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Original Investigation

Rapid automated blood sampling system for pharmacokinetics studies of cigarette smoking

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Abstract

Introduction: We developed an automated sampling system to allow multiple, discrete blood samples from a human participant to be collected rapidly and immediately following cigarette smoke exposure. We reported the details of the sampling system along with the results of a pilot study for evaluation of the system.

Methods: Components of the system include silastic tubing, solenoid pinch valves, a peristaltic pump, and a fraction collector. This system incorporates a smoking machine that allows precise delivery of cigarette smoke through a mouthpiece and intricate timing to correlate blood samples with smoke inhalation. All components are controlled via integration from a user interface and are fully customizable. We performed several tests to evaluate the equipment, including tubing dead volume, leakage tests, and sample reproducibility. We also performed a pilot study with 6 adult smokers, who received 6 controlled puffs of a research test cigarette. Each inhalation was followed by radial arterial blood collection (1 sample per second tapered to 1 sample every 4 s) for 1 min. Samples were evaluated for nicotine via liquid chromatography–tandem mass spectrometric methods.

Results: Sampling times and volumes were sufficient for nicotine analysis. No adverse effects were seen in the pilot study, and a 30-min washout period was deemed appropriate between puffs. A significant rise in plasma nicotine levels above baseline after inhalation of smoke was consistently detected in all participants.

Discussion: The unique advantage of this system is to allow rapid blood sampling after a puff of cigarette smoke, with the benefits of reproducibility, reduction in labor intensity, and high temporal resolution.

Introduction

Pharmacokinetic measurements of compounds, such as nicotine from cigarette smoke, are typically taken from surrogate body fluids such as blood, urine, or saliva due to the difficulties associated with determination of the compound's arrival directly at the target organ(s). In most cases, blood samples are taken intermittently by manual sampling at specific time intervals. This is a reasonable approach following oral or intramuscular administration, when plasma levels may take minutes to hours to reach peak levels. However, with intravenous (i.v.) or inhalation administration, levels in blood can increase from baseline within seconds, making it a challenge to measure with traditional blood sampling techniques.

Several studies have measured nicotine concentrations in arterial blood after smoking, with reported mean peak levels anywhere from 20 to 100 ng/ml (Gourlay & Benowitz, 1997; Henningfield, Stapleton, Benowitz, Grayson, & London, 1993). Rose, Behm, Westman, and Coleman (1999) sampled arterial blood after smoking or i.v. nicotine injection at a rate of one sample every 5 s, limited by practical and technical realities with manual sampling. However, to our knowledge, studies that characterize nicotine pharmacokinetics with a second-to-second temporal resolution are presently unavailable. These types of studies could yield important information about the absorption of nicotine immediately upon reaching the lungs.

Due to our need for a reliable, reproducible method to collect second-by-second arterial blood samples following cigarette smoke inhalation, we have developed an automated computer-controlled sampling system, which will allow multiple, discrete blood samples from a human participant. Our system is designed to minimize the amount of blood collection and maximize temporal resolution, with a capability of collecting a 1-ml blood sample every second, while also maximizing participant

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safety. In addition, to address the challenge of consistent dose delivery, this system incorporates a smoking machine that allows precise delivery of cigarette smoke through a mouthpiece and intricate timing to correlate blood samples with smoke inhalation. Other added features include the ability to present a computerized visual analog scale (VAS) for subjective responses, as well as bar code detection for samples and participant identification. Finally, several biomonitoring components serve as safety measures that can be observed by the investigator throughout the experimental procedure. This automated sampling system has the benefits of reproducibility, reduction in labor intensity, reduction in human error, temporal resolution, and protection of experimenters from exposure to blood.

Methods

System components

The blood sampling portion of the system design was based on a concept for a high-speed blood sampler for positron emission tomography (PET) studies in humans (Graham & Lewellen, 1993). Similar systems exist for blood sampling from small animals (DiLab, Inc., 2008; Graham, 1971; Graham & Abbott, 1984). A schematic that details the arrangement of our system components as designed is shown in Figure 1. Testing and evaluation of the system were done with saline in a laboratory setting, followed by a pilot study in human volunteers, as described below in Evaluation of System section.

As seen in Figure 1, individual blood samples from an indwelling arterial catheter (20 Ga., 4.45 cm; Hospira, Inc., Lake Forest, IL) are collected in test tubes that are advanced by a fraction collector (Gilson FC203B, Middleton, WI) contained within a plexiglass box fitted with a small carbon filter and fan to prevent sample contamination. Solenoid pinch valves, which are located along a network of sterile silicone tubing (Masterflex L/S; Cole-Parmer, Vernon Hills, IL), control the flow of blood and heparinized saline that are moved by a peristaltic minipump (Minipuls 3; Cole-Parmer). These valves were selected so as to fail in their native closed state, and the peristaltic pump is programmed to flow unidirectionally away from the participant at a preset speed of 1 ml/s. The valves, pump, and fraction collector are controlled by a Compact FieldPoint module (National Instruments, Austin, TX) onto which a custom-designed application, programmed in LabVIEW 7.1.1 (National Instruments), is downloaded. The software integrates all the electronic components and turns each on or off with precise timing based on an experiment-specific sequence defined by the investigator. These sequences are created in comma-separated value format and can be easily modified based on experimental needs without changing the main program. Program execution is monitored from two IBM ThinkPad notebook computers via the user interface. Bar code recognition allows input of participant identification or sample numbers that are stored with all other experimental data in a comma-separated value (.CSV)–formatted file.

A polypropylene stoppered waste flask collects any excess blood or saline pumped through the system and is replaced for each experiment. The custom-prepared silicone tubing set can be replaced with new sterile tubing for each participant, eliminating infection transmission between participants as well as contamination of samples. A heparinized 0.9% saline bag pressurized by an inflatable cuff allows flushing of the tubing as well as the catheter line for maintenance of patency. A pressure transducer monitors the amount of pressure (adjusted manually to maintain a pressure above the participant's systolic blood pressure) on the bag.

The biomonitoring equipment (MP150 System for Windows; Biopac, Inc., Goleta, CA) is an Ethernet-capable data acquisition and analysis system, consisting of a main acquisition unit and several interchangeable amplifier modules. The MP150 allows for simultaneous recording and display of the participant's vital signs (including heart rate, blood oxygen level, blood perfusion units, electrocardiogram), which can be monitored by the medical investigator during an experiment for any irregularities. In addition, preset upper and lower limits for physiological measures such as blood pressure and heart rate set within the programming provide an alert if the participant's vital signs are too high or too low, adding an extra measure of safety.

A smoking machine (RM102; Heinrich Burghart GmbH, Wedel, Germany) is incorporated into the system to provide a defined, precise amount of cigarette smoke for inhalation. Integrated control of the machine by the LabVIEW 7.1.1 software allows precise control of the timing and volume of the puff of smoke delivered. The smoke from a manually lit cigarette is inhaled by the RM102 upon a button press and then ejected through an exhaust port to a length of rigid Teflon tubing. This tubing conducts the puff of smoke from the RM102 to an adapter built onto a TSD117 medium-flow transducer and introduces the smoke into the air stream inhaled by the participant. A valve fitted opposite the mouthpiece of the transducer allows air to mix with the smoke at precise volumes and times as defined by the experimental sequence (Figure 1).

A flat-panel computer screen provides a visual display for the participant. An instruction to begin inhalation appears when the investigator presses a button. A scale is provided to show total inhalation volume and inhalation rate, which can be experiment-specific. The medium-flow transducer detects the beginning of inhalation by the participant (an investigator-defined threshold), signaling the blood sampler to begin collection. The volume and timing of the smoke bolus released from the smoking machine to the transducer are fully adjustable and can account for any smoke lost in the dead volume of the tubing. The smoke bolus is then mixed with clean air through the mouthpiece until a maximum inhalation volume is reached (investigator-defined in the experimental sequence; e.g., see Figure 2). The air valve closes and no more air can be inhaled. Blood collection then continues as defined for the remainder of the experimental procedure (again, investigator-defined) until all valves close and the pump shuts off. If desired, the flat-panel screen can then display a series of questions that can be answered via the VAS transducer by the participant, and all answers are recorded in the data file.

All the above components are housed in a stainless steel cart with lockable doors and wheels for security and portability. A vacuum hose with a filter and fan is inserted through the top of the cart to exhaust any excess smoke and safely duct it out of the building.

Programming/sequences

Precise integration of all components of the system is essential for execution of each experimental sequence. Sequence files defining each step of the experiment are created in Microsoft Excel and saved to the local network–attached storage device. The

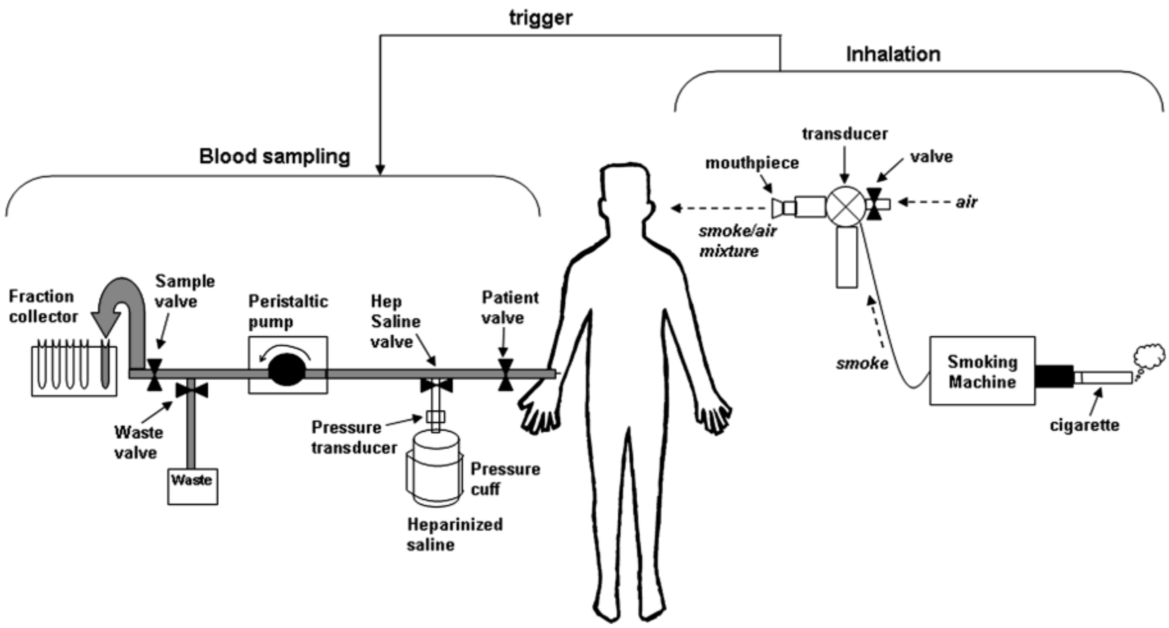

Figure 1. Schematic diagram of the blood sampling and smoke delivery components. All equipment is computer controlled via Compact Field-Point with LabVIEW 7.1.1 programming. The fraction collector is fully enclosed in a plexiglass box fitted with a carbon filter fan to prevent escape of aerosolized blood. The heparinized saline bag resides in a manually controlled pressure cuff, and the pressure is monitored by an in-line pressure transducer. The smoking machine prepuffs each cigarette and delivers the smoke to the mouthpiece at the appropriate time. The change in air velocity across the mouthpiece as the participant begins to inhale triggers the blood sampling machine.

investigator can then choose a particular sequence file prior to the start of each experiment. Each piece of equipment (valves, pump, fraction collector, smoking machine) receives a signal to turn on or off based on instructions from the sequence file. The step number and duration of each step in the sequence, rapidity of sample collection, and number of samples, as well as smoke delivery, can be fully customized by the investigator.

Data collected by the system include investigator log-in name and time, participant ID, biomonitoring data, inhalation start time, inhalation rate and volume, blood sample collection times, and subjective responses. All data are time-stamped and stored on the local network–attached storage device and can be copied to CD. The software allows operator and administrator permissions to be set so that all changes and operations are tracked and require a password. An administrator log-in allows changes to the hardware and software settings, changes to experimental sequences, updates to the user log-in list, running of the hardware on manual control, and other administrative tasks. An operator log-in only allows selection of an experimental sequence, entry of participant ID and demographics, and running of the experiment. Thus, there is a traceable audit trail of all activities within the software, as well as experimental data collection.

Evaluation of system

Prior to potential use in a research study setting, the following characteristics of the system were evaluated.

Tubing dead volume

In order to determine how much fluid would reside at any time throughout the length of the tubing set, 10 tubing sets were assembled and labeled, weighed, filled with deionized water, and reweighed. Tubing volume was determined based on weight difference and water density at the temperature the experiments were conducted (room temperature). Tubing sets consisted of the main tubing from the catheter to the fraction collector, the tubing to the waste container, and the tubing from the heparinized saline connected by polypropylene tubing connectors.

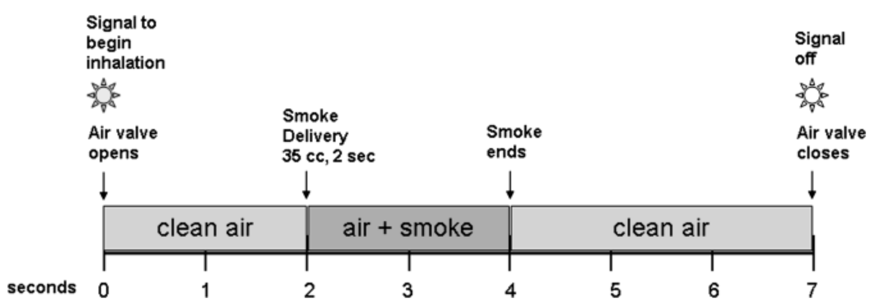

Figure 2. Smoke delivery procedure. When signaled, the participant begins inhalation of 2 s of clean air through the mouthpiece. Smoke (35 cc) is then mixed with the air for 2 s, followed by clean air for a maximum of 7 s or 900 ml total air inhalation, whichever is reached first.

Sampling reproducibility

To ensure consistency of sample collection within a single sampling cycle and from participant to participant, sampling volumes were measured. A sampling cycle was defined to collect one sample every second for 20 s, then one sample every 2 s for 10 s, and finally one sample every 4 s for 30 s, for a total of 60 s per cycle. This allowed the assessment of sample volume for various time settings. Saline was used in place of blood for these studies, with a small catheter inserted into a saline bag. A complete set of test tubes (40 test tubes per sampling cycle, six total cycles) was weighed before and after sampling. This test was repeated once a day for 5 days.

Contamination

Integrity of the tubing set is vital to avoid potential sample cross-contamination or exposure of the participant or the technical staff to biological fluids. The integrity of the tubing sets was tested by connecting a 1,000-ml bag of saline (injected with 10 ml of 50 mg/ml fluorescein in water) to one end of the tubing set. After each sampling cycle, tubing sets and all tubing connections were examined under black light illumination to check for any observed fluorescence outside the tubing system. This test was repeated once a day for 5 days.

Nicotine delivery

To ensure consistent delivery of a defined amount of smoke, a determination of nicotine delivery was performed. An in-line (between the smoke delivery port and the mouth piece) 19-mm Cambridge filter pad was used to collect the 4th puff from a control research model cigarette at three different puff volumes (35-, 45-, or 55-cc puff volume, 2-s puff duration). Following sample collection, the Cambridge filter pad was removed and weighed. The Cambridge filter pads were then extracted with 2 ml triethylamine-modified ethyl acetate, fortified with quinoline as an internal standard, and the extracts were analyzed by gas chromatography/mass spectrometry to calculate the nicotine concentration for each puff collected. A series of six cigarette puffs per day was run for 6 days.

Pilot study

A double-blind, multiple measurement pilot study was conducted to (a) evaluate the intrasubject and intersubject variability and (b) to determine the suitability of sampling times and sample volumes to characterize the full arterial blood nicotine concentration–time curve. The pilot study was conducted at the Center for Drug Studies, School of Pharmacy, at Virginia Commonwealth University. This study was conducted in accordance with Good Clinical Practices, as contained in the U. S. Code of Federal Regulations (Title 21, Parts 50 and 56), and was approved by Western Institutional Review Board prior to participant recruitment.

Participants were six healthy adult male and female smokers between the ages of 21 and 65 years. Participants had a history of smoking 10–30 cigarettes/day for at least 12 months prior to study initiation of manufactured nonmenthol cigarettes with a tar content of 7–12 mg tar/cigarette. Females who were pregnant, lactating, or intending to become pregnant during the course of the study were excluded. Use of any other type of nicotine-containing product other than manufactured cigarettes was prohibited. Participants with a history of drug or alcohol abuse were excluded from the study. No food or beverages containing alcohol were permitted for 48 hr prior to check-in. All participants were required to provide a medical and medication history, undergo a physical examination (including vital signs), and provide blood and urine for laboratory analysis. Written informed consent was obtained from each participant following explanation of the study procedures and any possible risks or benefits of participation.

Each participant underwent a training session after checking into the clinic to become familiar with the smoke inhalation paradigm. The participants were then confined to the clinic overnight with no smoking allowed after 11 p.m. Approximately 1 hr after a light breakfast, the Allen's (1929) test for integrity of the radial and ulnar arteries was performed on each participant. Based on results of the test, arterial puncture and insertion of an arterial catheter were performed. The participants were seated in a comfortable chair and biomonitoring detectors (pulse, blood oxygen, electrocardiogram, laser Doppler flow) were attached. Each participant received six single puffs from a research test cigarette during the experiment. These test cigarettes were manufactured at Philip Morris USA (Richmond, VA) for research purposes only, with Federal Trade Commission (FTC) parameters as follows: tar, 9.2 mg/cigarette, and nicotine, 0.81 mg/cigarette. Only the 4th puff from each test cigarette was delivered for inhalation—each cigarette was presmoked (three puffs) using the smoke delivery instrument (programmed to FTC smoking protocol: 2 s, 35-cc puff at 1-min intervals). Thirty seconds after a baseline blood sample was taken, the participant was instructed to inhale the 4th puff of cigarette smoke (35 cc), which was mixed with clean air through the mouthpiece of the smoke delivery instrument. Blood samples after each puff were collected at 0 s (preinhalation) and postinhalation at 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, 12.0, 13.0, 14.0, 15.0,16.0, 17.0,18.0, 19.0, 20.0, 22.0, 24.0, 26.0, 28.0, 30.0, 34.0, 38.0, 42.0, 46.0, 50.0, 54.0, 58.0, and 62.0 s. Between each of the six puff inhalations/arterial blood collections, there was a 30-min washout period during which no smoking was allowed and the arterial catheter was flushed every 10 min with heparinized saline. Approximately 300 ml of blood was collected during the entire study for nicotine analysis. Plasma samples were analyzed using a validated liquid chromatography–tandem mass spectrometric (LC-MS-MS) method (Byrd, Davis, & Ogden, 2005) with a limit of quantification of 2.5 ng/ml for nicotine. Pharmacokinetic parameters (area under the curve [AUC], peak nicotine concentration [C_{max}], and time-to-peak concentration [T_{max}]) were determined using a one-compartment model with lag time and first-order elimination via WinNonlin 5.0.1 (Pharsight Corp., Mountain View, CA), and estimates of the coefficient of variation (CV) and the intraclass correlations were obtained using a repeated measures analysis of variance.

Safety assessment

During the pilot study, each participant was closely observed by a physician and vital signs recorded via the biomonitoring system. Adverse events were documented and treatment, if necessary, was administered. The arterial catheter was also observed throughout the study to ensure that no leakage or patency issues occurred. Visual inspection of the heparinized saline backflush into the catheter was confirmed after each puff by the study nurse. The blood collection system was observed for any malfunctions or leaks throughout the experiment. After each puff, the fraction collector and plexiglass enclosure were examined for blood leaks or drops.

Results

Tubing dead volume

The mean weight of water captured in the tubing was 2.8091 ± 0.0625 g (95% *CI*). Using a value for the density of water of 0.99821 g/ml (measured at 20 °C and 101.3 kPa), the mean dead volume of a complete tubing set was 2.814 ± 0.065 ml (95% *CI*). Based on this measurement, the dead volume for the portion of the tubing set running directly from the catheter to the fraction collector was estimated to be 1.95 ml. This was the smallest volume achievable due to the design of the sampling system and allowed for each sample to be a reasonable measurement of each timepoint in the pilot study.

Sampling reproducibility

The mean saline collection volume per test tube during the one-sample every second saline sampling time was 0.939 ± 0.007 ml (95% *CI*), while the mean collection volume per test tube during the one-sample every 2-s and one-sample every 4-s sampling times combined was 1.090 ± 0.010 ml (95% *CI*). The average total volume of saline collected in the test tubes over six puffs/sampling cycles in 1 day was 234.1 ± 8.3 ml. There was no significant difference in average overall sample volume over the 5 days of measurements nor was there any significant difference in average total volumes for any set of test tubes within each day. Since the analytical methods used to measure nicotine in each potential blood sample from the pilot study would only require 200 μl of plasma, the collected volumes were deemed sufficient for future research studies.

Contamination

Under the test conditions utilized, fluorescein was not detected on the instrument or exterior of the tubing on any of the 5 days of testing. Thus, no leakage was observed.

Nicotine delivery

The mean nicotine levels (±95% *CI*) extracted from the Cambridge filter pads over a 6-day trial were 82.12 ± 4.69 μg/puff for the 35-cc puff, 93.84 ± 6.96 μg/puff for the 45-cc puff, and 115.31 ± 8.75 μg/puff for the 55-cc puff. As expected, nicotine concentrations reliably increased as puff volume increased. To assess puff-to-puff variability, the set of all 4th puff nicotine measurements made with a 35-cc puff volume (excluding Grubbs' test outliers) over the 6-day trial was evaluated. The percent relative *SD* (%RSD) was determined to be between 7.1% and 11.1%. The variation in nicotine deliveries from day to day was not statistically significant.

Pilot study

Fifteen volunteers were screened for the study to ensure that six participants (five males and one female) were enrolled in the pilot study. The six participants had a smoking history ranging from 12 to 20 cigarettes/day for a duration of 5–12 years. The data from the pilot study are presented in Figure 3. One participant was only able to complete one of the six total puffs due to problems with faulty tubing (data not shown). The nicotine AUC for each puff from each participant ranged from 194.8 to 766.3 ng/ml/s, with an overall mean of 429.2 ng/ml/s (±*SD* 148.1). Overall, the intrasubject variability for the AUC ranged from 19.5% to 25.6% CV, while the intersubject variability for AUC ranged from 21.1% to 48.7% CV. The C_{max} values in all participants ranged from 4.3 to 27.7 ng/ml, with an overall mean of 12.7 ng/ml (±4.9), indicating a significant rise in plasma nicotine levels above baseline after inhalation of smoke. Thus, cigarette smoke inhaled from the mouthpiece effectively reached the lungs. The time to peak nicotine concentration (T_{max}) ranged from 8 to 24 s. Statistical analysis determined that a sample size of 24 participants would be sufficient in future pharmacokinetic studies to detect a minimum difference of 20% between two test cigarettes with an 80% probability. The sampling times, ranging from one sample every second to one sample every 4 s, were well tolerated by the participants and yielded the expected number of samples. The volumes of the blood samples collected throughout the study were sufficient for analytical analysis (approximately 1 ml). The 30-min washout period was determined to be adequate for nicotine washout as plasma levels of nicotine returned to similar baseline levels prior to each successive puff.

Safety assessment

All adverse events reported during the pilot study were listed as mild in severity. The most commonly reported adverse events were redness, tenderness, and discomfort at the catheterization site and were determined by the investigator to be procedure-related but not study product-related. There were no serious adverse events (SAEs) reported during the study. At no time during the study did the participants' vital sign readings exceed the upper and lower safety limits determined by the physician. No leaks were observed throughout the blood sampling system or at the catheter site, and the heparinized saline provided sufficient flushing of the catheter with no patency problems.

Discussion

The system described here allows rapid arterial blood sampling following cigarette smoke inhalation. Furthermore, this type of sampler can be programmed to sample blood at any predetermined rate, for any preset sample volumes, timing, and number of samples. The smoking machine can be easily disconnected, allowing the sampler to be used in conjunction with more conventional dosing regimens.

Previous investigators have manually sampled arterial plasma every few minutes after cigarette smoking (Gourlay & Benowitz, 1997; Henningfield et al., 1993). However, it has been speculated that peak nicotine concentration may reach levels near 100 ng/ml within the first few seconds after cigarette smoke inhalation (Gourlay & Benowitz; Henningfield et al.), suggesting that faster sampling is necessary to detect such levels. Rose et al. (1999) sampled arterial blood manually every 5 s during cigarette smoking, the most rapid sampling regimen published thus far. With our safe automated sampling system, we have increased the temporal resolution past the capability of manual blood draws to one sample per second. This resolution is important to detect the true peak and rise time for rapidly absorbed product components such as nicotine. The increased number of datapoints gained from this rapid sampling technique allows us to visualize the shape and magnitude of the peak concentration in the arterial blood.

The advantages to such rapid automated sampling include the ability to collect blood samples safely and at high speeds

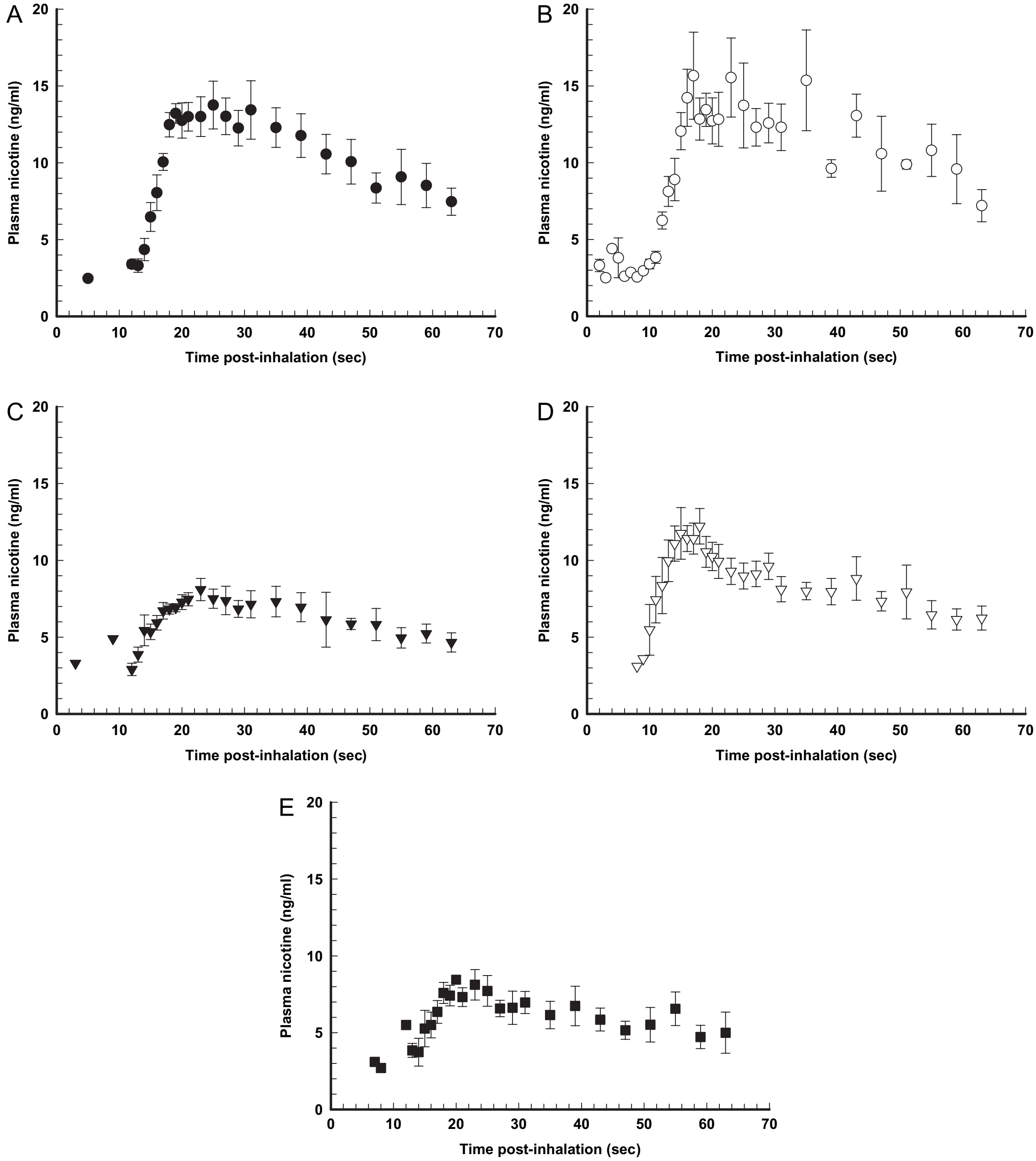

Figure 3. Individual pilot study data depicting plasma nicotine concentration profiles for five adult smokers (panels A–E) following inhalation of a single puff of cigarette smoke from a research cigarette. Each datapoint represents the mean ± *SEM* of six puff iterations. Error bars not shown are too small to be seen on the graphs. The participant in panel B completed only five puffs.

when manual blood draws are not feasible. This is critical with compounds that exert a very rapid effect on the body and/or brain as they reach the circulation within seconds of administration. In addition, human error is eliminated with such an automated system, and consistency of sampling is preserved. The ease of altering the sampling sequence to fit the experimental needs makes this system fully adaptable to many types of studies. Discrete blood samples yield exact sampling time correlations so that a time–concentration curve can be constructed. Due to the slight but possible risk with arterial catheterization and blood draw, the design of the system incorporates many safety features as described earlier. The ability to automatically flush the tubing and catheter with heparinized saline adds in another level of safety, as well as potentially allowing longer experiment duration without discomfort or complications. The tubing can easily be replaced between participants to eliminate potential transmission of infection. These added features improve upon a system previously built for blood sampling during PET studies (Graham & Lewellen, 1993).

The sampler consistently provided saline samples around 1 ml that, when extrapolated to blood, is more than sufficient in volume to extract plasma and analyze for nicotine and/or its metabolites by LC-MS-MS. The total amount of saline collected over six puffs/sampling cycles was approximately 235 ml, a lower volume than that collected from a typical blood donation. Thus, the experiment is well designed to prevent excess or dangerous amounts of blood collection from the participants. No leaks were found after vigorous testing of the tubing sets, assuring that the connections between segments of tubing hold fast when fluid is flowing. The smoking machine provided reliable puff volumes and nicotine delivery as programmed, as shown by nicotine measurements on the Cambridge filter pad in-line with the mouthpiece.

From the pilot study, we concluded that this system was able to accurately and reproducibly collect human blood samples in the timeframe selected. The volume of each of the blood samples was as expected (approximately 1 ml), and no leaks were observed in the blood sampling system. No SAEs were reported, and only mild discomfort at the catheter insertion site was noted in some participants. We also concluded that changes in nicotine concentrations in arterial blood within the first minute after inhalation of cigarette smoke were readily detectable when collected by this device. We saw consistent concentration–time curves for nicotine in arterial blood with relatively low intrasubject variability for the six puffs (Figure 3). The five participants did differ somewhat in their C_{max} values, but all showed a rapid rise in nicotine, peaking at around 15–20 s and declining in a similar fashion. As seen in Figure 3, the data from just five participants are extremely consistent as compared with other studies (specifically, nicotine pharmacokinetic studies) reported in the literature (Gourlay & Benowitz, 1997; Henningfield et al., 1993; Rose et al., 1999) and prompted a subsequent full-scale study (McKinney et al., 2010). We believe that most of the variability in the pilot study can be attributed to the range of inhalation volume/velocity (150–250 ml/s) but not differences in the blood collection or device function. Compared with the variability one might see when collecting discrete blood samples by hand (Rose et al.), the data presented herein are at least equivalent, if not tighter. The most important finding in this report is that the device as built is able to safely collect blood samples as defined and deliver accurate boluses of smoke upon command.

Sample size calculations for future studies were based on the 80/20 rule to estimate the sample size of future studies that would be large enough to provide at least an 80% probability of detecting a minimum difference of 20% between the means of two treatments (e.g. two different test cigarettes). Based on these estimates, a sample size of 24 individuals was considered sufficient for statistical comparison of pharmacokinetic parameters between two treatments in future studies. The sample size in the present report reflects a pilot study to ascertain the function and applicability of the device. In addition, restrictions on what an institutional review board would allow for a method development study (first-time use of a new device) would prohibit us from using a larger sample size in this situation. We have recently completed a full-scale double-blinded crossover study with 28 participants looking at nicotine pharmacokinetics after smoking cigarettes with two different levels of ammonia in smoke (McKinney et al., 2010).

In summary, we have successfully designed, built, and tested a rapid automated sampling system, which will permit the study of immediate pharmacokinetics of cigarette smoke in human arterial blood samples. Further adaptations to the system could allow for measurement of any compound delivered in any fashion. The reliability and reproducibility of this sampling device make it an essential research tool for characterizing the absorption of fast-acting compounds and their potential delivery to target organs.

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Declaration of Interests

None declared.

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