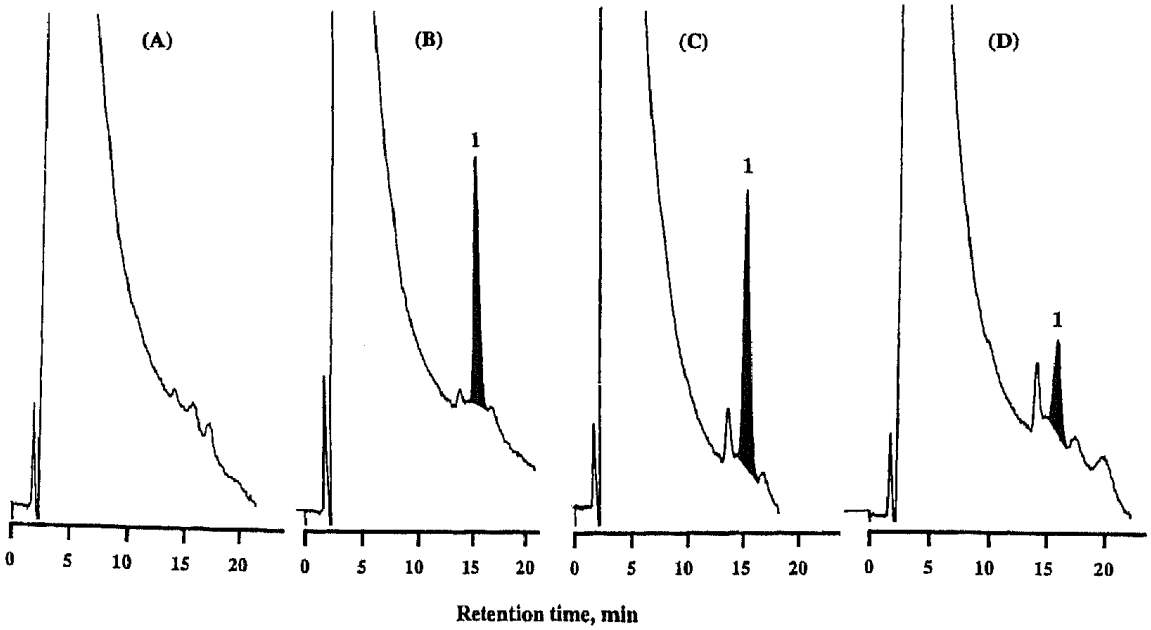

Figure 2. Typical chromatograms of rat brain microdialysate samples obtained from a single i.v. administration of BPA (10 mg/kg). (A) Sample collected before administration; (B) sample spiked with 5.0 ppb PBA; (C) sample containing BPA (5.6 ppb) collected 20 min after BPA administration; (D) sample containing BPA (1.1 ppb) collected 180 min after BPA administration. **1**, BPA.

The precision (relative standard deviation, RSD) was calculated from the observed concentrations as follows [equation (4)]:

$$\text{RSD (\%)} = [\text{standard deviation (SD)}/C_{obs}] \times 100 \quad (4)$$

Oral bioavailability

The oral bioavailability (F) of BPA was calculated from the doses and respective values of area under the concentration time curve (AUC) following i.v. and oral administrations, according to equation (5) (Upmeier *et al.*, 2000):

$$F = \frac{AUC_{oral} Dose_{i.v.}}{Dose_{oral} AUC_{i.v.}} \quad (5)$$

The AUC was calculated by numeral integration using a linear trapezoidal formula and extrapolation to infinite time based on a monoexponential equation (Yamaoka *et al.*, 1978).

RESULTS AND DISCUSSION

The column switching HPLC fluorescence detection method was applied to the determination of BPA in rat brain microdialysates. A column switching technique was employed as an on-line clean-up procedure for removing excess fluorescent labeling reagent after the derivatization reaction. Isocratic separation of BPA derivative from interference peaks in the brain microdialysate was achieved with an optimal mobile phase composed of acetonitrile–0.1 M acetate buffer (pH 3.0)–methanol = 35:10:55 (v/v). The retention time of BPA was about 15.5 min on the separation column. Calibration curves obtained from rat brain dialysates spiked with BPA over a concentration ranging from 1.0 to 10 ppb showed good linearity (Table 1). A simple HPLC fluorescence detection method with one-step liquid–liquid extraction procedure was performed for determination of BPA in rat plasma. By using the mobile phase of acetonitrile–H_2O–methanol–tetrahydrofuran (55:10:35:2.5, v/v) at a flow rate of 0.15 mL/min, BPA could be completely separated from interference peaks on a semi-micro column. The retention time under this condition was about 12 min. Good linearity was obtained from rat plasma spiked with BPA ranging from 0.3 to 10.0 ppm and BPA could be detected at 4.6 ppb [signal-to-noise ratio (S/N) = 3]. These data are also shown in Table 1. Figure 2 showed the representative chromatograms obtained from blank rat brain microdialysate [Fig. 2(A)], brain microdialysate spiked with 5.0 ppb of BPA [Fig. 2(B)], brain microdialysate containing BPA (5.6 ppb) collected 20 min after BPA administration [Fig. 2(C)] and brain microdialysate containing BPA (1.1 ppb) collected 180 min after BPA administration [Fig. 2(D)]. Typical chromatograms obtained from rat plasma blank [Fig. 3(A)], plasma spiked 7.5 ppm of BPA [Fig. 3(B)] and plasma containing BPA (2.4 ppm) collected 5 min after a single i.v. administration of 10 mg/kg of BPA [Fig. 3(C)] are shown in Fig. 3.

The validation of the column switching HPLC for rat brain microdialysate and simple HPLC fluorescence detection for rat plasma assay were also performed. As shown in Table 2, accuracy (percentage recovery) was

Table 1. Linearity, LOD of BPA in different volumes of rat brain dialysates and plasma

Sample volume (μL)	BPA nominal concentration	Equation	Correlation coefficient (r)	LOD[a] (ppb)
In rat brain dialysate				
20	1.0–10 ppb	$y = 1.044x + 0.263$	0.996	0.7
30	1.0–10 ppb	$y = 1.615x + 0.186$	0.998	0.6
60	1.0–10 ppb	$y = 3.329x + 0.836$	0.998	0.3
In rat plasma				
50	0.3–5.0 ppm	$y = 12.893x + 0.1065$	1.000	4.6

[a] LOD, limit of detection at a signal-to-noise ratio of 3.

Table 2. Intra- and inter-day accuracy and precision for BPA assay in rat brain microdialysates and plasma

Nominal concentration (ppb)	Observed concentration (ppb)	Intra-day assay ($n = 4$)		Inter-day assay ($n = 6$)
		Recovery (%)	% RSD	% RSD
In rat brain microdialysate				
2.0	1.95 ± 0.24	97.6	10.9	6.9
5.0	4.89 ± 0.34	97.8	7.0	5.5
In rat plasma				
500	497.5 ± 10.6	99.5	2.2	6.3
2000	2020 ± 21.9	101.0	1.0	5.6

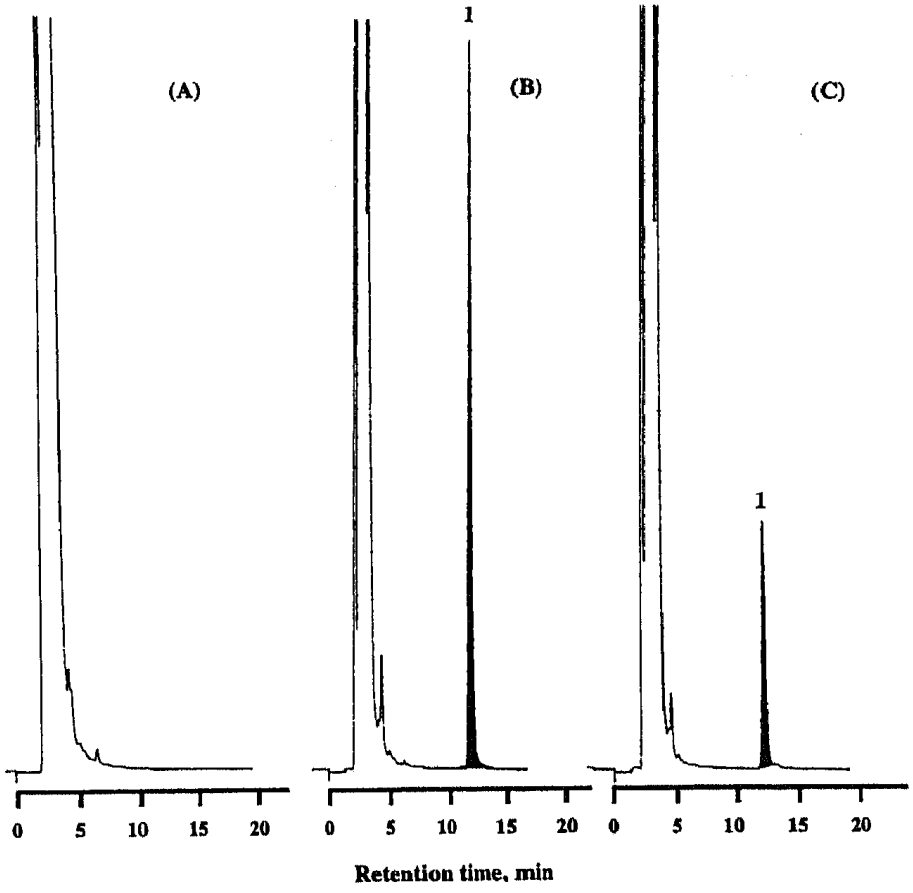

Figure 3. Typical chromatograms of rat plasma samples obtained from a single i.v. administration of BPA (10 mg/kg). (A) Plasma collected before administration; (B) plasma spiked with 7.5 ppm PBA; (C) sample containing BPA (2.4 ppm) collected 5 min after BPA administration. **1**, BPA.

higher than 97.6 and precision (% RSD) was less than 10.9 obtained from intra-day assay; precision (% RSD) for inter-day assay was less than 6.9.

The recovery of microdialysis probe could be affected by certain factors, such as the material of the probe, the perfusion rate and temperature. Since the destination compound, BPA, is a monomer used in the manufacture of polycarbonate, use of microdialysis probes made of polycarbonate were avoided. *In vitro* recovery and delivery were estimated at different perfusion rate (1.0 and 2.0 μL/min). Higher recoveries were obtained at a perfusion rate of 1.0 μL/min, thus it was adopted for the following experiment (Table 3). Generally, similar values were observed from *in vitro* recovery and delivery, as shown in Table 3. Subsequently, the *in vivo* recovery of BPA was calculated to be 17.2% as follows:

$$\text{Recovery}_{in\ vivo} = \text{Delivery}_{in\ vivo} \times (\text{Recovery}_{in\ vitro}/\text{Delivery}_{in\ vitro}) \quad (6)$$

The proposed method was applied to the determination of BPA in rat brain microdialysate and plasma after a single i.v. or oral administration. The brain has its special regions (such as the hypothalamus) responsible for expressing ER and uptake of estrogens in a variety of vertebrates (Guerriero *et al.*, 2000; Osterlund *et al.*, 2000). Hence, the microdialysis probe was inserted into hypothalamus of the rat brain. BPA microdialysate concentrations were converted to BPA concentrations in brain extracellular area. Figure 4 shows the time courses of BPA concentrations in rat brain and plasma after a single i.v. administration (10 and 20 mg/kg). Concentrations of BPA in brain increased rapidly, and the maximum concentration appeared within 20 min of

Table 3. Recovery and delivery of BPA from the microdialysis probe

Flow rate (μL/min)	Recovery (%)	Delivery (%)
In vitro		
1.0	17.6 ± 2.4	18.1 ± 1.6
2.0	8.6 ± 0.1	9.8 ± 5.5
In vivo		
1.0	—	17.7 ± 2.2

Data are expressed as mean ± SD ($n = 5$).

administration, indicating quick penetration of the BBB by BPA. Thereafter, BPA concentrations decreased rapidly in plasma and brain. However, BPA could still be detected 8 h after administration both in rat plasma and brain microdialysate. The ratio of AUC of BPA in brain to that in plasma for 10 and 20 mg/kg was observed to be 3.8%.

Figure 5 shows the time course of BPA concentrations in brain and plasma after oral administration (200 mg/kg, i.v.) to rats. The initial absorption of BPA was different in individual animals. The same result was also obtained in brain. The maximum concentration of BPA in brain (19.70 ppb) appeared within 60 min of administration, while in rat plasma, the peak concentration of BPA (1.6 ppm) appeared at 30 min after administration. After 5 h of administration, BPA could not be detected in brain microdialysate. The ratio of AUC of BPA in brain to that in plasma was about 3.0%, which reached a similar order of magnitude but a little lower than that of i.v. administration. Based on AUC values of BPA in rat plasma, the oral bioavailability of BPA was calculated as a mean value of 4.4% for the 200 mg/kg oral dose. This is in good agreement with the result in the literature that the oral bioavailability of BPA for the dose of 100 mg/kg was

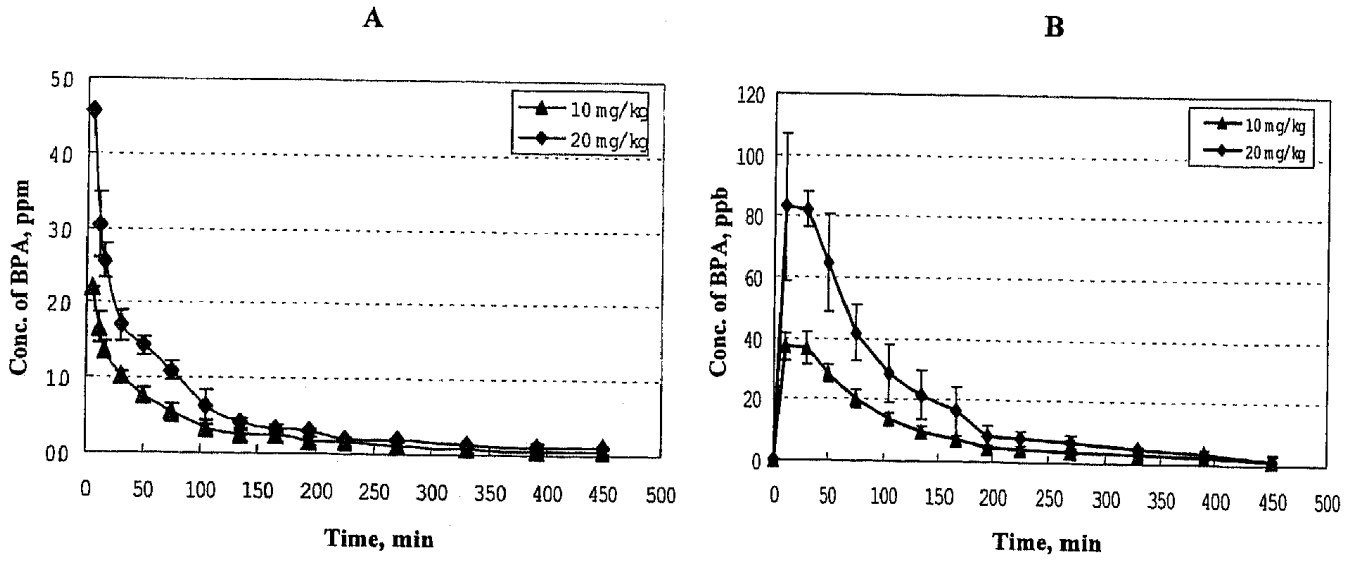

Figure 4. Time courses of concentrations of BPA in brain and plasma after a single i.v. administration (10 and 20 mg/kg) in rat. (A) Plasma; (B) brain. Data are shown as mean ± SD ($n = 3$) for each dose.

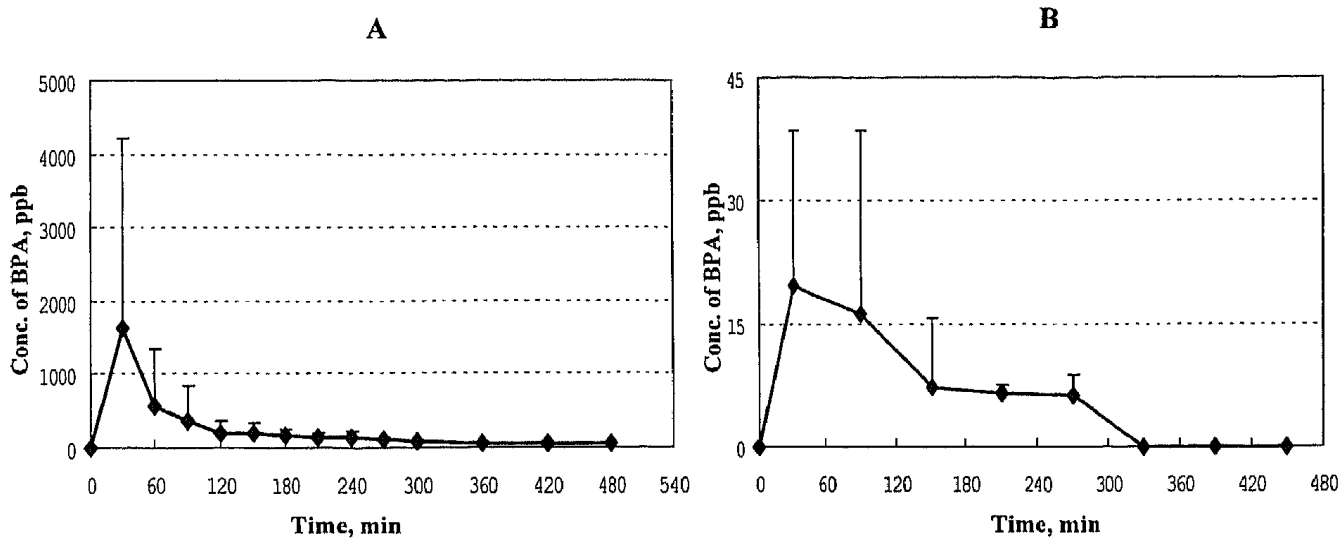

Figure 5. Time courses of concentrations of BPA in brain and plasma after a single oral administration (200 mg/kg) in rat. (A) Plasma; (B) brain. Data are shown as mean ± SD ($n = 3$).

observed to be 5.6%. There is a tendency that the higher dose being used will result in the lower oral bioavailability (Upmeier *et al.*, 2000). Pharmacokinetic parameters of BPA in rat plasma and brain are summarized in Table 4.

Glucuronidation is the major metabolic pathway of BPA to form the more water-soluble BPA-glucuronide in animal experiments (Pottenger *et al.*, 2000). The UDP-glucuronosyltransferase (UGT) isoform (UGT2B1) has been proved to be responsible for catalyzing glucuronidation of BPA (Yokota *et al.*, 1999). UGTs are mainly located in liver and, because of the effective glucuronidation pathway, BPA was removed rapidly from the body circulation. Recently, extrahepatic tissues including kidney, gastrointestinal tract and brain have been identified as expressing UGT isoforms (King *et al.*, 2000). This might be one of the explanations of the rapid decreasing of BPA concentration in extracellular areas of the brain after i.v. administration.

To the best of our knowledge, until now no paper has been published describing the analysis of BPA in rat brain by using microdialysis, mainly due to the lack of a sensitive analytical method. Compared with other biological sample pretreatment procedures prior to HPLC or GC (Yoo *et al.*, 2000; Miyakoda *et al.*, 2000), the microdialysis simplifies sample pretreatment, as well as minimizing biological fluid loss during sampling. Therefore it is widely applied in the monitoring of drugs in organs, blood vessels and brain tissues (de Lange *et al.*, 1997).

In this study, doses are much higher than the value of acceptable daily intake (ADI) of BPA of 0.05 mg/kg/day (Environmental Protection Agency, 2000). Nevertheless, the main purpose of this study is to clarify whether BPA could pass through the BBB. The positive result obtained could be regarded as one of the properties of BPA whatever the dosage amount is. Since, from the results of this study, dose dependence of BPA was observed in rat brain between 10 and 20 mg/kg i.v. administration, the extrapolation is that even trace levels of BPA from environmental exposure could penetrate the BBB. It has been reported that exposure to BPA affects behavior in

Table 4. Pharmacokinetic parameters of each dose studied

Dose (mg/kg)	AUC (μg.min/mL)[a]	C_{max} (ppb)[b]	T_{max} (min)[c]
i.v. administration			
Plasma 10	141.30 ± 22.50	2200.0 ± 20.0	5
20	258.73 ± 23.67	4600.0 ± 44.0	5
Brain 10	5.40 ± 0.62	37.3 ± 4.5	10
20	9.90 ± 1.50	82.9 ± 24.3	10
Oral administration (200)			
Plasma	128.63 ± 105.44	1623.7 ± 2599.3	30
Brain	3.83 ± 2.56	19.7 ± 18.7	30

[a] AUC, area under the concentration–time curve.
[b] C_{max}, maximum concentration (mean ± SD, $n = 3$).
[c] T_{max}, time of maximum concentration.

experimental animals (Farabollini *et al.*, 1999); our results hypothesize that BPA penetrating the BBB might interfere with and causes abnormalities in the CNS.

CONCLUSIONS

An effective method for determination of BPA in rat brain by coupling microdialysis and HPLC-fluorescence detection has been developed. Microdialysis technique simplified sample clean-up steps compared with other conventional sample pretreatment procedures. The proposed method is sensitive and can detect BPA at 0.3 ppb in rat brain microdialysate and 4.6 ppb in rat plasma with an S/N of 3.

In this study, BPA could be determined in rat brain microdialysate as well as in plasma after single oral or i.v. administration, and thus it was proved that BPA penetrates the BBB. However, the concentration of glucuronidated BPA metabolite in rat brain and plasma remains unknown in this study and needs to be investigated further.

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