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Design and development of a suitable adsorbent to capture theophylline for non-invasive therapeutic drug monitoring with exhaled breath

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The possibility of using exhaled breath as a substitute for blood/plasma in areas of therapeutic drug monitoring was investigated. Theophylline was used as a model chemical of non-volatile drug and the adsorbents suitable for drug capture from exhaled breath were studied. Two macro-porous adsorbents with different monomer compositions were prepared by a suspension copolymerization. The capture properties of the synthesized adsorbents and two conventional gas adsorbents packed in cartridge were evaluated by both aerosol generation and rat expiration. The efficiency of drug capture was not improved by decreasing the pore size, namely by increasing the specific surface area. A hydrophilic region was found to be necessary for holding the aerosol particles on the adsorbent surface, but a hydrophobic region is also necessary for the adsorption of theophylline. The materials were applied to the intravenous bolus experiment of rats. The amount of theophylline collected from exhaled breath was in the range of 0 - 0.02 ng min^{-1}, and that varied similarly to the concentration in blood, which was in the range of 0 - 37 μg mL^{-1}.

Introduction

Blood is the conventional sample used for therapeutic drug monitoring (TDM). However, being an invasive means of sample collection, it is associated with large patient compliance problems. TDM can also be done non-invasively by using biological fluids, such as saliva, sweat, and tears [1-3], although further studies are needed to show that these fluids become alternatives to plasma/serum in this purpose.

Ancient physicians tried to recognize diseases by the specific smell of exhaled breath. Modern breath testing began in the 1970s when Pauling et al., detected around 200 different volatile organic compounds (VOCs) in exhaled breath by gas chromatography [4]. More than 3000 volatile compounds have been detected in exhaled breath [5], and many researchers have investigated them as potential biomarkers of airways disease [6]. The finding that alcohol concentrations in breath and blood are well correlated [7] raises the possibility of using exhaled breath for real-time monitoring of volatile drugs. Indeed, several studies have shown that the concentration of volatile anesthetic drug propofol in breath reflects the concentration in blood [8-10]. Recently, modern on-line and real time monitoring techniques for the analysis of propofol in exhaled breath has been reported [11]. If a precise TDM technique using breath can be established, an epoch-making diagnoses technique as compared with traditional diagnostic techniques will be offered. Breath sampling, in addition to being non-invasive, does not require skilled medical staff. The matrix of exhaled breath is less complex than that in blood or other body fluids [5]. However, disadvantages of this method are that the majority of drugs are non-volatile and the drug concentrations in breath are very low compared to the concentrations in blood and other body fluids.

Exhaled breath has so far not been considered as testing matrix for drug monitoring except for abused drugs, for example, tetrahydrocannabinol (THC), the active ingredient in cannabis was detected in breath following cannabis smoking in the 1980s [12]. Subsequently exhaled breath was found to contain not only low-volatile amphetamine [13] but also the less volatile methadone [14]. It has been a doubtless fact that non-VOCs are exhausted in breathing. A comparison of the proteins in bronchoalveolar lavage and in exhaled breath strongly indicate that the proteins originate from the respiratory tract lining fluid [15], whose composition is the same as that of blood [16]. Aerosol particles in sub-micrometer size are assumed to carry non-volatile drugs [17]. Although the mechanism and exact location of particle formation in the airways are unclear, exhaled breath would be proposed as a new possible matrix for TDM of non-volatile drugs.

The detection of very low drug levels is easier today with advanced analytical instruments and technologies. The level of tetrahydrocannabinol carboxylic acid, the main metabolite of THC, in breath was undetectable in the 1980s [12], but it is now is detectable by LC-MS/MS [18]. However, LC-MS/MS may not be sensitive enough to detect many other drugs. The concentrations of drugs in breath are very low, so some preconcentration is required prior to MS detection. Solid-phase extraction (SPE) can improve the sensitivity and precision of an exhaled breath analysis. SPE was developed in the late 1960s for sampling hydrophobic solutes and for air quality analyses [19].

SPE avoids the need for complicated sample preparation, especially when the samples are unstable. However, exhaled drugs are thought to be dissolved in or adsorbed to aerosol particles, and collection of the target drug from breath is greatly different from the traditional concept of SPE.
Here, we synthesized two polymeric adsorbents designed to adsorb a non-volatile drug (theophylline) in aerosol and breath samples and evaluated their adsorption properties.

Experimental

Reagents

All reagents for synthesizing polymeric adsorbents were purchased from Wako Pure Chemical Industries (Osaka, Japan). Glycidylmethacrylate (GMA) as a functional monomer, and divinylbenzene (DVB) and ethyleneglycoldimethacrylate (EGDM) as cross-linkers, were utilized without removal of stabilizer. As an initiator of polymerization, 2,2-azobis (isobutyronitrile) was utilized. Polyvinylalcohol (PVA, n=500) and methyl cellulose were used as suspension stabilizer.
Acetonitrile of HPLC grade was obtained from Merck (Darmstadt, Germany). Theophylline and formic acid were purchased from Wako Pure Chemical Industries. Stock solution (1 mg mL^{-1}) of theophylline was prepared in acetonitrile and was stored at -20°C. All aqueous solutions were prepared using water obtained from a Toray (Tokyo, Japan) LV408 extra-pure-water production unit. Using the stock solution three calibration standards were prepared in ultra-pure-water at the following concentrations: 0.3, 1.0, 10.0 μg L^{-1}.
Neophylline®, medicinal preparation for injection of theophylline was obtained from Eisai (Tokyo, Japan).
Two column packing materials for gas chromatography, Porapak T (80-100 mesh) and Tenax TA (60-80 mesh) were purchased from GL Sciences (Tokyo, Japan).

Instruments

An Agilent Technologies (Santa Clara, CA, USA) 1100 series HPLC system equipped with a 6140 single quadrupole mass detector was used for the determination of theophylline. Operating conditions were as follows: column, InertSustain C18 (GL Sciences, 3 μm, 250 × 2.1 mmi.d.); flow rate, 0.2 mL min^{-1}; column temperature, 50°C; injection volume, 10 μL; mobile phase, acetonitrile / 10 mM formic acid (3+7). The electrospray ionization source was operated in the positive mode at the following conditions: capillary voltage of +4.0 kV, desolvation N_2 gas temperature of 350°C and flow-rate of 10.5 L min^{-1}. MS detection was performed using the SIM acquisition mode with a scan time of 0.6 sec. Data processing was performed using LC/MSD ChemStation 3.01 software. A Bruker Daltonics (Billerica, MA, USA) maXis TOF-MS detector was used at a mass number of 181.072 to qualify theophylline in the breath samples.
Aerosols were produced with an Omron (Kyoto, Japan) NE-U17 ultrasonic nebulizer.

Synthesis of polymer adsorbents

Two macro-porous adsorbents for the aerosol samples were prepared by a suspension copolymerization as described previously [20] except for the oil composition. Two blended oils (BG15 and BE50) were prepared. BG15 contained 15 g of GMA, 85 g of EGDM, 40 g of butylacetate and 60 g of 2-methylbutanol. BE50 contained 50 g of GMA, 50 g of EGDM, 100 g of butylacetate and 25 g of 2-methylbutanol. The protocol for synthesizing the adsorbents is shown in Fig. 1. The copolymerization was performed at 70°C (BG15) or 80°C (BE50) for 7 hr with stirring. The obtained polymers were classified into various particle sizes by standard wire sieves. The epoxy group on the BG15 polymer was opened into diol group by reaction in 10 mM H_2SO_4 / acetonitrile for 3-4 hr at 40°C.

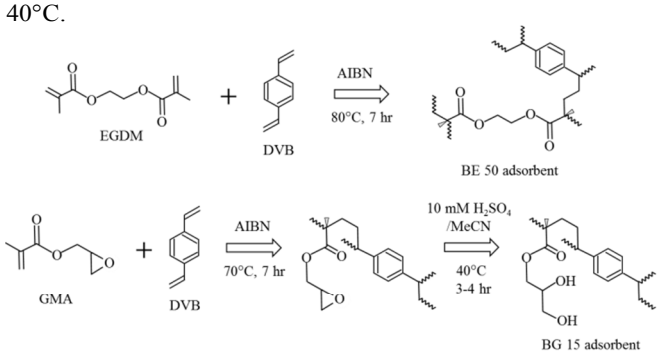

Fig. 1. Protocol for synthesis of the adsorbents for aerosol sample

The specific surface areas and average pore diameters of the adsorbents were measured using a Beckman Coulter (La Brea, CA, USA) SA3100 Surface Area Analyzer.
SPE cartridges (3-mL, BondElut Reservoir, Varian) were packed with 75 mg of adsorbent for the rat expiration experiment. The adsorbent was fixed by O-rings and wire meshes to maintain breathability (refer to Fig. 2). The same cartridges packed with 50 mg of the adsorbents were used for the nebulizer experiment. The packed cartridges were washed with methanol and then stored in a desiccator under vacuum until use.

Sampling of breath and blood

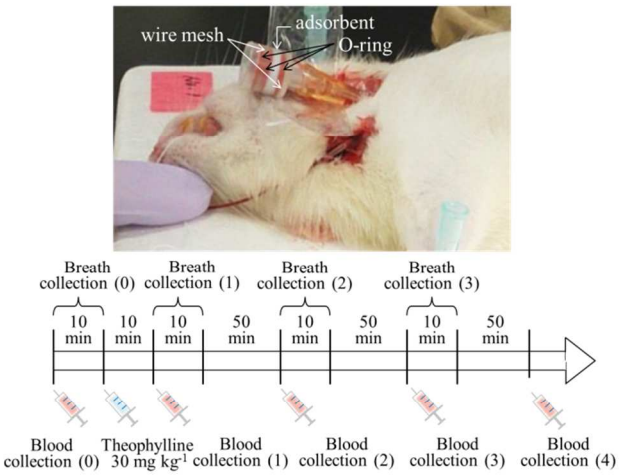

Fig. 2. Spontaneous collection of rat breath and sampling profiles

Ten-week-old male Sprague-Dawley (SD) rats with body weights ranging from 319 to 352 g were purchased from Japan SLC (Hamamatsu, Japan). Animal experiments were approved by the Animal Experiment Committee of Nagoya University Graduate School of Medicine. All procedures were performed in accordance with the Guidelines for Animal Experimentation of Nagoya University. Anesthesia was induced by intraperitoneal injection with 25 mg per kg body weight of sodium pentobarbital. The trachea was

cannulated with polyethylene tubes for breath collection. The right jugular vein was also cannulated for drug administration and blood collection. In the intravenous bolus study, theophylline was administered at a dose of 30 mg per kg body weight via a jugular vein cannula. Breath samples were collected for 10 min through the trachea cannula to which the SPE cartridge was attached. Blood samples were drawn through the jugular vein cannula. Breath and blood samples were collected before administration of theophylline and at 1-hr intervals starting at 10 min after administration (Fig. 2).

Theophylline measurements

Theophylline in the rat breath retained on the adsorbent in the SPE cartridge was eluted with 1 mL of methanol. The effluents were evaporated in vacuo. The dried analytes were reconstituted with 100 µL of water. The rat serum was diluted 100 times with water, and ultrafiltrated with an Amicon Ultra-4 centrifugal filter unit (Merck Millipore, Tullagreen, Ireland). Theophylline in these sample solutions were measured by HPLC-MS.

Results and discussion

Detection of a non-volatile drug in exhaled breath

Theophylline has been used as potent bronchodilators and itspharmacokinetics has been discussed in detail [21]. The blood concentrations of theophylline in rats remained almost constant between 10 to 60 min after an intravenous infusion. Evaluation of the adsorption characteristics of the adsorbents has been performed by using non-volatile drug theophylline. Two adsorbents, we synthesized, and two adsorbents on the market, Tenax TA and Porapak T, which are separating resin for gas chromatography, were packed into SPE cartridges and rat breath was passed through them successively in every 10 min after 10 min from administration. The response of the MS detector at m/z=181 was linear from 0.3 to 10 µg theophylline L^{-1}. Detection limit for theophylline in exhaled breath at signal-to-noise ratio of 3:1 was about 2 pg min^{-1}. Although SIM chromatograms are not shown here, no endogenous peaks interfered with the detection of theophylline.

Table 1. Physical properties and adsorption characteristics of various adsorbents

	Porapak T	Tenax TA	BG15	BE50
Specific surface area	530 m^2 g^{-1}	15-18 m^2 g^{-1}	700 m^2 g^{-1}	550 m^2 g^{-1}
Particle size	150-180 µm	180-250 µm	180-250 µm	250-300 µm
Pore size	8.5 nm	9-11 nm	8.4 nm	10.2 nm
Ratio of aromatic monomer	0%	100%?	85%	50%
Polar group	ester	oxide	diol	ester
Capture quantity of theophylline (n=3)	5 ± 4 pg min^{-1}	11 ± 4 pg min^{-1}	15 ± 6 pg min^{-1}	13 ± 5 pg min^{-1}

Physical properties of adsorbents used here and their adsorption characteristics are summarized in Table 1. Of the four adsorbents used in the SPE cartridge, the captured quantities of theophylline were in order BE50 ≈ BG15 > Tenax TA > Porapak T. The specific surface of Tenax TA is an order of magnitude smaller than the specific surface of the other materials. Relation between capture quantity and specific surface cannot be precisely discussed here for unevenness of the particle size, but we have assumed that the capture quantities do not depend on the specific surfaces of adsorbents and theophylline requires an adsorbent with a hydrophobic surface because of its aromatic rings. If non-volatile drugs are transported by aerosol particles in exhaled breath, the minute pore on the adsorbent will not be active on the adsorption of theophylline in the aerosol because the aerosol particle size is much larger than the pore size. Although the adsorbent must be wettable to capture the aerosol particles, a hydrophobic region is also necessary to adsorb the theophylline dissolved in the aerosol. A delicate balance between the hydrophobic and hydrophilic regions on the adsorbent surface is necessary to adsorb theophylline effectively. It is expected that theophylline adsorption will depend on the monomer compositions of the synthesized adsorbents.

Theophylline concentrations in the eluate from the adsorbent were verified by the measurement with LC-TOFMS at m/z = 181.072. Since the values of theophylline measured by TOFMS and quadrupole MS almost agreed with the regression equation of TOFMS = 0.80 QMS + 0.03 and the correlation coefficient of 0.988 (n=6), the exhaled breath clearly contains theophylline, derived from circulating blood.

Evaluation of adsorption characteristics of adsorbents for aerosol sample

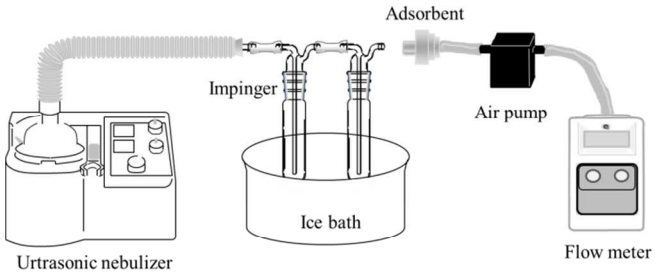

Fig. 3. Schematic representation of nebulizer experiment

Air-borne aerosol particle size in exhaled breath varies widely but approximately 85% of particles have diameters < 1 µm [16]. We assembled a device to prepare an invisible small aerosol particle as depicted in Fig. 3. The influences of particle size of the adsorbent and the suction speed of the aerosol on the adsorption efficiency of theophylline were evaluated with this device. BE50 adsorbent which gave a good result by a rat expiration experiment was applied on this nebulizer experiment. A BE50 cartridge packed with 50 mg of wide-ranged size particle and another BE50 cartridge with small particle ranged between 63-90 µm were connected in series. The adsorption efficiency was calculated from the ratio of capture quantities of theophylline between the two cartridges. A hundred mg L^{-1} of theophylline aqueous solution was sprayed with a nebulizer and the generated aerosol was sucked up into the cartridges for 10 min. The minute ventilation of SD rat was about 27 mL min^{-1} per 100 g [22]. The suction rate was decided in 100 mL min^{-1} equivalent to a minute ventilation of rat and 500 mL min^{-1} equivalent to 5 times as much as the ventilation. The capture efficiency was clearly inversely related to both the suction rate and the particle size (Table 2). It is of great convenience to use adsorbents of small particle size for aerosol adsorption because a practical adsorption area is wide. However, if the size is too small, the resistance to air flow increases and making it difficult for rats to breathe. Consequently, the adsorbents with particle size of 150-180 µm, on which the capture efficiency of theophylline exceeds 90% at the suction rate equivalent to a minute ventilation of rat, were used at following rat expiration

experiment.

Table 2. Effects of particle size and suction rate on the capture efficiency on BE50 adsorbent (n=4)

	100 mL min^{-1}	500 mL min^{-1}
250-300 μm	62.7±9.2%	37.7±12.4%
180-250 μm	74.4±14.8%	57.1±15.4%
150-180 μm	94.0±4.4%	74.1±8.2%
63-90 μm	90.0±6.3%	-

Correlation of theophylline concentrations in breath and blood

Time-profiles of theophylline concentrations in breath and blood were determined. The amount of adsorbent was increased from 50 mg to 75 mg to improve the capture efficiency. Approximately 0.2 ng of theophylline per 10 min was captured in the exhaled breath of rats until two hours after dosage, while the theophylline concentration in the serum was initially 37.2 μg mL^{-1} and dropped at about 5 μg mL^{-1} per hour (Fig. 4). The blood concentrations of theophylline were similar to previously reported values [21]. Although theophylline concentrations determined in blood slightly varied, the values of individual rat showed same tendency to decrement. But time profiles of theophylline quantity in breath did not show a constant tendency in the rat individual. Since the collection time for the breath was precisely fixed for 10 min, unstable results observed in breath would come out as changes in the expiration volume based on the depth of anesthesia. Also the lower drug level in exhaled breath would decrease the analytical precision. The use of the ventilator may be necessary to obtain the robust correlations between theophylline concentration in breath and blood.

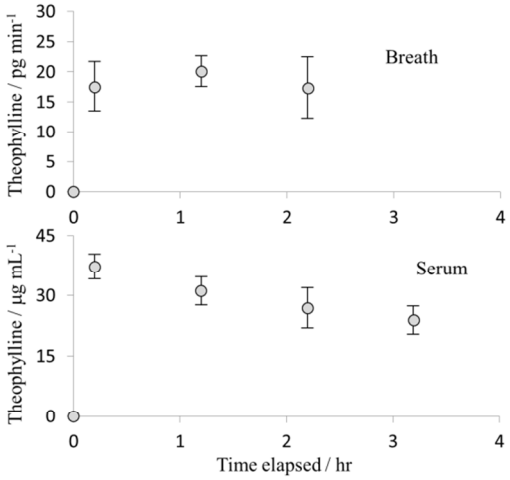

Fig. 4. Concentration-time profiles of theophylline in breath and serum. Each point of time is the mean of four rats (±SD).

Conclusions

Suitable adsorbents to capture theophylline for non-invasive TDM with exhaled breath were prepared. The adsorbent must be hydrophilic to capture the aerosol particles and be hydrophobic to adsorb the theophylline dissolved in the aerosol. The optimized adsorbent was offered for a rat expiration experiment. Time-profiles of theophylline concentrations in breath and blood showed slightly different tendency.

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Notes and references

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