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Simultaneous blood and brain microdialysis in a free-moving mouse to test blood-brain barrier permeability of chemicals

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ABSTRACT

Neurotoxic chemicals that pass through the blood-brain barrier (BBB) can influence brain function. Efficient methods to test the permeability of the BBB to specific chemicals would facilitate identification of potentially neurotoxic agents. We report here a simultaneous blood and brain microdialysis in a free-moving mouse to test BBB permeability of different chemicals. Microdialysis sampling was conducted in mice at 3–5 days after implantation of a brain microdialysis probe and 1 day after implantation of a blood microdialysis probe. Therefore, mice were under almost physiological conditions. Results of an intravenous injection of lucifer yellow or uranine showed that the BBB was functioning in the mice under the experimental conditions. Mice were given phenyl arsenic compounds orally, and concentration-time profiles for phenyl arsenic compounds such as diphenylarsinic acid, phenylarsonic acid, and phenylmethylarsinic acid in the blood and brain dialysate samples were obtained using simultaneous blood and brain microdialysis coupled with liquid chromatography-tandem mass spectrometry. Peak area-time profiles for linalool and 2-phenethyl alcohol (fragrance compounds or plant-derived volatile organic chemicals) were obtained using simultaneous blood and brain microdialysis coupled with gas chromatography-mass spectrometry in mice given lavender or rose essential oils intraperitoneally. BBB function was confirmed using lucifer yellow in these mice, and results indicated that the phenyl arsenic compounds, linalool and 2-phenethyl alcohol, passed through the BBB. The present study demonstrates that simultaneous blood and brain microdialysis in a free-moving mouse makes it possible to test the BBB permeability of chemicals when coupled with appropriate chemical analysis methods.

1. Introduction

The blood-brain barrier (BBB) restricts transport of chemicals from the blood into the brain. The primary basis of the BBB is endothelial cells lining the brain capillaries [1]. Given that the brain capillary endothelial cells are highly sealed together by tight junctions, the brain endothelial cells restrict movement of materials including ions, nutrients, hormones and endogenous solutes, larger molecules such as proteins, cells and xenobiotics between the blood and the brain interstitial fluid [2]. Limited kinds of materials and chemicals can pass through the BBB principally via passive diffusion (or the lipid-mediated transport mechanism) or carrier (or transporter)-mediated specific transport systems [2–4]. Chemicals can influence brain function after passing through the BBB. Assessment of neurotoxicity requires information regarding the toxicokinetics of chemicals in both the brain and blood. Conventional methods measuring concentrations of chemicals in blood/plasma and brain tissue are resource-intensive in terms of animals, time, and costs. Alternative methods that allow efficient testing of the permeability of the BBB to chemicals would facilitate exploration of potentially neurotoxic chemicals among the large number of chemicals that have not been studied. A variety kinds of in vivo methods have been developed to assess the BBB transport of chemicals. The methods include the brain uptake index method, the intravenous injection method, the in situ brain perfusion method, the brain efflux index method, the percentage of the injected dose method, the cerebrospinal fluid sampling method, and the positron emission tomography method [5].

Microdialysis is a well-established technique for in vivo measurement of exogenous and endogenous substances in both plasma and tissues [6]. In terms of toxicokinetic studies of chemicals in the brain, intracerebral microdialysis is the only technique that offers the possibility of continuously monitoring the BBB permeability of unbound chemicals in animals tested under physiological conditions [7].

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Microdialysis allows continuous collection of a high number of samples over short time periods in the same animal, making determination of concentration–time profiles with high time resolution possible. In addition, microdialysis allows sampling from multiple sites of the same animal [8–10]. Simultaneous and continuous sampling from multiple sites is made possible without affecting toxicokinetic profiles by implanting more than one probe in the same animal. Simultaneous and continuous sampling from multiple sites in an animal can reduce the number of animals needed for the toxicokinetic studies. As a microdiaysis probe consists of a semipermeable membrane with a molecular mass cut-off ranging from 5000 to 50,000 Da, the dialysis membrane is only permeable to protein-unbound small molecules. Thus, microdialysis samples do not require purification prior to chemical analysis [11]. This is another advantage of using microdialysis to conduct toxicokinetic studies.

One of the disadvantages of microdialysis is that probe implantation may damage the BBB [5]. Thus, microdialysis studies that examine the permeability of the BBB need to wait for recovery of brain damage after implantation of the brain probe. In addition, because anesthetic agents affect the function of the BBB [12], microdialysis sampling should be done in non-anesthetized animals to allow for examination of BBB permeability under physiological conditions.

We report here a method for simultaneous microdialysis sampling from the blood and brain in a free-moving mouse. The method involved tested fluorescent chemicals that barely permeate the BBB [13–15]. Then, we examined whether the method was applicable for testing BBB permeability of chemicals by also performing chemical analysis methods. Whether or not phenyl arsenic compounds and fragrance compounds (plant-derived volatile organic chemicals [VOCs]) permeate the BBB was tested using simultaneous blood and brain microdialysis coupled with liquid chromatography-tandem mass spectrometry (LC/MS/MS) or gas chromatography-mass spectrometry (GC/MS) in mice in which the BBB function was examined using the fluorescent chemicals.

2. Materials and methods

2.1. Subjects

Male ICR strain mice (Clea Japan, Tokyo, Japan) aged 10–20 weeks and weighing 40–50 g at the start of experiments were used. Mice were housed in aluminum cages (3 mice/cage) with a stainless-steel mesh top and containing wood shavings as bedding material. Commercial solid food (Clea Japan) and tap water were provided *ad libitum.* The cages were placed in a room artificially illuminated by fluorescent lamps on a 12 -h light/dark cycle (light period: 07:00−19:00) and a room temperature of 25 ± 1 °C. All experiments were conducted during the light phase.

The Committee for Experimental Animals of the National Institute for Environmental Studies, Japan, approved all experiments.

2.2. Agents

Phenyl arsenic compounds including diphenylarsinic acid (DPAA), phenylarsonic acid (PAA), and phenylmethylarsinic acid (PMAA), and their stable isotope-labelled internal standards were obtained from Hayashi Pure Chemicals Ind. Ltd. (Osaka, Japan). Essential oils of lavender and rose were provided by Maggie Tisserand Ltd. (Brighton, UK). Lucifer yellow, uranine, mannitol, linalool, and 2-phenethyl alcohol were purchased from Nakalai Tesque (Kyoto, Japan). Lucifer yellow, uranine, and mannitol were dissolved in physiological saline (0.9 % NaCl solution), and administered intravenously. Phenyl arsenic compounds were dissolved in pure water by adjusting the pH to around 7 via the addition of diluted ammonium hydroxide solution; these compounds were administered orally. Essential oils were diluted in olive oil (Nakalai Tesque) and administered intraperitoneally. The administration volume was 1 mL/100 g body weight for all agents except for the 25 % mannitol solution.

2.3. Microdialysis probe implantation

Mice were anesthetized using 50 mg/kg pentobarbital (Somnopentyl®, Kyoritsu Seiyaku Co., Tokyo, Japan) with 4 mg/kg carprofen (Rimadyl®, Zoetis Japan, Tokyo, Japan) and fixed in a stereotaxic apparatus equipped with a mouse adapter (David Kopf, CA, USA). As previously reported [16], a brain microdialysis probe (D-I-6-02, cutoff 50,000 Da, Eicom, Kyoto, Japan) was stereotaxically implanted into the striatum (AP: +0.1 mm; L: +2.0 mm; V: -2.8 mm from bregma) according to a mouse brain atlas [17] and fixed with dental cement. After surgery, the animals were housed in individual cages and left to recover.

Two to 4 days after implantation of the brain probe, mice were anesthetized using 50 mg/kg pentobarbital with 4 mg/kg carprofen, and the blood microdialysis probe (TP-145-03, cutoff 50,000 Da, Eicom) was implanted in the right jugular vein. The blood probe was passed under the skin to the outside of the back of the mice and fixed using an adhesive bandage. Mice were placed in a cage for the microdialysis experiment immediately after surgery, and the blood probe was connected to a syringe pump. The blood probe was perfused with Ringer's solution (147 mM Na^+, 4 mM K^+, and 2.3 mM Ca^{2+}, 155.6 mM Cl^-) at a flow rate of 0.3 μL/min. Food and water were available *ad libitum* in the cage.

2.4. Microdialysis experiments

Microdialysis experiments were conducted 1 day after implantation of the blood probe. The microdialysis system consisted of a syringe pump (ESP-64, Eicom) and a fraction collector (EFC-82, Eicom) with an electronic cooler (EFR-82, Eicom). The brain and blood probes were connected to the syringe pump, and the probes were perfused with Ringer's solution at a flow rate of 2 μL/min. Microdialysis samples from the blood and brain were collected every 25 min. The collected samples were kept at 4 °C during the sampling and kept at −80 °C immediately after the end of the sampling.

2.5. Fluorescence measurement

The fluorescence intensity of 40 μL dialysate samples was measured using a multifunctional microplate reader FLUO star Optima (BMG Labtechnologies, Offenburg, Germany) with Ex405 nm and Em 544 nm filters for lucifer yellow and Ex485 nm and Em 520 nm filters for uranine.

2.6. Analysis of phenyl arsenic compounds by LC/MS/MS

Phenyl arsenic compounds in microdialysis samples were analyzed using LC/MS/MS as reported previously [18]. In brief, 15 μL of stable, isotope-labeled internal standard mixture, ^{13}C-DPAA, ^{13}C-diphenylmethylarsine oxide (^{13}C-DPMAO), ^{13}C - -PMAA, ^{13}C-phenyldimethylarsine oxide (^{13}C-PDMAO), and ^{13}C-PAA (2 ppb each), were added to 15 μL of each fraction of dialysate sample.

Agilent 1200 Series (Agilent Technologies, Santa Clara, CA) coupled with the 4000 QTRAP LC–MS/MS System (AB Sciex, Framingham, MA) were used in this study. Chromatographic separation was achieved using an Atlantis T3 column(150 × 2.1 mm inner diameter, 3-μm thickness, Waters Corporation, Milford, MA) at 40 ℃. Four μL of each sample was injected into the column and separated using a linear gradient of 0.1 % formic acid in water [A] with methanol [B] as follows: 80 to 10 % [A]: 20–90% [B] (7.5 min), 10 % [A]: 90 % [B] (2-min hold). The flow rate was maintained at 0.2 mL/min. Electrospray ionization was performed in the positive ionization mode. Detailed analytical conditions are provided in Table 1 in the supplementary material.

2.7. Analysis of fragrance compounds (plant-derived VOCs) by GC/MS

The solid phase micro extraction (SPME) method was used to extract fragrance compounds (plant-derived VOCs) from the dialysate samples. The SPME fiber was coated with 65 μm polymethylsiloxane/divinylbenzene (PDMS/DVB) (Sigma-Aldrich, Tokyo, Japan). The SPME fiber was incubated in a glass tube containing the dialysate sample at 30 °C for 15 (lavender oil sample) or 30 (rose oil sample) min.

GC/MS analysis was performed using an MStation JMS-700KII mass spectrometer (JEOL, Tokyo, Japan) equipped with a 6890 N gas chromatograph (GC; Agilent Technologies, Santa Clara, CA) [19]. The GC was fitted with a fused silica capillary column (DB-5MS; 0.25 mm inner diameter, 30 m) coated with 5% phenyl methyl silica. The column temperature was increased from 40 °C to 280 °C at 5 °C/min increments. The SPME fiber was inserted into the GC at 250 °C for 1 min in the splitless mode. The injection and separator temperatures were 250 °C and 280 °C, respectively. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Mass spectral analysis was performed in the electron impact ionization mode. Linalool and 2-phenethyl alcohol were confirmed by the retention times and mass spectra of authentic standard compounds obtained using the GC/MS analysis.

3. Results

3.1. Validation of the simultaneous blood and brain microdialisys technique as a method for testing the BBB permeability of chemicals

Microdialysis experiments were conducted in mice 3–5 days after brain probe implantation and 1 day after blood probe implantation. The BBB function in the mice was examined using a fluorescent dye lucifer yellow that barely passes through the BBB. When 25 mg/kg lucifer yellow was intravenously administered to mice, fluorescence intensity in blood dialysate samples significantly increased immediately after injection, whereas fluorescence intensity in the brain dialysate samples barely changed (Fig. 1(a)). In order to validate this finding, the BBB function in different mice was tested using another fluorescent dye uranine that also barely passes through the BBB. When 10–25 mg uranine was administered intravenously, fluorescence intensity in blood dialysate samples significantly increased in a dose-dependent manner immediately after injection, but fluorescence intensity in brain dialysate samples barely changed (Fig. 1(b)). We next examined whether these results were due to the BBB function. We used hyperosmotic mannitol to opens the BBB. Fluorescence intensity in brain dialysate samples barely

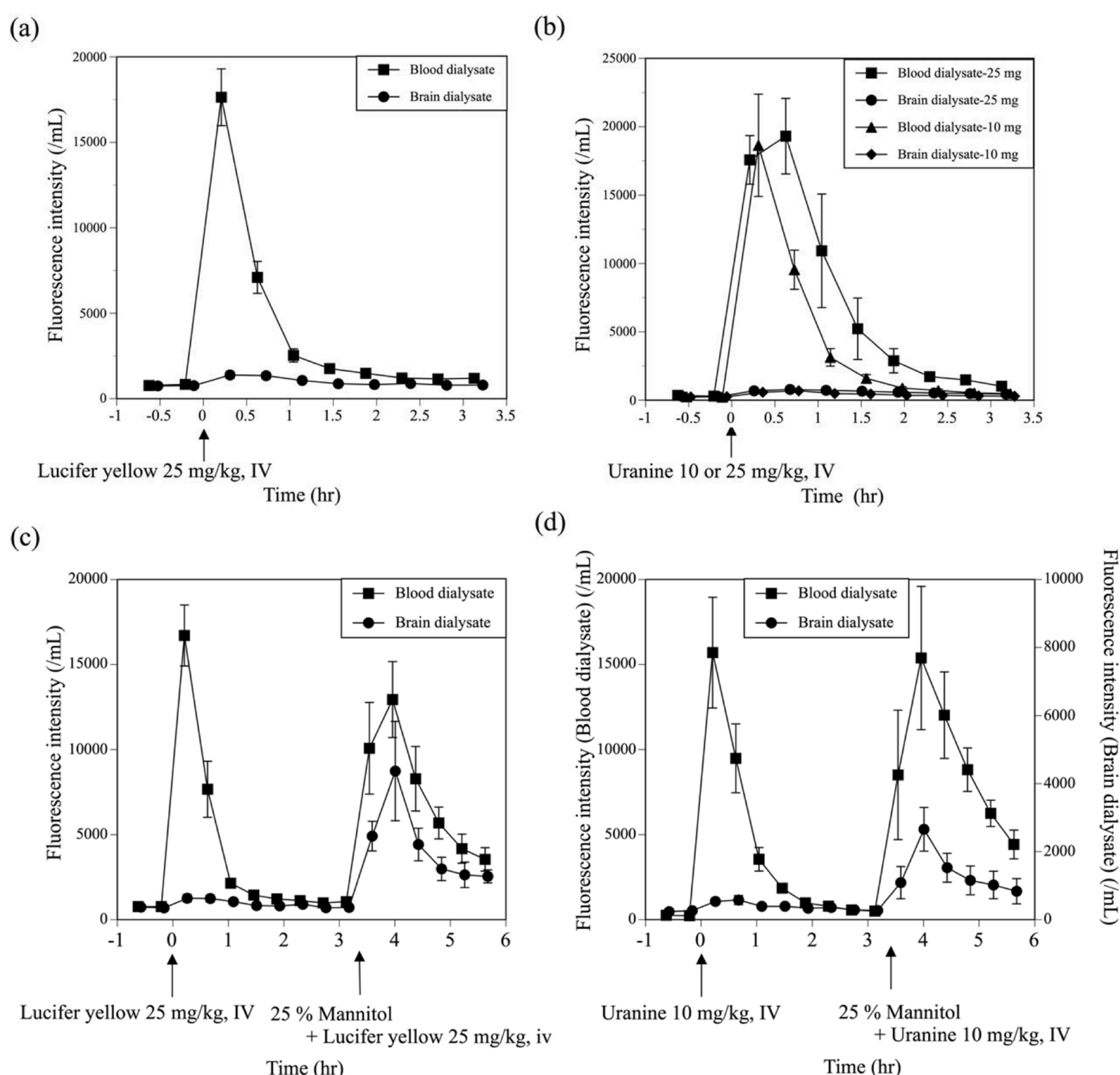

Fig. 1. (a) Alterations of fluorescence intensity in blood and brain microdialysis samples after intravenous (IV) administration of (a) 25 mg/kg lucifer yellow or (b) 10 or 25 mg/kg uranine. (c) Alterations of fluorescence intensity in blood and brain dialysate samples after single IV administration of 25 mg/kg lucifer yellow, 3 h later followed by IV co-administration of 25 mg/kg lucifer yellow with 1 mL of 25 % mannitol solution. (d) Alterations of fluorescence intensity in blood and brain dialysate samples after single IV administration of 10 mg/kg uranine, 3 h later followed by IV co-administration of 10 mg/kg uranine with 1 mL of 25 % mannitol solution. Symbols indicate mean values and vertical lines denote standard errors of the mean (SEM). Number of mice: (a) N = 7, (b) N = 2-4, (c) N = 3, (d) N = 3.

changed after single administration of 25 mg/kg lucifer yellow or 10 mg/kg uranine. However, in the same mice, fluorescence intensity in the brain dialysate samples remarkably increased after co-administration of those fluorescent chemicals with 1 mL of 25 % mannitol solution (Fig. 1 (c), (d)). Thus, no significant changes in fluorescence intensity in the brain dialysates after single administration of lucifer yellow or uranine demonstrated that the BBB was normally functioning.

3.2. The simultaneous blood and brain microdialisys coupled with LC/MS/MS for testing the BBB permeability of phenyl arsenic compounds

We examined whether the simultaneous blood and brain microdialysis was applicable to test the BBB permeability of phenyl arsenic compounds such as DPAA, PAA and PMAA. LC/MS/MS was used to measure phenyl arsenic compounds in dialysate samples.

DPAA at 12. 5 mg/kg was administered to a mouse after confirmation of the BBB function using lucifer yellow (Fig. 2(a)). After the DPAA administration, DPAA was detected in both blood and brain dialysate samples obtained from the mouse using LC/MS/MS analysis (Suppl.

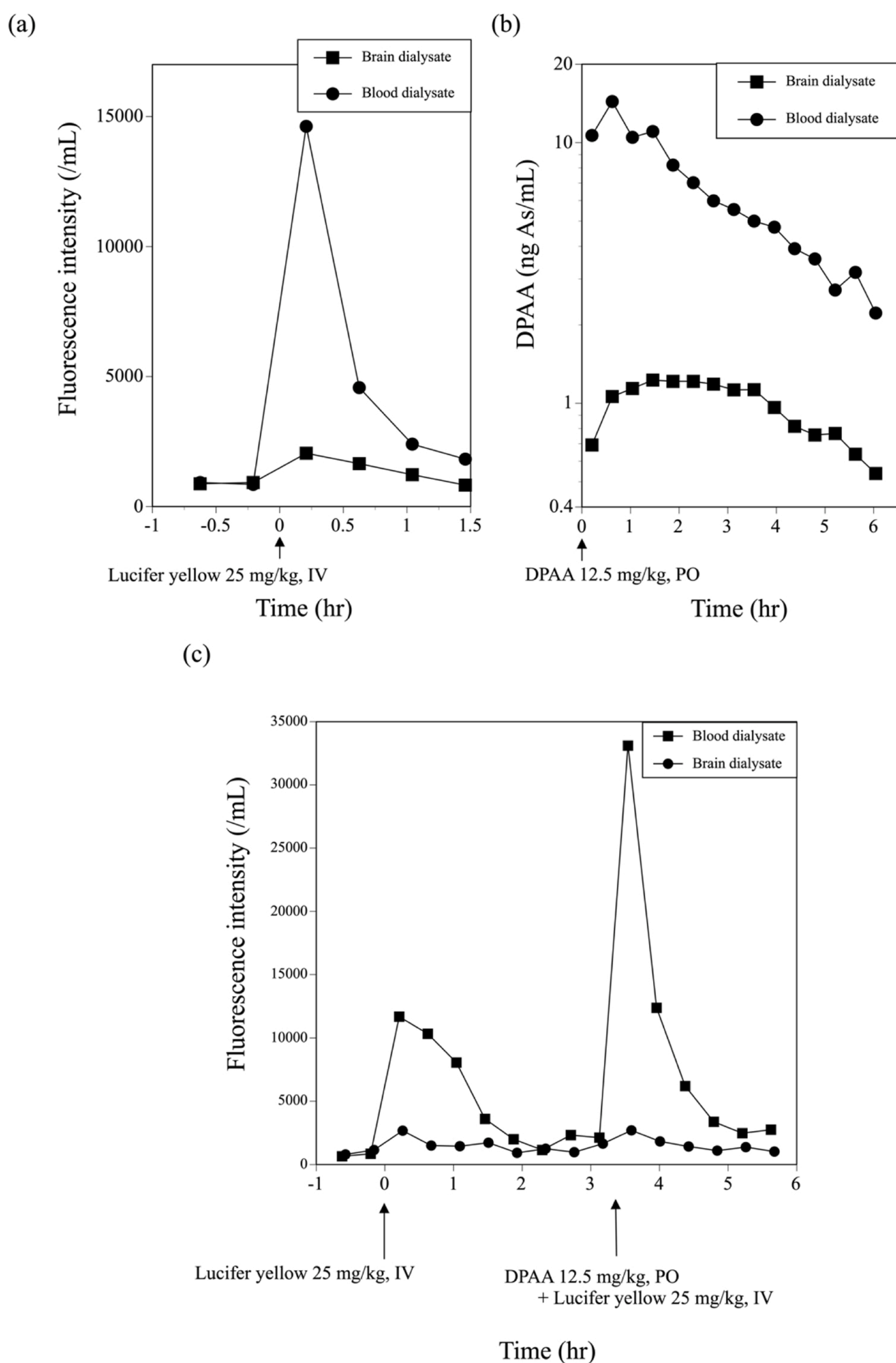

Fig. 2. (a) Alterations of fluorescence intensity in blood and brain dialysate samples after intravenous (IV) administration of 25 mg/kg lucifer yellow. (b) Alterations of concentrations of diphenylarsinic acid (DPAA) in blood and brain dialysate samples after oral (PO) administration of 12.5 mg/kg DPAA. DPAA was administered 3 h after the lucifer yellow administration. (c) Alterations of fluorescence intensity in blood and brain dialysate samples after single IV administration of 25 mg/kg lucifer yellow, 3 h later followed by co-administration of 25 mg/kg lucifer yellow (IV) with 12.5 mg/kg DPAA (PO). Number of mice: (a), (b) N = 1, (c) N = 1.

Fig. 1). The concentration-time profiles of DPAA in the blood and brain dialysate samples are shown in Fig. 2(b). DPAA appeared immediately after oral administration in blood dialysate samples. DPAA was also detected in brain dialysate samples within 25 min after oral administration. The blood and brain concentrations of DPAA rapidly reached maximum levels, followed by a decrease in concentration over time. The concentration of DPAA in brain samples was about 1 order of magnitude lower than that in blood samples. Then, we examined whether 12.5 mg/kg DPAA would impair the BBB function. After confirming that fluorescence intensity in the brain dialysate samples barely changed after single administration of lucifer yellow, lucifer yellow was co-administered with 12.5 mg/kg DPAA. DPAA did not influence fluorescence intensity in the brain dialysate samples (Fig. 2(c)), suggesting that 12.5 mg/kg DPAA did not affect the BBB impermeability of lucifer yellow.

After confirmation of the BBB functions (Fig. 3(a), (d)), 12.5 mg/kg PAA or 12.5 mg/kg PMAA were orally administered to mice. LC/MS/MS analysis detected PAA and PMAA in both blood and brain dialysate samples (suppl. Fig. 2,3). Fig. 3 (b) and (e) show the concentration-time profiles of PAA and PMAA in blood and brain dialysate samples, respectively. PAA and PMAA appeared in blood dialysate samples immediately after oral administration. PAA and PMAA were also detected in the brain dialysate sample within 25 min after oral administration. PAA and PMAA rapidly reached maximum concentrations and then decreased in both blood and brain dialysate samples. The concentrations of PAA and PMAA in the brain samples were about 1 order of magnitude lower than those in the blood samples. Similar to DPAA, 12.5 mg/kg PAA and 12.5 mg/kg PMAA did not affect the BBB impermeability of lucifer yellow, respectively (Fig. 3(c), (f)).

3.3. The simultaneous blood and brain microdialisys coupled with GC/MS for testing the BBB permeability of fragrance compounds (plant-derived VOCs)

We next examined whether the simultaneous blood and brain microdialysis was applicable to test the BBB permeability of fragrance compounds (plant-derived VOCs). GC/MS was used to measure plant-derived VOCs in dialysate samples.

The BBB function of mice was first confirmed using lucifer yellow (Fig. 4(a), (c)). Then, 1600 mg/kg lavender or rose essential oils were administered intraperitoneally to the mice. A significant amount of linalool was detected by GC/MS analysis in both blood and brain dialysate samples after administration of the lavender essential oil (suppl. Fig. 4). A significant amount of 2-phenethyl alcohol was observed in both blood and brain dialysate samples after administration of the rose essential oil (suppl. Fig. 5). Fig. 4(b) shows the peak area-time profiles of linalool in blood and brain dialysate samples after administration of the lavender essential oil. Linalool was detected in blood dialysate samples immediately after the administration of the essential oils. Linalool also appeared in the brain dialysate sample immediately after administration

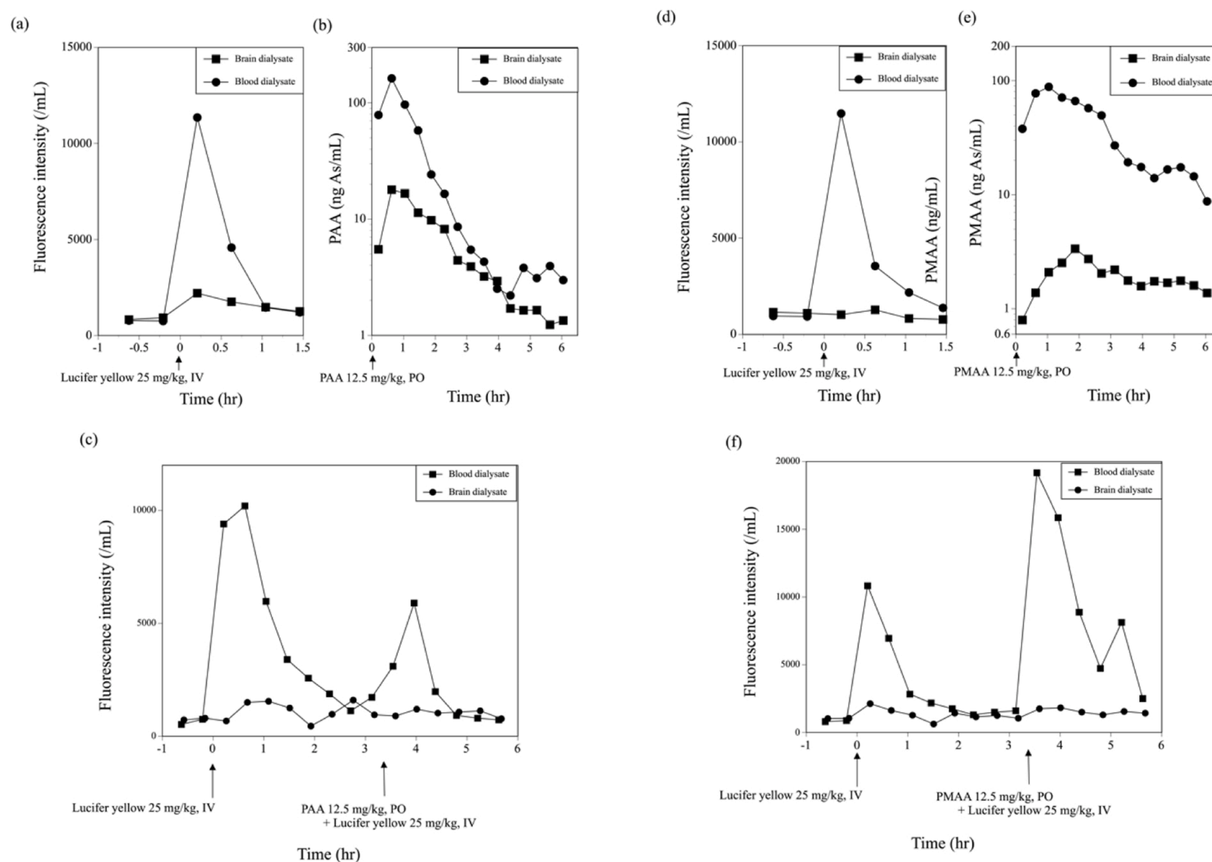

Fig. 3. (a), (d) Alterations of fluorescence intensity in blood and brain dialysate samples after intravenous (IV) administration of 25 mg/kg lucifer yellow. (b) Alterations of concentrations of phenylarsonic acid (PAA) in blood and brain dialysate samples after oral (PO) administration of 12.5 mg/kg PAA. PAA was administered 3 h after the lucifer yellow administration (a). (e) Alterations of concentrations of phenylmethylarsinic acid (PMAA) in blood and brain dialysate samples after PO administration of 12.5 mg/kg PMAA. PMAA was administered 3 h after the lucifer yellow administration (d). (c) Alterations of fluorescence intensity in blood and brain dialysate samples after single IV administration of 25 mg/kg lucifer yellow, 3 h later followed by co-administration of 25 mg/kg lucifer yellow (IV) with 12.5 mg/kg PAA (PO). (f) Alterations of fluorescence intensity in blood and brain dialysate samples after single IV administration of 25 mg/kg lucifer yellow, 3 h later followed by co-administration of 25 mg/kg lucifer yellow (IV) with 12.5 mg/kg PMAA (PO). Number of mice: (a), (b) N = 1, (d), (e) N = 1, (c) N = 1, (f) N = 1.

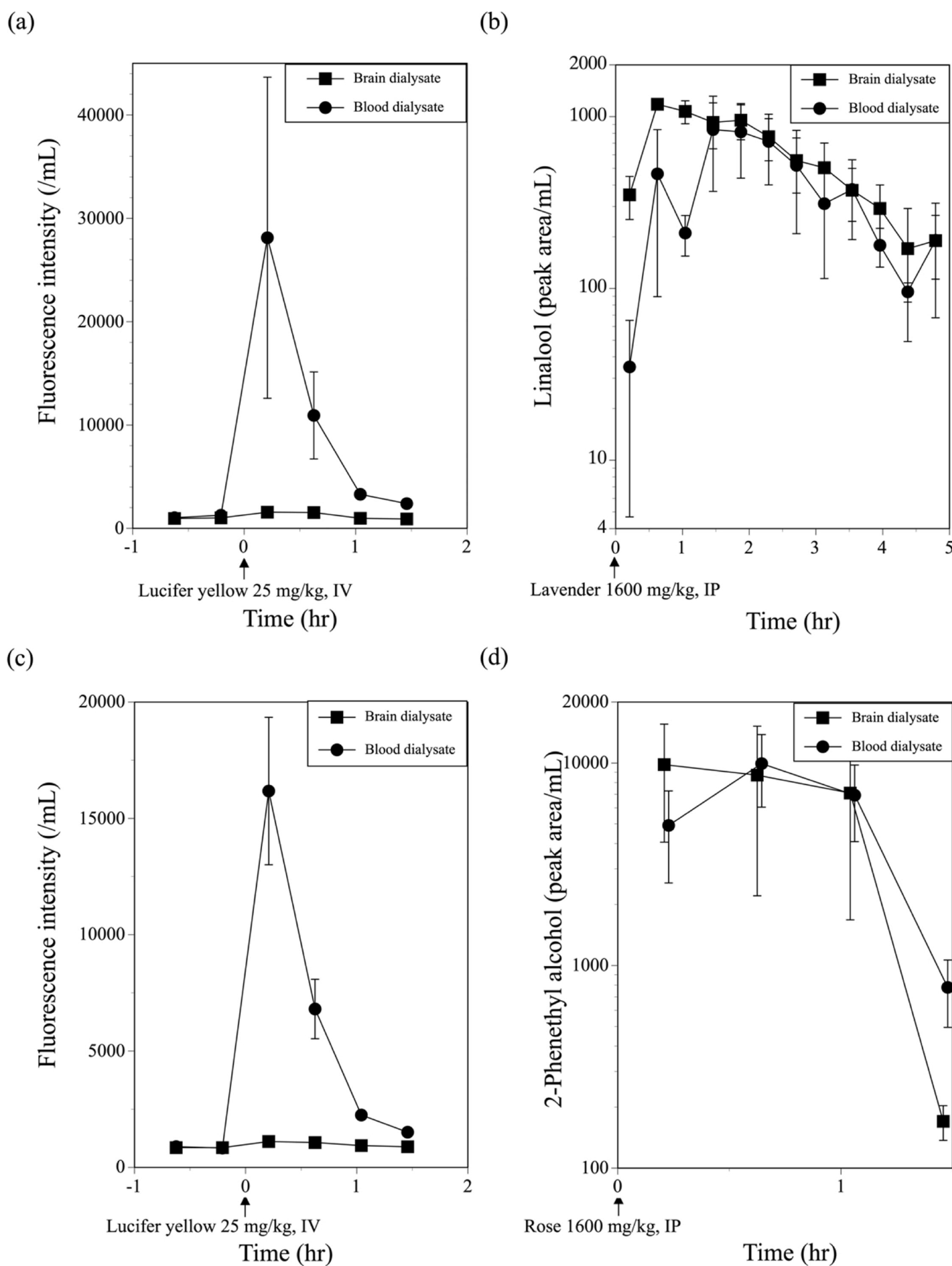

Fig. 4. (a), (c) Alterations of fluorescence intensity in blood and brain dialysate samples after intravenous (IV) administration of 25 mg/kg lucifer yellow. (b) Alterations of peak area of linalool in blood and brain dialysate samples after intraperitoneal (IP) administration of 1600 mg/kg lavender oil. Lavender oil was administered 3 h after the lucifer yellow administration (a). (d) Alterations of peak area of 2-phenethyl alcohol in blood and brain dialysate samples after intraperitoneal (IP) administration of 1600 mg/kg rose oil. Rose essential oil was administered 3 h after the lucifer yellow administration (c). Number of mice: (a), (b) N = 3, (c), (d) N = 4.

of the lavender essential oils. Peak areas of linalool early after administration were lower in the blood dialysate samples than in the brain dialysate samples, followed by comparable peak area-time profiles both samples. The concentration of unbound linalool in blood may be low during the early period. Fig. 4 (d) shows the peak area-time profiles of 2-phenethyl alcohol in the blood and brain dialysate samples after administration of rose essential oil. 2-Phenethyl alcohol appeared in both blood and brain samples immediately after administration of the rose essential oil. 2-Phenethyl alcohol showed almost comparable peak area-time profiles in both blood and brain samples, but it appeared to decrease more rapidly in the brain dialysate sample than in the blood dialysate sample. 2-Phenethyl alcohol could be detected up to 1.5 h after

administration of the rose essential oil.

4. Discussion

We established the simultaneous blood and brain microdialysis method in a free-moving mouse. Our data showed that the BBB functioned almost normally under the experimental condition and thus the method would allow to test the BBB permeability of chemicals. In fact, the method was applicable to test the BBB permeability of phenyl arsenic compounds and plant-derived VOCs when coupled with LC/MS/MS or GC/MS.

Use of chemicals that do not pass through the BBB is a way to test the BBB function. In the present study, two different fluorescent chemicals that barely pass through the BBB were used to validate the BBB function of mice under the experimental condition. Fluorescence intensity in brain dialysate samples barely changed after single administration of lucifer yellow or uranine, suggesting that the BBB was functioning in the mice. This interpretation was further supported by the present results that fluorescence intensity in the brain dialysate samples remarkably increased after co-administration of those fluorescent chemicals with 1 mL of 25 % mannitol solution. Hyperosmotic mannitol causes dehydration, shrinks endothelial cells of brain capillary vessels, tears tight junction between the endothelial cells, and thus opens the BBB [20]. Accordingly, no significant increase of the fluorescence intensity in the brain dialysate samples after the single administration of the fluorescent chemicals demonstrated the healthy BBB function. Chen et al. [21] report that intravenous mannitol does not increase the BBB permeability to Evans blue and uranine in adult rats. In their study, a single bolus intravenous administration of 20 % mannitol (dose; 0.5 g/kg) is made, immediately followed by intravenous administration of 0.5 mL saline that dilutes the concentration of mannitol in blood. In our study, 1 mL of 25 % mannitol solution was intravenously administered. The dose was calculated to be about 6.3 g/kg. Given that the total blood volume in a mouse is much smaller than that in a rat, mannitol in brain capillary could reach the concentration enough to open the BBB in mice in the current study.

DPAA is a ground water pollutant in Japan [22–24], and is suspected to have caused health problems in a group of citizens who used polluted well water. Major symptoms observed in the patients were neurologic in nature such as staggering, gait disturbances, hand tremors, weakness in extremities, titubation, scanning speech, myoclonus, impairment of ocular movement, insomnia and/or nightmares and memory impairment [25,26]. DPAA was detected in the cerebrospinal fluid of the patients [27]. Oral administration of DPAA produces various behavioral effects in rodents [28–30]. The highest level of arsenicals are detected in the brain compared with other organs in DPAA-administered rats [31]. Similarly, the concentration of DPAA in the brain is higher than that in the other tissues in DPAA-administered rats [18]. These observations suggest that DPAA passes through the BBB and affects brain function. The results of the simultaneous blood and brain microdialysis coupled with LC/MS/MS demonstrated that DPAA was absorbed into the blood and distributed to the brain through the BBB within 25 min after oral administration. Although DPAA rapidly entered the brain after oral administration, the behavioral effects of DPAA in rodents have been shown to become apparent after prolonged administration (the Ministry of the Environment, 2006; [29]). DPAA acutely alters gene and protein expressions in cultured astrocytes and cells [32,33,34,35], although some unknown processes that take a longer time to occur may also cause behavioral effects.

PAA and PMAA are also found in environmental and biological specimens [23,24,36], and in urine and serum of the patients exposed to the polluted well water [27,37]. However, it remained unclear whether PAA and PMAA affected the brain. As our LC/MS/MS analysis method was able to detect not only DPAA but also PAA and PMAA, we tested whether PAA and PMAA entered the brain in mice after oral administration. The results of the simultaneous blood and brain microdialysis coupled with LC/MS/MS demonstrated for the first time that PAA and PMAA were absorbed into the blood and distributed to the brain through the BBB within 25 min after oral administration.

The current study revealed that phenyl arsenic compounds such as DPAA, PAA and PMAA were rapidly distributed to the brain through the BBB after oral administration.. The phenyl arsenic compounds did not affect the BBB impermeability of lucifer yellow when administered together, suggesting that the passage of these phenyl arsenic compounds into the brain occurred within the intact permeability capacity of the healthy BBB. The BBB permeability of chemicals correlates with their octanol/water partition coefficients (log Pow) [38]. Phenyl groups increase the log Pow value of arsenic compounds (arsenic acid, -3.14; DPAA, 1.2; PAA, 0.06) [39,48], therefore, phenyl-groups may be involved in the BBB permeability of the phenyl arsenic compounds

As many plant-derived VOCs produce a variety of subjectively pleasant odors, they are used as fragrance compounds in food, beverages, and other products used daily such as cosmetics, cleaning products, and air fresheners. Similar to man-made VOCs such as anesthetics and organic solvents, fragrance compounds are known to influence brain function [40–43]. Lavender and rose essential oils, mixtures of plant-derived VOCs obtained from the flowers, produce behavioral effects such as antianxiety-like effects in mice in the conflict tests after intraperitoneal administration [44,45]. Linalool and 2-phenethyl alcohol are the major constituents of lavender and rose essential oils, respectively. Given that linalool and 2-phenethyl alcohol also produce antianxiety-like effects in the conflict tests in mice after intraperitoneal administration, it is suggested that linalool and 2-phenethyl alcohol enter the brain after the intraperitoneal administration of the lavender and rose essential oils to produce the behavioral effects ([44], 2006). The results of the simultaneous blood and brain microdialysis coupled with GC/MS demonstrated that linalool and 2-phenethyl alcohol were rapidly absorbed into the blood and distributed to the brain through the BBB after intraperitoneal administration of lavender and rose essential oils. The results suggest that linalool and 2-phenethyl alcohol easily permeate the BBB like man-made VOCs.

One advantage of the method reported here is that blood and brain microdialysis is conducted in a mouse under almost physiological conditions. The micorodialysis was conducted 3–5 days after brain probe implantation, which could be enough for the damaged BBB to recover. Sampling was conducted 1 day after anesthesia for implantation of the blood probe; therefore, the BBB was not affected by the anesthesia. Confirmation using fluorescent compounds ensured BBB function in mice. In the present study, the blood and brain microdialysis made it possible to test the BBB permeability of test compounds when coupled with appropriate chemicals analysis methods. Various methods for determining recovery rate have been developed. In vivo recovery estimations for test compounds would allow quantitative toxicokinetic analyses using data obtained by the blood and brain microdialysis. Toxicokinetic analyses using mathematic models can provide information about not only the extent of transport but also estimates of influx and efflux clearances at the BBB [5]. Magnetic resonance imaging (MRI) can be used to evaluate the BBB permeability in vivo [46], but it is difficult for the MRI method to identify specific compounds. Positron emission tomography (PET) is also used to assess a unidirectional influx rate constant for specific compounds in vivo [47]. The PET method can trace isotope-labeled specific compounds, however, correction for the decay of the isotope and subtraction of the radioactivity signals from the metabolites are necessary to obtain reliable results. Due to their spatial resolution, the MRI and PET methods are difficult to apply small animals such as mice. In addition, both the MRI and PET methods require immobilization of subject animals. Although quantitative toxicokinetic analyses were not conducted in the present study, the present study showed that semi-quantitative data were also useful to examine whether or not test compounds permeated the BBB.

Standard neurotoxicity tests using animals are laborious, time-consuming, and expensive. Simultaneous blood and brain

microdialysis sampling in a free-moving mouse would help with efficient exploration of BBB permeable chemicals that may trigger neurotoxicologic effects.

5. Conclusion

In the present study, we established the simultaneous blood and brain microdialysis in a free-moving mouse. Validation tests using the chemicals that barely permeate the BBB showed that the BBB was functioning in the mice under the experimental conditions. The BBB permeability of phenyl arsenicals was observed using the simultaneous blood and brain microdialysis coupled with LC/MS/MS. The BBB permeability of fragrance compounds was observed using the simultaneous blood and brain microdialysis coupled with GC/MS. Thus, the present study demonstrated that the simultaneous blood and brain microdialysis in a free-moving mouse made it possible to test the BBB permeability of chemicals when coupled with appropriate chemical analysis methods. The simultaneous blood and brain microdialysis would help with efficient exploration of potentially neurotoxic chemicals among the large number of chemicals that have not been studied.

CRediT authorship contribution statement

Toyoshi Umezu: Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Writing - review & editing. **Tomoharu Sano:** Methodology, Investigation, Resources. **Junko Hayashi:** Investigation. **Yasuyuki Shibata:** Methodology, Investigation, Resources, Funding acquisition.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.10.023.

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