Letter

Online Real-Time Monitoring of Exhaled Breath Particles Reveals Unnoticed Transport of Nonvolatile Drugs from Blood to Breath

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ABSTRACT: We used online secondary electrospray ionization mass spectrometry to measure venlafaxine (VEN), a nonvolatile drug, in the exhaled air of mice intraperitoneally treated with VEN. The breath pharmacokinetic (PK) profile of VEN was recorded, which was in good agreement with that of the blood. Combined with online collection of exhaled breath particles (EBPs), it was shown that VEN existed as part of EBPs rather than gas molecules in the breath. Linear free-energy relationship analysis confirmed that almost completely ionized VEN at physiological conditions unlikely partition from the lung lining fluid (LLF) into breath air. This implies that the occurrence of VEN in exhaled air accompanies the formation of EBPs from the LLF. By comparison with the low breath signals of VEN metabolites, passive membrane permeability and lung/blood partition coefficient are suggested as the main influencing factors for the levels of drugs in the breath. This study advances our knowledge on the mechanism by which nonvolatile drugs are transferred from blood into exhaled breath, providing guidance for breath test-based therapeutic drug monitoring.
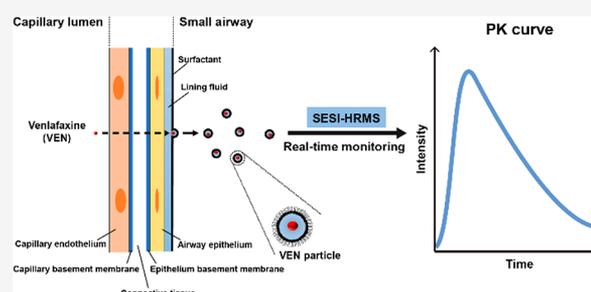
Therapeutic drug monitoring (TDM) refers to the practice of measuring drugs with a narrow therapeutic window in the blood. The breath test has been regarded as one of the most promising methods for TDM, because of noninvasiveness and little restriction in sampling volume and frequency. Detection of drugs in exhaled air can be traced back to nearly 30 years ago when the anesthetic gas isoflurane was monitored by sensors online.[1] Since then, a number of drugs, such as sevoflurane, valproic acid, propofol, ketamine, methadone, and fentanyl, have also been detected in breath air,[2,3] demonstrating the potential of breath test in TDM. However, the mechanism by which drugs, in particular nonvolatile compounds, are transferred from blood into exhaled breath remains undefined.

Recently, dozens of nonvolatile drugs were observed in exhaled breath particles (EBPs) through offline particle collection followed by liquid chromatography—mass spectrometry (LC−MS) analysis.[4−6] It delivers us an important message that nonvolatile drugs can be transported via exhaled particles. Thus, sensitive and robust methods for detecting compounds in EBPs can facilitate the monitoring of nonvolatile drugs in breath air. Secondary electrospray ionization-mass spectrometry (SESI-MS) is an ESI-based ambient MS technique and has the capacity for detecting gas and aerosol samples in real time.[7−9] Gu et al. reported rapid analysis of several aerosol drugs, in which aerosol drugs were manually introduced into a SESI source and directly ionized without sample pretreatment.[10] This suggests that SESI-MS may be applied for the

detection of nonvolatile drugs in the EBPs. In fact, our previous work has validated the potential of SESI-MS for monitoring the pharmacokinetics (PK) of nonvolatile drugs in real time in mice, using ketamine and its metabolites as examples.[3] However, at that moment, we supposed that these compounds existed as gas molecules in the breath air of mice.

Venlafaxine (VEN) is one of the most commonly used antidepressants worldwide. However, recent studies showed that patients treated with VEN exhibited large interindividual variability in drug efficacy. The TDM of VEN thus plays an increasingly important role in the individualization of VEN dosage regimens.[11,12] In this study, VEN was used as a model nonvolatile drug. A homemade SESI source coupled to a high-resolution quadrupole-orbitrap mass spectrometer (SESI-HRMS)[13] was used to monitor VEN in the exhaled air of mice, as illustrated in Figure S1. In combination with the online collection of EBPs, the origin of VEN in exhaled air was investigated (Figure 1). The transport of VEN from blood to exhaled air was analyzed by model calculation using linear free-energy relationship (LFER) equations. The aims of this study
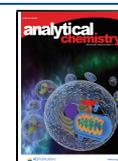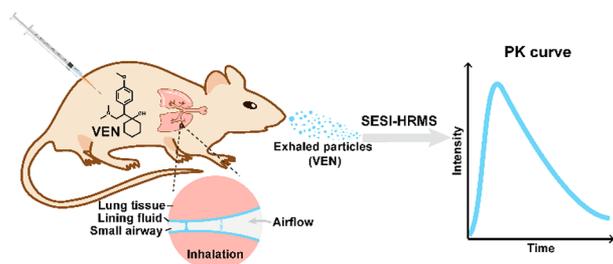
Figure 1. Schematic of real-time monitoring of nonvolatile VEN in breath.

are to figure out the nature of the airway pharmacokinetics of nonvolatile drugs and to provide some guidance for the application of breath test in the TDM of nonvolatile drugs.

■ RESULTS AND DISCUSSION

VEN was detectable in mouse breath immediately after the mouse was treated intraperitoneally with VEN at a single dose of 100 mg kg^{-1}. The protonated molecule of VEN ([M + H]$^+$ at m/z 278.2115) and its isotopic peaks in MS spectrum are consistent with that of standard VEN (Figure 2). By further
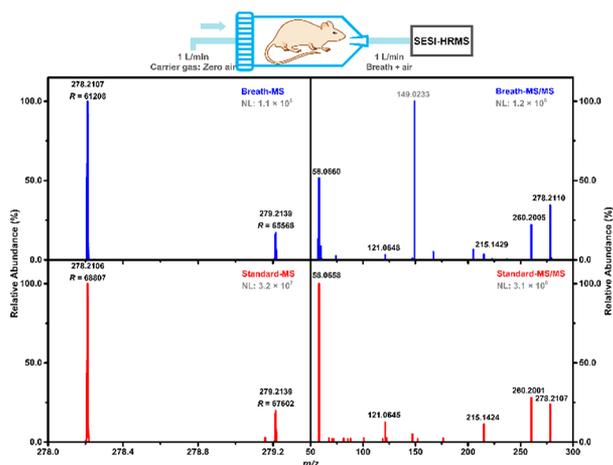
Figure 2. MS and MS/MS spectra of exhaled VEN (blue line) and VEN reference (red line).

performing MS/MS analysis, fragment ions at m/z 260.2005, 215.1429, 121.0648, and 58.0660 are produced from [M + H]$^+$ of VEN, which exactly match with the standard MS/MS spectrum (Figure 2). For all the control mice, no VEN was found in the breath during the whole monitoring period. To investigate the contribution of gaseous VEN and particle-bound VEN to the signal detected using SESI-HRMS, we applied the SensAbues device (SensAbues AB, Vasteras, Sweden) in the breath sample delivery line connected to the SESI source to collect particles from mouse breath (Figure 3). The VEN signal disappeared immediately after the SensAbues device was used; in contrast, there was no significant change in the signal intensity of trimethylamine (TMA, Figure 3), which is a systemic volatile organic compound resulting from the metabolism of dietary nutrients (such as phosphatidylcholine, choline, and carnitine) by gut microbes.[14] To further confirm that compounds in the gas phase cannot be trapped in the SensAbues device, the device was tested by a gas mixture of standard acetone, 2-butanone, 2-pentanon, isoprene, and α-terpiene (2 parts per billion by volume); similar to TMA, there
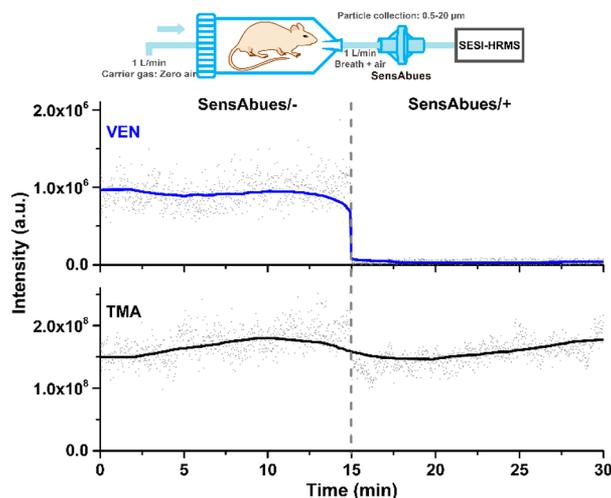

Figure 3. Intensity–time variations of VEN (blue line) and TMA (dark gray line) without or with the SensAbues device in the sample delivery line. The gray spots represent the raw data, and smoothed interpolation lines are added to help visualize the trends.

is no variation in the signal intensity of all the compounds (Figure S2). Moreover, the exhaled particles collected by SensAbues were eluted and analyzed by UHPLC−HRMS using the method of Beck et al;[15] VEN was detected and identified by comparison with the MS isotopic pattern and MS/MS spectrum of its standard (Figure S3). It is thus concluded that VEN in exhaled breath is present in the particle phase and little or none of it in the gas phase.

Moreover, one peak at m/z 264.1958 was found in the eluent, indicating the possible existence of VEN metabolites, O-desmethylvenlafaxine (ODV) and/or N-desmethylvenlafax-ine (NDV) in the EBPs. ODV and NDV are isomers, leading to the protonated molecules at the same m/z (264.1958). By means of UHPLC−HRMS, ODV and NDV were confirmed using the standards in terms of retention time and MS and MS/MS spectra (Figure S4). However, neither of the metabolites were observed in real time. It may well be that their breath levels were lower than the detection limit of the present SESI-HRMS method.

Temporal profiles of breath VEN were plotted over 120 min for three mice treated intraperitoneally with 100 mg kg^{-1} of VEN (Figure 4a and Figure S5). Meanwhile, at predefined time points, groups of mice (n = 6 for each group) were sacrificed and the blood samples were collected to conduct UHPLC−HRMS analysis. The breath curves resulted in a mean half-life ($t_{1/2}$) of 57.9 min, which is in good agreement with the value (58.1 min) deduced from the blood PK curve (Figure 4b). However, the time of the peak concentration (T_{max}) of breath VEN showed a delay of about 17 min compared to blood VEN. A similar phenomenon was observed for ketamine in mice as reported previously.[3] This could be attributed to the transport of VEN from blood to the lung and breath air.

We explored the transport process of VEN at a deeper level. The transport of VEN from blood to breath can be dissected into permeation of VEN from blood to the lung lining fluid (LLF) and transfer from the fluid to breath air (Figure S6). VEN is a weak base (pK_a = 9.4)[16] and thus is >99% ionized under the physiological pH (7.4 for the blood;[17] ∼6.9 for LLF[18]). VEN is essentially a nonvolatile organic compound with a water/air partition coefficient ($K_{w/a}$) of 4.82 × 10^8,
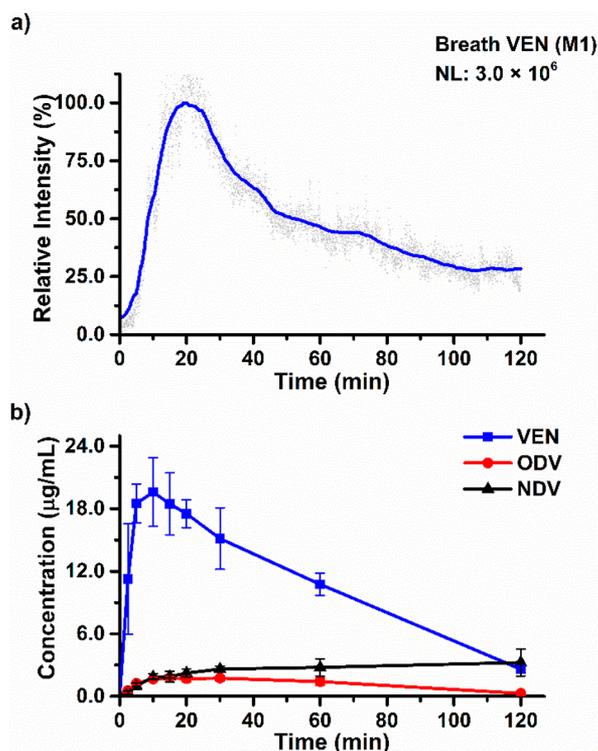
Figure 4. Pharmacokinetic profiles of VEN in breath and blood: (a) relative intensity−time curve of breath VEN and (b) blood concentration−time curves of VEN and its metabolites. Each data point represents the mean ± SD, $n = 6$. The gray spots represent the raw data, and a smoothed line is added to aid in the visualization of the underlying trends.

which was calculated using a LFER equation provided by Abraham et al.,[19] and the almost complete ionization makes it more unlikely partition into breath air.[20] This agrees well with our experimental observation that VEN hardly existed in the breath gas phase. These findings imply that the occurrence of VEN in the EBPs is caused by the formation of EBPs, which has been explained as being a rupture process of LLF films created by the reopening of small airways (internal diameter <2 mm) during inhalation.[21] In this study, we found no significant difference on the respiratory function (such as respiratory frequency, tidal volume, and minute volume) of mice after VEN treatment (Figure S7), so the amount of EBPs generated per breath was supposed to stay constant. That is to say, the breath level of VEN is closely connected with its level in the LLF, which depends on the permeation flux from blood to LLF.

The blood-to-LLF permeation flux is determined by blood concentration and permeation rate. The latter may be influenced by transport across plasma membranes and the tissue retention which results from the binding of VEN to components in the capillary endothelium and airway epithelium as well as the connective tissues between them. Given the similarity of passive membrane permeation, we here used brain permeation as an estimate for the permeation across plasma membranes in the capillary endothelium and airway epithelium. The brain permeation coefficient (PC_{BBB}) was also calculated through LFER.[22] The predicted PC_{BBB} of neutral and ionized VEN are 9.9×10^{-4} cm s^{-1} and 3.3×10^{-4} cm s^{-1}, suggesting that the ionization slows down the transfer of VEN from the blood to the fluid layer by a factor of 3.0. However,

both forms of VEN permeates not much slower than carbon dioxide ($PC_{BBB} = 4.4 \times 10^{-3}$ cm s^{-1}),[23] which can diffuse freely through plasma membranes. It suggests that VEN readily diffuses across the biological membranes. Note that the apparent PC_{BBB} of VEN at pH 7.4 is 3.4×10^{-4} cm s^{-1}, which was calculated based on the ionization degree and the PC_{BBB} of neutral and ionized forms, whereas those of ODV and NDV are 3.9×10^{-5} cm s^{-1} and 1.6×10^{-5} cm s^{-1}. This, coupled with the low blood levels of ODV and NDV (Figure 4b), may result in low levels of ODV and NDV in the LLF. Now it is easily understood why ODV and NDV were not detected in real time. However, if we use the lung/blood partition coefficient ($P_{lung/blood}$) to evaluate the tissue binding of drugs, it is found that the predicted $P_{lung/blood}$ of VEN is 11.69, which is higher than those of ODV and NDV (2.52 and 5.71). Thus, the effect of the tissue retention on the permeation from blood to LLF need to be further studied.

■ CONCLUSION

In conclusion, SESI-HRMS provides a robust way for online real-time monitoring of nonvolatile drugs in exhaled breath. More importantly, our results indicated that nonvolatile drugs enter into breath air via EBPs generated from LLF rather than the partition process. Therefore, a drug, in theory, can be detected by breath test as long as it can effectively diffuse into LLF, regardless of its volatility. However, when breath test is applied in TDM, we need to take into consideration two preconditions: (1) the drug should not produce side effects on the respiratory function, in particular the production of EBPs in the case of nonvolatile drugs and (2) the drug can be rapidly transferred from blood to LLF, with less tissue impedance or retention effect. It may be characterized by passive membrane permeability and $P_{lung/blood}$. The LFER models can certainly be used for other drugs in the field. All that is required is that the descriptors for a compound lie within the range of the descriptors used in the data set for modeling. We believe that this work advances our understanding on the transport of nonvolatile drugs from blood to breath air, offering a helpful guidance for applying breath test in TDM.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.1c00509.

> Experimental details of animals, materials and reagents, SESI-HRMS analysis of exhaled VEN, EBP collection, SESI-HRMS analysis of standard gas mixture, EBP offline analysis, UHPLC−MS/MS analysis of blood VEN, LFER calculations, and methodological validation of SESI-HRMS analysis; experimental setup for real-time monitoring of exhaled breath (Figure S1); signal intensities of gas standards detected with and without using SensAbues (Figure S2); MS/MS identification of VEN in EBP (Figure S3); MS/MS identification of ODV and NDV in EBP (Figure S4); PK profile of breath VEN for another two mice (Figure S5); schematic diagram of VEN transport from blood to breath air (Figure S6); respiratory function tests (Figure S7); linear correlation between VEN concentration in aerosol particle and signal intensity (Figure S8); nominal concentrations of calibration standards and quality control samples used in the UHPLC−MS/MS method

(Table S1); solute descriptors and predicted properties of VEN and its metabolites (Table S2); apparent permeability across the blood-brain barrier (Table S3); and coefficient of variation of intra- and interday measurements (Table S4) (PDF)

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Author Contributions

○Xing Chen and Keda Zhang contributed equally. Xing Chen conducted the investigation, formal analysis, and writing of the original draft preparation. Keda Zhang worked on the methodology, formal analysis, and writing of the original draft preparation and funding acquisition. Zhihong Yin conducted the investigation. Mingliang Fang performed the writing as well as review and editing. Weidan Pu worked on the writing and review and editing. Zhening Liu worked on the manuscript writing as well as the review and editing and writing of the funding acquisition. Lei Li provided the resources. Pablo Sinues worked on the methodology and performed the writing as well as review and editing. Robert Dallmann worked on the methodology and performed the writing as well as review and editing. Zhen Zhou provided the resources. Xue Li worked on the conceptualization, methodology, resources, writing and review and editing, supervision, and funding acquisition.

Notes

The authors declare the following competing financial interest(s): P.S. is cofounder of Deep Breath Initiative A.G. (Switzerland), which develops breath-based diagnostic tools.

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