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A System for Administering Quantified Doses of Tobacco Smoke to Human Subjects: Plasma Nicotine and Filter Pad Validation

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GILBERT, D. G., R. A. JENSEN AND C. J. MELISKA. *A system for administering quantified doses of tobacco smoke to human subjects: Plasma nicotine and filter pad validation.* PHARMACOL BIOCHEM BEHAV 31(4) 905–908, 1988.—A new, automated system for administering quantified doses of cigarette smoke to human subjects is described and results of two studies demonstrating the reliability and validity of the system are presented. To overcome the large variability in nicotine and tar delivery associated with previous means of controlling smoke delivery, an automated quantified smoke delivery system was constructed. The system increases the precision and reliability of the smoke and nicotine dose delivered to human subjects. The quantified smoke delivery system was found to deliver doses of nicotine with a substantially greater degree of precision than procedures typically used in previous laboratory studies of smoking behavior.

Smoking Nicotine delivery Plasma nicotine Quantified smoke delivery

THE characterization of dose-response relationships and the assessment of individual differences in response to smoking have been seriously inhibited by lack of a method for delivering accurately quantified doses of nicotine via the smoking route. Previous attempts to quantify nicotine delivery have generally achieved only limited success. To date, the most commonly employed technique is to have experimental subjects smoke cigarettes with different Federal Trade Commission (FTC) smoking-machine-estimated nicotine deliveries. However, the quantity of nicotine actually obtained from a cigarette with a given FTC-estimated delivery varies considerably from smoker to smoker (6,7). For example, evidence suggests (15) that only 25% of the individual differences in blood-nicotine concentrations after smoking can be attributed to the measured nicotine delivery from a given type of cigarette. This variability results from large between-smoker differences in puff volume, puff frequency, and depth of inhalation (15). In one study (28), puff volumes varied from 17 to 83 ml and puff flow rates varied from 5.6 ml/sec to 81 ml/sec. Puff durations ranged from 0.9 to 3.2 sec, while puff intervals varied from 22.0 to 72.0 sec. In addition, the manner in which individuals smoke depends in part on the type of cigarette being smoked (1, 2, 9, 10, 22).

FTC nicotine-delivery estimates for a given type or brand of cigarette are based on data obtained from machine smoking of these cigarettes with a series of 35-cc sinusoidal-shaped puffs taken at one-minute intervals. That procedure is continued until the char line reaches a point 3 mm from the filter overwrap, or to a 30-mm butt length in the case of unfiltered cigarettes. Even subtle deviations from these parameters create substantial changes in nicotine delivery (8,23).

Recognizing the importance of another factor, puff-frequency, in determining the amount of nicotine delivered to a subject, some investigators (12,18) have instructed subjects to puff in response to a signal, such as a tone, presented at predetermined intervals. Other investigators have monitored, but not controlled, variables such as interpuff interval, puff duration, and/or puff volume (19). Multiple regression equations using puff volume, puff number, interpuff interval, FTC machine-estimated nicotine delivery, and several inhalation parameters may be able to predict actual nicotine delivery to a relatively high degree, and a large percentage of the variance in nicotine delivery can be accounted for using these predictors (15). However, while such estimates of nicotine delivery are useful, most researchers would prefer to specify and control nicotine delivery, rather than simply monitor it.

In a preliminary attempt to control nicotine delivery more precisely (21), a manually operated syringe was used to take a series of 35-cc puffs from cigarettes. The smoke from the syringe was then injected into a plastic bag filled with 2000 cc of air. Subjects then inhaled the air and smoke mixture from the bag. While this method represents an improvement over ad lib smoking procedures, it still presents a number of problems when applied in quantified smoking research settings. One problem is that the speed and shape of the puff are entirely dependent on manual movement of the syringe

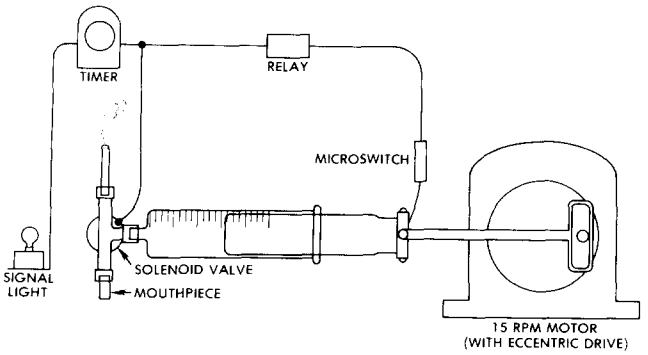

FIG. 1. Schematic representation of Quantified Smoke Delivery System.

plunger. Another is that it is difficult to measure the amount of nicotine actually delivered to an experimental subject with this system. Another manually operated system (17) involved injecting smoke directly into the subject's mouth. However, this system was not evaluated for mean nicotine delivery and for variability. Furthermore, the determination of delivery of nicotine by this system, as with the system described above (21), is difficult and only approximate since it is manually operated and because slight variations in puff shape (8) and smoke residence time in such systems are likely to alter smoke and nicotine delivery substantially. Variation in residence time in the system is likely to create variance in nicotine delivery because of smoke particle deposition on system surfaces and because smoke particle size rapidly increases from second to second (16). Larger particles are more likely to impact in the mouth and upper respiratory tract (21), from which nicotine is much less rapidly and completely absorbed than from the lungs (4).

We recently developed a motorized and automated quantified smoke delivery system (QSDS) that overcomes many of the shortcomings associated with other ways of controlling nicotine delivery. Data on the effects on heart rate of quantified doses of smoke delivered by the QSDS demonstrate that the physiological effects of smoking with this system are similar to those observed during natural smoking, and that the magnitude of these effects varies as a function of the FTC machine-estimated deliveries of the cigarettes used (11). For example, cigarettes with FTC nicotine deliveries of 0.0, 0.6, and 1.2 mg produced mean changes in heart rate of −0.82, +6.99, and +10.86 beats per minute, respectively, during the time period from 6 to 7 minutes after completion of smoking.

In Experiment 1 of the present report, data on the calibration of the fully-automated QSDS are presented. Experiment 2 reports the effects of quantified doses of smoke delivered by the QSDS on plasma nicotine concentrations measured five minutes after completion of smoking.

EXPERIMENT 1: SYSTEM CALIBRATION

The QSDS is depicted in Fig. 1. Consistent with FTC standards for machine smoking, the system takes 2-second-duration, 35 cc, sinusoidal-shaped puffs drawn at 60-second intervals by a 50-cc glass syringe with a 6.4 mm diameter opening. The plunger of the syringe is moved by a mechanical linkage attached to a 15 rpm motor. The cigarette is connected to the syringe by means of a 2.0 cm piece of Penrose drain tubing (7.9 mm diameter) and a Teflon® lined, two-way solenoid valve with an internal orifice diameter of 6.4 mm. The filter end of the cigarette is placed approximately 8 mm into one end of the drain tubing, while the other end of the tubing is connected to the valve.

TABLE 1

MEAN QSDS NICOTINE DELIVERIES FOR CIGARETTES WITH TWO DIFFERENT FTC-ESTIMATED NICOTINE DELIVERIES

FTC-Estimated Delivery (mg/cigarette)	Measured Nicotine (mg/cigarette)	N	S.D.
0.6	0.46	20	0.08
1.1	0.85	20	0.11

Using the system involves the following steps: 1) the two-way valve connecting the cigarette and mouthpiece to the syringe is automatically positioned so that the path from the cigarette to the syringe is open; 2) the motor connected to the plunger of the syringe is started by pressing the puff button as the cigarette is lit at the onset of the first puff; 3) after the plunger has been withdrawn 35 cc over a two-second period, closure of a switch turns on a signal light and activates the two-way solenoid valve to provide a pathway between the syringe and the mouthpiece; 4) the smoke is immediately injected into the mouth of the smoker who has been previously trained to suck the smoke through a straw directly into his mouth as it is ejected; this ejection occurs over a period of two seconds as the motor pushes the piston forward; 5) the smoker then inhales and holds the smoke in his lungs, holding his mouth open so that the experimenter can verify inhalation until the signal light automatically turns off, five seconds after the beginning of inhalation. (Preliminary tests showed subjects had no difficulty performing these operations consistently.)

For calibration studies, cigarettes of two different FTC machine-estimated nicotine deliveries (0.6 and 1.1 mg) were used. For each cigarette, a Cambridge filter assembly (weighed to the nearest 0.1 mg) was placed in series with the mouthpiece of the system. The system was then operated so that a series of 35-cc puffs were delivered, once per minute, through the filter to a vacuum source attached to the mouthpiece. Degree of vacuum was adjusted to "inhale" the smoke through the filter over a two-second period. Approximately 10 "puffs" were used to "smoke" the cigarette to a point where the char line was within 3 mm of the filter overwrap. Then the Cambridge filter assembly was removed from the system and reweighed to the nearest 0.1 mg, yielding a measure of total moisture and particulate matter. The filter pad was then removed from the assembly, individually sealed in a labeled vial, and refrigerated until shipped by air express on ice for analysis by the Kentucky Tobacco and Health Research Institute, Lexington, KY. Methanol rinses from each filter were analyzed for nicotine content by gas chromatography. Each type of cigarette tested was given twenty smokings, each on a separate filter.

RESULTS AND DISCUSSION

Table 1 shows measured nicotine deliveries obtained by gas chromatographic analysis of the Cambridge filter pads. As these data indicate, measured values of nicotine delivered by this system are approximately 77% of the FTC machine-

estimated value for each type of cigarette. The relatively small standard deviations associated with these values suggest that cigarette-to-cigarette variability was small. Hence, with QSDS, measured nicotine deliveries, while somewhat lower than those obtained with FTC smoking machines, were quite reliable from cigarette to cigarette.

EXPERIMENT 2: EFFECTS OF SMOKING VIA THE QSDS ON PLASMA NICOTINE CONCENTRATIONS

Subjects

Six Caucasian males, aged 21 to 47 years (Mean=29.8, S.D.=9.9 years), and weighing 76.4 to 93.2 kg (Mean=82.1, S.D.=6.4) were each paid $40 for their participation. Each was a habitual daily smoker of not fewer than 10 cigarettes of not less than 0.7 mg FTC nicotine delivery. Potential subjects who reported chronic use of CNS- or ANS-active substances, or who reported chronic medical problems were excluded from the study.

Cigarettes

Cigarettes with two different FTC machine-estimated nicotine deliveries were used. They were 85-mm, University of Kentucky Smoking and Health Research Institute research cigarettes: 1A3 (1.28 mg FTC-estimated nicotine; 29.0 mg tar) and 2R1 (2.45 FTC-estimated nicotine; 36.0 mg tar). Prior to use, cigarettes were conditioned at 60% relative humidity for a minimum of 48 hours in a humidor containing a standard 2:1 (vol/vol) mixture of glycerin and water (26).

Procedure

Subjects participated in one orientation session and two experimental sessions. During orientation the goals of the study were explained, an informed consent form was signed, and subjects practiced smoking one 2.45-mg FTC-estimated nicotine delivery cigarette via the QSDS. The next day each subject came to the laboratory after overnight smoking abstinence, which was verified by measuring exhaled carbon monoxide. Each subject's arm was cleaned with alcohol and a baseline 10-cc blood sample was drawn into a heparinized Vacutainer® by a professional phlebotomist. The arm was then wrapped in clear plastic film to prevent contamination of the skin with cigarette smoke particles and the subject was escorted to the room containing the QSDS. Each subject then smoked one of the Kentucky Reference Cigarettes (either 1.28 mg or 2.45 mg FTC nicotine delivery); order of cigarette presentation was counterbalanced across subjects. Five minutes after completion of the cigarette, a second blood sample was taken. The entire procedure was repeated on the following day with the other cigarette.

Blood samples were stored on ice for a minimum of 20 min and a maximum of 1 hour. They were then centrifuged at 750×g for 20 min. The plasma was decanted, transferred to a sterile capped tube, and stored at −90°C until shipped on dry ice to Dr. Neil Benowitz's laboratory in California for nicotine assay (14).

RESULTS

Plasma nicotine concentrations after smoking with the QSDS are reported for each individual subject in Table 2. The ranges and standard deviations of plasma nicotine concentrations were relatively small for each of the two FTC cigarettes. A *t*-test for related measures confirmed that the two cigarettes produced substantially different plasma nicotine concentrations, as expected, $t(5)=8.91$, $p<0.001$. In addition, the ratio of mean plasma nicotine concentrations of 2.26 for the two cigarettes closely approximates the ratio of FTC machine-estimated nicotine deliveries of 1.91.

TABLE 2

PLASMA NICOTINE CONCENTRATIONS (ng/ml) FIVE MINUTES AFTER SMOKING WITH THE QUANTIFIED SMOKE DELIVERY SYSTEM

	1.28 mg FTC Nicotine		2.45 mg FTC Nicotine	
Subject	Baseline	Post-Smoking	Baseline	Post-Smoking
1	<1.0*	8.0	<1.0	14.5
2	<1.0	8.4	<1.0	16.8
3	<1.0	6.0	<1.0	17.8
4	<1.0	7.3	<1.0	18.5
5	<1.0	8.6	<1.0	22.4
6	<1.0	9.0	<1.0	17.1
Mean	<1.0	7.9	<1.0	17.8
S.D.	—	1.1	—	2.6

*All presmoking baseline values were determined to be below the limit of sensitivity of the gas chromatographic assay of 1.0 ng/ml.

DISCUSSION

The few studies reporting data on the relationship between FTC estimated nicotine delivery and plasma nicotine concentrations after smoking illustrate the wide variability in results obtained with ad lib smoking. For example, one study (13) reported mean (±SD) increases in plasma nicotine concentrations of 24.01 (17.14) ng/ml after 10 subjects smoked four cigarettes with estimated mean (±SD) nicotine deliveries of 0.94 (0.18) mg. Both absolute and percent change in pre- and postnicotine blood concentrations were not significantly related ($p>0.05$) to nicotine yield of cigarette, number of puffs per cigarette, mean puff duration, or machine-estimated nicotine intake. Another study (27) reported a somewhat smaller mean increase in plasma nicotine of 20.5 ng/ml (SD=5.6) in 6 subjects who smoked two 1.5 mg nicotine delivery cigarettes, "consecutively." But using roughly half that dose in a single, 1.46-mg FTC-estimated nicotine delivery cigarette (20) produced a mean elevation in blood nicotine concentration of 16.8 ng/ml (range 7.9–25.7 ng/ml, S.D.=6.73, N=6). Seyler and colleagues reported mean peak plasma nicotine levels of 7.8 ng/ml (24) and 8.4 ng/ml (25) after subjects smoked two 0.48 mg FTC-estimated nicotine delivery cigarettes within 15 minutes. These values are comparable to the mean value of 7.9 ng/ml we obtained when subjects smoked a single cigarette whose total nicotine delivery (1.28 mg) was similar to the combined total of the two 0.48 mg cigarettes. However, the earlier study (24) reported a range of plasma nicotine concentrations of 1–17 ng/ml with 10 subjects (S.D.=5.4). The range and S.D. for our six subjects smoking via the QSDS were 6.0–9.0 ng/ml and 1.1, respectively. Thus, the QSDS delivers quantified doses of nicotine with considerably less intersubject variability than found with ad lib smoking procedures.

Individual differences in plasma nicotine concentrations following smoking are not attributable entirely to differences in nicotine ingestion. Two different smokers inhaling identi-

cal amounts of nicotine may differ in their plasma nicotine concentrations due to individual differences in tissue distribution, metabolism, and excretion of the drug (3). For example, plasma nicotine concentrations were measured in five subjects during and after 30-minute, intravenous nicotine infusions (5). Nicotine infusions of 60 μg/kg elevated mean plasma nicotine concentrations to approximately 28 ng/ml. But even with constant infusion rates of 2 μg/kg/min, standard deviations for the observed plasma nicotine values were in the order of 10–20 ng/ml during the infusion period. Thus, even tightly regulated, controlled intravenous nicotine infusions can produce considerable subject-to-subject variability in plasma nicotine concentration.

The QSDS is currently being used in our laboratory to establish dose-response relationships for smoking-delivered nicotine and a variety of physiological and psychological dependent measures. Because the system makes it possible to precisely control the dose of nicotine administered, problems arising from individual differences in puff frequency, volume, and topography (6,15), and from deviations from FTC machine-smoking standards (8) can be eliminated. Furthermore, since it provides a degree of reliability and precision of dose delivery which cannot be obtained with ad lib smoking, the QSDS makes it possible to assess and evaluate individual differences in physiological and subjective reactions to given doses of nicotine with far greater accuracy than has heretofore been possible. Thus, we believe the development and validation of this automated QSDS is an important new methodology that we encourage other investigators to incorporate in their smoking research.

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