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Investigation on the influence of time-of-day on benzene metabolic pharmacokinetics by direct breath analysis in mice

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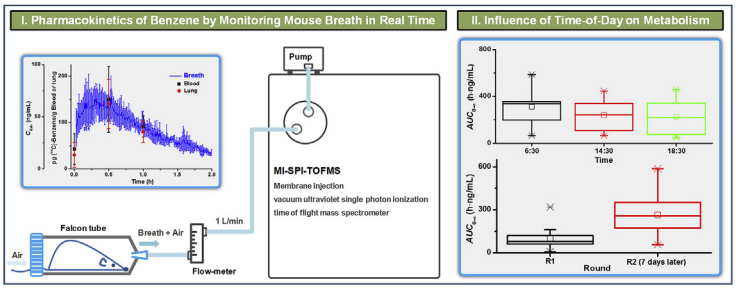
HIGHLIGHTS

- Exhaled benzene in mouse breath was monitored in real time.
- Pharmacokinetic parameters were obtained with high temporal resolution data.
- Damage in liver due to benzene exposure was reflected from exhaled benzene.

GRAPHICAL ABSTRACT



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ABSTRACT

Benzene, well known as a ubiquitous environmental pollutant, can lead to increasing risk of cancer, bone marrow failure as well as other serious diseases. Benzene has been classified as carcinogenic to humans with no recommended safe level of exposure. In this study, the influence of time-of-day on benzene metabolism has been tentatively explored in a mouse model based on direct real-time breath analysis by using membrane inlet single photon ionization time of flight mass spectrometry (MI-SPI-TOFMS). The exhaled breath of eight mice was monitored at a time resolution of less than 20 s after intraperitoneal (I.P.) injection of benzene in the morning, afternoon and evening on different days, and two rounds of experiments were carried out in total. The pharmacokinetic parameters such as total exposure $AUC_{0\text{-}\infty}$ (h ng/mL), peak level C_{max} (ng/mL), time of peak level t_{max} (h), and terminal half-life $t_{1/2z}$ (h) were calculated and discussed. The values of individual parameter varied greatly among the eight mice, e.g., $AUC_{0\text{-}\infty}$ in the morning of the first round of experiment ranged from 10.66 to 162.17 h ng/mL and the mean ± SD was 103.72 ± 99.72 h ng/mL ($n = 8$). Significant difference has also been observed between two rounds of experiments, implying the damage in the liver caused by the benzene exposure. However, there is no significant difference among the results from the morning, afternoon and evening for each

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round of the experiment. In our follow-up study, the influence of time-of-day will be further investigated, in which the metabolites of benzene as well as endogenous metabolites will be considered.

1. Introduction

Benzene, a ubiquitous environmental pollutant, can be produced from both anthropogenic and natural processes, such as wild fires, synthesis of chemicals, fabrication of rubbers, etc (ATSDR, 2007a). Benzene has been classified as carcinogenic to humans with no recommended safe level of exposure (IARC, 1987; McMichael, 1988). People can be exposed to benzene through tobacco smoke, emissions from automobile service stations, exhaust from motor vehicles, and industrial emissions, which lead to increasing risk of cancer, bone marrow failure as well as other serious diseases. The inhaled benzene is rapidly absorbed through the lungs, quickly distributed all over the body, and primarily metabolized in the liver. At higher exposure levels, a portion of absorbed benzene is excreted as parent compound in exhaled air due to the saturation of benzene in the metabolic pathways (Snyder and Hedli, 1996).

The exhaled breath has been characterized with the advantage of non-invasiveness and proven to be a promising bio-sample for cancer diagnosis (Haick et al., 2014), metabolomics (García-Gómez et al., 2016; Li et al., 2015; Martínez-Lozano Sinues et al., 2017), environmental exposure (Benoit et al., 1985; Pleil et al., 2011). Furthermore, with breath analysis the small animal (e.g., mouse) can be used repeatedly as long as it is recovered from the last administration/exposure experiment, and this is almost impossible when blood or tissue samples are analyzed. The reuse of animals will not only save the cost but also reduce the deviation caused by individual differences.

For breath analysis, gas chromatography mass spectrometry (GC-MS) has been reported most commonly (Manolis, 1983; Lourenço and Turner, 2014; Pleil et al., 2014), partly due to the easy access to the instrument for different labs all over the world while partly due to the well-established reference method, *i.e.*, the GC-MS method for atmospheric volatile organic compounds (VOCs) released by EPA in 1970s (Pellizzari, 1977; Gordon et al., 1985). With emerging direct MS techniques, it is possible to perform breath analysis at a time resolution as high as several seconds, and this real-time data has brought unparallel information to the knowledge on metabolic pharmacokinetics (Li et al., 2015; Martínez-Lozano Sinues et al., 2017) as well as other relevant fields. The direct MS-based techniques mainly include trace atmospheric gas analyzer (TAGA) (Benoit et al., 1985), proton transfer reaction MS (PTR-MS) (Kushch et al., 2008), selected ion flow tube MS (SIFT-MS) (Storer et al., 2014), secondary/extractive electrospray ionization MS (SESI/EESI-MS) (Gamez et al., 2011; Li et al., 2015) and active capillary plasma ionization MS (Bregy et al., 2014).

Membrane inlet vacuum ultraviolet single photon ionization mass spectrometry (MI-SPI-MS) can also be used for direct analysis of VOCs. The analytes are directly introduced into the mass spectrometer through a gas permeable hydrophobic membrane, such as polydimethylsiloxane (PMDS) (Gao et al., 2013). The membrane removes most of the carrier gas and also much of the water, leading to higher sensitivity. The SPI source allows efficient and selective ionization of compounds that with ionization potentials (IP) lower than the photon energy. As only a small amount of energy exceeds the IP, mass spectra mostly present molecular ions rather than fragment ions (Hua et al., 2011; Mühlberger et al., 2007), which facilitates the identification of compounds and also increases sensitivity. The on-line detection limit (LOD) of SPI quadrupole MS for benzene was down to 32 ppbv (Mühlberger et al., 2007), showing its potential for on-line monitoring of trace benzene with dynamic fluctuations. Recently, the development of portable MI-SPI time-of-flight mass spectrometer (MI-SPI-TOFMS) (Gao et al., 2013) has further enhanced its application in field online measurements.

The time-of-day of drug administration is closely related with drug pharmacokinetics and efficacy/toxicity (Dallmann et al., 2012, 2014; 2017; DeBruyne et al., 2014), and thus it is very likely that time-of-day plays a similar role in benzene exposure. Moreover, it was found lately that the influence of time-of-day on metabolism is possible to be tracked by breath analysis (Martínez-Lozano Sinues et al., 2017). Therefore, in this study, real-time monitoring of benzene metabolic pharmacokinetics by using MI-SPI-TOFMS has been demonstrated and the influence of time-of-day on benzene metabolism was tentatively explored in a mouse model. The exhaled breath of mouse was monitored at a time resolution of <20 s after benzene injection in the morning, afternoon and evening on different days; two rounds of experiments were carried out in total. Pharmacokinetic parameters including total exposure $AUC_{0\text{-}\infty}$ (h ng/mL), peak level C_{max} (ng/mL), time of peak level t_{max} (h), and terminal half-life $t_{1/2z}$ (h), etc. were calculated, compared and discussed.

2. Methods

2.1. Reagents, materials and animals

5.8 ppmv (20.22 ng/mL) and 5 ppmv (17.43 ng/mL) of benzene calibration gases were purchased from Shanghai Weichuang Standard Gas Analytical Technology Co. Ltd. and Guangzhou Shiyuan Gas Co. Ltd., respectively. Benzene (Liquid, analytical reagent grade) was supplied by Guangzhou Guangpeng Scientific Instrument Co. Ltd. High purity nitrogen gas (N_2, 99.99%) was obtained from Guangzhou Shiyuan Gas Co. Ltd. The specific pathogen free (SPF) ICR female mice (30–34 weeks old, 32–34 g) were provided by Guangdong Medical Laboratory Animal Center (Guangdong, China) and kept under standard conditions in the Laboratory Animal Center of JNU. Before experiments, the mice were acclimated to the laboratory for at least one week and exposed to 12 h of light per day (LD 12:12) with *ad libitum* access to water and feed.

2.2. Sample preparation

10–5000 ppbv (0.03–17.43 ng/mL) or 11.6–5800 ppbv (0.04–20.22 ng/mL) of calibration standards (Gas) were prepared in a 3-L Tedlar bag by serially diluting 17.43 ng/mL or 20.22 ng/mL of benzene calibration gas with high purity N_2 (99.99%) by a automatic gas distribution system (DSC-1000, Guangzhou Hexin Instrument Co. Ltd.).

Before breath analysis, the mouse was administrated with 5 μL of liquid benzene (~133 μg/kg) by intraperitoneal (I·P.) injection. There were two rounds of experiments (R1 and R2), and for each round of experiment, individual mouse was injected at three time points on three different days, i.e., 6:30 (Morning), 14:30 (Afternoon) and 18:30 (Evening); the next injection was scheduled 3 days