Nicotine pharmacokinetics and its application to intake from smoking

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1 Five subjects were given 25 µg/kg nicotine intravenously over 1 min, before and after a loading period involving the smoking of six cigarettes.

2 Plasma nicotine concentrations declined in a biphasic manner, the half-lives of the initial and terminal phases averaging 9 min and 133 min respectively.

3 Terminal half-lives before and after the loading period were essentially the same suggesting the absence of saturation kinetics at nicotine concentrations that build up during smoking.

4 The plasma clearance of nicotine and the volume of distribution were very high averaging 915 ml/min and 173 l, respectively.

5 Two approaches were used to calculate the nicotine intake from smoking. The average dose of nicotine absorbed from one cigarette was 1.06 mg which was 82% of the standard machine-smoked yield of 1.3 mg.

6 To illustrate their potential use in 'nicotine titration' studies, these approaches were used to compare nicotine intake from smoking a high (2.4 mg) and low (0.6 mg) nicotine cigarette. The dose of nicotine absorbed averaged 1.14 mg and 0.86 mg per cigarette respectively, being 48% and 143% of the machine-smoked yields.

Keywords nicotine smoking pharmacokinetics

Introduction

Knowledge of the disposition and fate of nicotine in humans is important for understanding better the nature of the smoking habit and its effects on health. In two early studies the half-life of nicotine in plasma was found to be about 30 min (Armitage *et al.*, 1975) or less (Isaac & Rand, 1972). Both these studies derived their estimates from the first cigarette of the day in smokers who had abstained overnight and were based on blood concentrations from both initial and terminal phases which were not differentiated. We have shown

that with repeated dosage accumulation of nicotine occurs with an apparent corresponding increase in half-life (Feyerabend & Russell, 1978; Russell *et al.*, 1977). This could be due to saturation of the metabolic enzymes or to the gradual elimination of the initial phase. This question was not considered in two further recent kinetic studies based on intravenous administration of nicotine. Moreover, the terminal plasma half-life of nicotine was somewhat different in these studies, averaging 0.8 h in one (Kyerematen *et al.*, 1982) and 2 h in the other

(Benowitz *et al.*, 1982), but in the former case calculations were based on a less precise one-compartment model.

Most detailed kinetic studies involve the intravenous route of administration. This is because the dose reaching the systemic circulation is known. In the case of nicotine, however, knowledge derived from this form of administration needs to be applied to the natural form of intake, namely the inhalation of tobacco smoke. We report here an approach to estimate the dose of nicotine absorbed from a cigarette based on a principle, often used in bioequivalence testing, that the area under the plasma concentration *vs* time curve (AUC), during one dosing interval at steady state on repeated dosing is equal to the AUC to infinite time for a single dose.

We describe four experiments undertaken to answer these questions by investigating the pharmacokinetics of nicotine after intravenous injection and cigarette smoke inhalation in habitual smokers.

Methods

Experiment 1

The subjects were five healthy men, aged 24 to 41 years, who were habitual smokers. Their average cigarette consumption was 25 per day (Table 1). They were studied in the morning after at least 12 h abstinence from smoking. To standardise for the effect of urinary pH on nicotine excretion, the subjects were given 2 g ammonium chloride orally four times on the day before the experiment.

At the start of the experiment each subject received a dose of 25 µg/kg nicotine base made up in 10 ml of buffered normal saline. It was given intravenously over a period of 1 min. One hour after the intravenous dose of nicotine, the subjects smoked their usual brand of cigarette at a rate of one per 0.5 h to a total of six cigarettes, each one being smoked over a 5 min period. A second intravenous injection of nicotine was then given 25 min after the last cigarette, the dose being the same as for the first injection (Figure 1). Blood samples (10 ml) were taken predose and at 2, 5, 10, 15, 20, 25, 30, 45 and 60 min after the first intravenous dose of nicotine. The same sampling times, with the omission of the 25 min sample, were used after the second intravenous dose, with additional sampling at 90, 120, 150 and 180 min after the injection. Blood samples were also

Figure 1 Plasma nicotine profiles of five subjects following three intravenous nicotine injections (i.v.) and smoking six cigarettes (C), plotted on a semi-logarithmic scale. Subject identification is as follows: ●—● PR, *—* BW, ○—○ JL, ■—■ KK, □—□ SS

taken at 2, 10 and 25 min after finishing each cigarette, apart from the first subject (JL) who was sampled at only 2 and 25 min. The same dose of nicotine was injected a third time 3 h after the second dose and blood samples were taken at the same times up to 2 h.

All blood samples were collected via an indwelling cannula inserted into a forearm vein which was never from the same arm as used for the intravenous injections. The blood samples were centrifuged and the plasma fractions stored at -20°C until analysed for nicotine. Saliva was collected continuously at 5 min intervals for the duration of the intravenous phases of the study. Total voided urine was collected at hourly intervals throughout the study period. The volumes and pH were measured immediately and aliquots were stored at -20°C until analysed for nicotine.

Experiment 2

Following the first intravenous dose in Experiment 1, the true log-linear decline of nicotine in plasma was not always reached within the 1 h sampling period. To enable the terminal half-life of nicotine to be determined more accurately the intravenous injection was repeated, on a different day, in four of the subjects and the sampling period was extended to 2 h. Conditions such as overnight abstinence and urine acidification were identical with Experiment 1. Blood sampling times were also the same over the first hour but at 75, 90, 105 and 120 min thereafter.

Experiment 3

To estimate the nicotine intake per cigarette when smoking heavily one of the subjects (PR) smoked his usual brand of cigarette (1.3 mg nicotine) over a 5 min period three times per hour for a total of 7 h. Blood samples were taken just before and 2 min after each cigarette.

Experiment 4

To compare nicotine intake from a high and low nicotine cigarette, a final experiment was done with one of the subjects (JL). After overnight abstinence and urine acidification as in Experiment 1, the subject smoked a low nicotine cigarette (0.6 mg) on one day and on a subsequent day a high nicotine cigarette (2.4 mg). Blood was sampled before and at 2, 5, 10, 15, 20, 25, 30, 45 and 60 min after the first cigarette. On each of the days the subject then smoked 12 further cigarettes at 0.5 h intervals. Blood was sampled at 2, 10 and 25 min after each cigarette, and at 2, 5, 10, 15, 20, 30, 45, 60, 90 and 120 min after the last one. Each cigarette was smoked over a restricted 5 min period.

Nicotine analysis

The plasma concentrations of nicotine were measured by gas chromatography as previously reported (Feyerabend & Russell, 1980) except that the internal standard was replaced by ethylnornicotine and the column packing by 2% Carbowax 20M/5% KOH on chromosorb W-HP 80/100 mesh. Sensitivity of this assay was 0.1 ng/ml with a coefficient of variation of 2% at a concentration of 5 ng/ml.

Pharmacokinetic analyses

Each of the plasma profiles of nicotine from the second intravenous dose (Experiment 1) and from the separate single intravenous dose (Experiment 2) were analysed by the method of residuals to fit a sum of exponentials (equation 1) to the data using linear regression. Curve-fitting of the saliva data was also attempted.

$$C = \sum_{i=1}^n C_i e^{-\lambda_i t} \quad \text{Equation 1}$$

where C is the plasma concentration at time t , C_i and λ_i are the coefficient and exponential constants, respectively, to the i th term.

The half-life ($t_{1/2}$) of nicotine for each decline phase was calculated from the exponential constant using equation 2.

$$t_{1/2} = \frac{0.693}{\lambda_i} \quad \text{Equation 2}$$

The area under the curve (AUC) calculations of the first intravenous dose and the area under the curve for the sixth cigarette (AUC_7) were calculated by the trapezoidal rule.

The area under the curve measurements for the first intravenous dose were, in turn, used to calculate the plasma clearance (CL) of nicotine using equation 3.

$$\text{CL} = \frac{\text{Dose}}{\text{AUC}} \quad \text{Equation 3}$$

The volume of distribution of nicotine based on area (V) and the initial distribution volume (V_1) were calculated from equations 4 and 5 respectively.

$$V = \frac{\text{Dose}}{\text{AUC} \cdot \lambda_z} \quad \text{Equation 4}$$

where z is the terminal exponential constant.

$$V_1 = \frac{\text{Dose}}{\sum_{i=1}^n C_i} \quad \text{Equation 5}$$

Estimation of smoking dose

The calculation of the amount of nicotine inhaled and which reached the systemic plasma of each subject was determined using a modification of the calculation normally employed to determine absolute bioavailability (equation 6).

$$\text{Fraction of nicotine dose (F) inhaled and absorbed} = \frac{\text{Dose of nicotine i.v.} \times \text{AUC}_\tau}{\text{AUC}} \quad \text{Equation 6}$$

where AUC_τ is the area under the curve for one dosing interval.

An alternative approach was based on the fact that at steady-state the rate of nicotine entering the body equals that leaving it (equation 7).

$$\begin{aligned} \text{rate in} &= \text{rate out} \\ &= \text{CL} \cdot C_{\text{av}} \end{aligned} \quad \text{Equation 7}$$

where C_{av} is the mean steady-state plasma concentration.

The plasma clearance used was that calculated from the intravenous data. The C_{av} in the case of Experiment 1 was taken as the mean plasma concentration of the first three samples (2, 10 and 25 min) after the sixth cigarette. For Experiment 3, it was taken as the mean of the plasma concentrations over the plateau region of the plasma concentration vs time curve (from 3 h to 7 h). Where appropriate, statistical comparisons were made by a two-way analysis of variance.

Results*Intravenous administration*

The plasma nicotine concentration 2 min after the first intravenous dose averaged 23 ± 14.7 ng/ml. The individual plasma nicotine profiles for Experiment 1 are shown in Figure 1 and the kinetic data are summarised in Table 1. After each intravenous dose of nicotine, the plasma concentrations declined in a biphasic manner. However, close inspection of the plasma profiles showed that the true log-linear decline of the compound from plasma had not always been attained within the 1 h period of sampling after the first dose and thus the terminal half-life of nicotine could not be accurately determined from these data alone. Experiment 2 was therefore undertaken to obtain the necessary terminal half-lives.

Curve-fitting analysis of the plasma data from the second intravenous dose, when the plasma levels were higher and the sampling was con-

tinued for longer (3 h), showed that for four of the five subjects, the plasma concentrations of nicotine declined in a biphasic manner. The initial decline was not observed in the fifth subject after any of the three injections and was also absent after each cigarette (Figure 1). The shapes of the curves following the three injections varied greatly between subjects but were consistent within subjects (Figure 1).

The half-life for the initial decline phase averaged 8.9, 9.0 and 9.1 min for the three injections respectively, and the terminal half-lives averaged 2.1, 2.2 and 2.2 h respectively. Very little of the drug was eliminated in the initial phase ($6 \pm 4\%$) as opposed to the terminal phase when determined from the ratio of the area under the curve for each respective phase to the total area under the curve. The values for the three injections were 6.0, 7.0 and 5.0%, respectively. The AUC for the first dose, measured to infinite time using the terminal half-life determined from the second intravenous dose of nicotine, averaged 32.07 ± 5.44 ng ml⁻¹ h. This resulted in a high mean plasma clearance value of 915 ± 146 ml/min. The two measures of volume of distribution of nicotine, again determined from the first intravenous dose, were also comparatively high with mean $\pm$ s.d. values of 173 ± 23 l and 79 ± 45 l for volume of distribution based on area and the initial distribution volume respectively.

Nicotine intake from cigarettes

When six cigarettes were smoked at half-hour intervals starting 1 h after the first intravenous dose of nicotine, there was a steady accumulation of nicotine until, after approximately the fifth cigarette, steady-state conditions were reached (Figure 1). The average peak concentrations of nicotine at steady-state were 51.4 ± 20.0 ng/ml with trough levels of 28.6 ± 8.5 ng/ml. Using the area under the curve for the total 30 min period of the sixth cigarette, it was calculated that on average 1.04 ± 0.36 mg of nicotine was inhaled and absorbed into the systemic plasma of the subjects (Equation 6). The alternative method (Equation 7) gave almost identical values. These represented, on average, $82 \pm 32\%$ of the machine-smoked yields of the cigarettes (Table 1). The values for JL are a slight overestimate due to insufficient sampling (only two samples per cigarette) in his case.

In Experiment 3, after smoking three cigarettes per hour for 7 h, the plasma concentration of nicotine accumulated steadily until about 3 h when a steady-state was reached at an average concentration of 49 ng/ml (Figure 2). The dose

Table 1 Smoking habits, nicotine dosage and pharmacokinetic data of the five subjects

	<i>PR</i>	<i>BW</i>	<i>JL</i>	<i>KK</i>	<i>SS</i>	<i>Mean ± s.d.</i>
Daily cigarette consumption	25	25	30	30	15	25 ± 6.1
Nicotine yield of usual cigarettes (mg/cigarette)	1.3	1.3	1.2	1.3	1.4	1.3 ± 0.1
Body weight (kg)	71.0	80.2	71.2	62.0	62.0	69.3 ± 7.6
Dose of intravenous nicotine (mg)	1.78	2.01	1.78	1.55	1.55	1.73 ± 0.19
Initial $t_{1/2}$ (h)	0.12	0.16	0.15	0.15	—	0.15 ± 0.02
Terminal $t_{1/2}$ (h)	1.99	2.56	1.77	2.51	2.23	2.21 ± 0.34
% elimination in first phase	6	8	10	6	0	6 ± 4
% elimination in terminal phase	94	92	90	94	100	94 ± 4
AUC intravenous (ng ml ⁻¹ h)	37.78	37.25	25.72	31.85	27.75	32.07 ± 5.44
AUC _r (cigarette) (ng ml ⁻¹ h)	26.56	10.22	21.64	20.44	16.31	19.03 ± 6.13
CL (ml/min)	783	897	1153	811	931	915 ± 146
V_1 (l)	29	103	43	77	141	79 ± 45
V (l)	135	199	176	177	180	173 ± 623
* F (mg) (Equation 6)	1.25	0.55	1.50	1.00	0.91	1.04 ± 0.36
(Equation 7)	1.31	0.58	1.49	1.01	0.92	1.06 ± 0.35
% of cigarette yield (mean)	98	43	125	77	65	82 ± 32

* F is the systemic availability of nicotine inhaled from a cigarette.

of nicotine absorbed was calculated from the clearance and steady-state plasma concentrations of nicotine (Equation 7) to give a value of 0.77 mg per cigarette. This is less than the values of 1.25 mg and 1.31 mg per cigarette calculated for this subject in Experiment 1 and is also less than the machine-smoked yield of his cigarette (1.3 mg).

In Experiment 4 one of the subjects (JL) smoked a high nicotine cigarette (2.4 mg) on one day and a low nicotine cigarette (0.6 mg) on another day. The plasma nicotine concentrations are shown in Figure 3 and can be compared with this subject's data from Experiment 1. It can be seen that the concentrations on the low nicotine cigarette tended to be lower but the differences are small compared with the four-fold differences in machine-smoked yields. The plasma nicotine concentrations and AUCs for the first hour after the first cigarette can be compared with each other and with those after the first intravenous injection (1.78 mg nicotine) in Experiment 1. The amount of nicotine

absorbed from the first cigarette on each occasion was 1.01 and 0.75 mg for the high and low nicotine brands respectively (Figure 3). Using the two methods described for Experiment 1 (but applied to the seventh cigarette in this case) the estimated nicotine intakes were 1.17 and 0.90 mg (Equation 6) and 1.24 and 0.95 mg (Equation 7). Using the average steady-state value based on all the data from 3 to 7 h the average nicotine intake per cigarette was 1.13 and 0.82 mg for the high and low brands, respectively (Equation 7). Averaging all the measures, the nicotine intake from the high yield cigarette was 1.14 mg compared with 0.86 mg from the low yield cigarette, 48% and 143%, respectively, of their machine-smoked yields.

Urinary excretion

By controlling the urine at an acid pH, the urinary elimination of nicotine was maximised. Nevertheless, at an average of $4.18 \pm 1.39 \mu\text{g}$

Figure 2 Plasma nicotine concentrations in one subject (PR) just before and 2 min after each cigarette when smoking three cigarettes per hour for 7 h, plotted on a semi-logarithmic scale.

Figure 3 Plasma nicotine concentrations of one subject when smoking high (2.4 mg, ●) and low (0.6 mg, ○) nicotine cigarettes. The smoking period for each cigarette was restricted to 5 min as shown (■).

over the 7 h period it was a small proportion of the administered dose. Moreover due to the frequent changes in dosing conditions (intravenous dosing followed by cigarettes then by

another intravenous dose), combined with the lag between clearance by the kidney and actual urine collection, it was not possible to calculate renal clearance reliably.

Figure 4 Average plasma (---) and salivary (—) nicotine concentrations of five subjects before and after the first dose of intravenous nicotine, 25 $\mu\text{g/kg}$ given over 1 min.

Salivary excretion

Salivary concentrations of nicotine were much higher than those in plasma (Figure 4). The average ratio over the hour after the first injection ranged from 3.9 to 8.9 with a mean of 5.8. Although salivary concentrations closely paralleled the plasma concentrations for the first 30 min, accurate assessment of the terminal half-life was not possible due to the erratic decline in saliva concentrations after 30 min.

Discussion

The plasma profiles and half-lives of nicotine after the three intravenous injections were similar although the second injection was given after a steady-state had been attained by loading dosage from smoking. This suggests that saturation does not occur at nicotine concentrations that build up during smoking. There was no evidence that the initial phase was diminished or altered by repeated smoking. The initial phase half-life was similar after all three injections as was the proportion of the AUC attributable to the initial phase.

The decline of nicotine was biphasic. The average half-life of the initial phase was 0.15 ± 0.02 h and the terminal half-life averaged 2.21

± 0.34 h. Although the elimination of nicotine was followed for only one half-life in the present study, our estimate is in good agreement with that of Benowitz *et al.* (1982), who employed longer sampling times. Attempts were also made to use saliva measurements of nicotine to determine its half-life after intravenous dosing. With the slightly acidic pH of saliva, levels of the compound would be expected to be considerably higher than those in plasma and should enable nicotine determinations to be carried on for longer. This was confirmed experimentally with a saliva to plasma ratio of approximately 6 and although initial results were encouraging (sampling times up to 30–60 min), the later samples proved to be rather variable and it was not possible to measure half-life with sufficient precision.

The average plasma clearance of nicotine (915 ± 146 ml/min) obtained in this study was similar to that reported by Rosenberg *et al.* (1980) and Benowitz *et al.* (1982). This high value might suggest that nicotine could be subject to first pass metabolism, since the clearance value approaches the hepatic blood flow. To confirm this, however, would require data on renal clearance and a correction factor for the distribution of nicotine into erythrocytes. The volume of distribution based on area (173

± 23 l) was fairly high indicating extensive distribution into tissues.

Many studies have demonstrated the plasma nicotine concentrations produced by cigarette smoking, but only one has estimated the actual dose of nicotine absorbed from a cigarette which averaged 1.04 mg nicotine per cigarette in subjects who were smoking an average of 35.6 cigarettes per day (Benowitz & Jacob, 1984a). We used two methods of estimating nicotine intake. They gave similar values which averaged 1.05 mg per cigarette in our five subjects. Both methods rely on steady-state conditions being achieved. One method used clearance and mean steady-state plasma concentrations of nicotine, while the other related area under the curve over one 'dosing' interval to the area under the curve of a known intravenous dose of nicotine. We have also shown that the half-life and plasma profiles after an intravenous dose are remarkably similar from one day to the next. This being so, once i.v. data have been gathered on given subjects, they could be used in 'nicotine titration' studies of smoking behaviour provided blood sampling was carried out in the afternoon when steady state conditions would apply.

Nicotine intake varied widely between subjects and bore little relation to the standard machine-smoked yields of their cigarettes. This was not unexpected since it is well-known that the plasma nicotine and cotinine concentrations of smokers also vary greatly and have little correlation with the nicotine yields of the cigarettes they smoke (Russell *et al.*, 1980; Ebert *et al.*, 1983; Benowitz *et al.*, 1983). When

one subject smoked at a higher rate, three cigarettes per hour, he absorbed only 0.77 mg nicotine per cigarette compared with 1.28 mg per cigarette when smoking two per hour. Steady-state plasma nicotine concentrations were thus maintained at a similar level. Likewise, another subject, by 'undersmoking' a high nicotine cigarette absorbed only 48% of its machine-smoked yield and by 'oversmoking' and taking in 143% of the yield of a low nicotine cigarette, maintained fairly similar plasma nicotine concentrations despite a four-fold difference in the yields of the two brands.

These findings confirm previous work showing that smokers tend to regulate their nicotine intake by modifying their pattern of puffing and inhalation. In this way they tend to maintain a fairly constant intake whether the cigarettes they smoke have high or low nicotine yields (Benowitz & Jacob, 1984b). Previous nicotine titration studies have relied mainly on plasma nicotine concentrations as a valid dependent variable from which intake has been inferred. We have demonstrated how measurement of the actual dose of nicotine absorbed from a cigarette could be used in future nicotine titration studies. Such measurement could be especially useful as an intake marker of other smoke components of relevance to health that cannot be measured directly.

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