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Cigarettes labeled with [^{11}C]nicotine: formulation and administration for PET inhalation

S. M. Apana[a] and M. S. Berridge[a,b,c*]

The ultimate goal of this work was to relate nicotine kinetics in the brain after cigarette smoking to a feature of sensitization in drug addiction. To do this required a positron emission tomography study to measure the regional cerebral biodistribution kinetics of cigarette-smoked nicotine. This in turn required a cigarette formulated with carbon-11 labeled nicotine suitable for administration by single bolus inhalation. Here we report the development and validation of cigarettes formulated with [^{11}C]nicotine that were successfully used for single bolus administration by smoking. We also report measurements of nicotine delivery from smoked cigarettes.

Keywords: carbon-11; nicotine; cigarettes; PET

Introduction

The effects of many drugs, including nicotine, on the brain[1–3] are enhanced by the rate at which the drug is delivered to the brain[4–6] ('rate of rise') independently of the peak drug concentration. The case in support of this hypothesis as it relates to cocaine addiction was elegantly demonstrated using a series of positron emission tomography (PET) scanning studies by the group of Volkow, Fowler, and Ding at Brookhaven National Laboratory.[5–13] There is also anecdotal support in the observation that smoked forms of drugs are generally more abused than other forms. However, differences in the administration route or effects of components of tobacco smoke might also affect the amount of drug delivered to the brain. Brain kinetics after intravenous delivery of [^{11}C]nicotine,[14–18] after inhalation with a vapor inhaler,[19,20] and after nasal administration[3] have been measured, while brain kinetics after smoked delivery have not. Potential interactions of smoked nicotine in the lung or mucosa might have a significant effect. Examination after smoked delivery will test the hypothesis that the rate of rise of nicotine in the brain is rapid enough to affect the neuropharmacology and behavioral psychology of smoking. To do this it is necessary to obtain PET scans of subjects after a single inhalation from a cigarette radiolabeled with [^{11}C]nicotine. In order to perform such a study, this work was undertaken to develop methods, based on prior work with nicotine and with PET of inhaled formulations,[21–27] to produce a radiolabeled cigarette and use it to perform administrations suitable for PET scanning. In order to minimize variability and produce generally informative data, it was desired to perform a single-puff, bolus, administration, the results of which could then be generalized to any desired multi-puff administration protocol.

To obtain a single-puff inhaled cigarette administration of [^{11}C]nicotine, formulation methods and a smoking apparatus were required to allow efficient administration of a dose in one inhalation while keeping the cigarette outside of the camera's field of view and avoiding hazards to participants and equipment. Air flow rate and flow resistance behavior similar to a normally smoked cigarette was also required to minimize apparatus effects on the smoking technique of a subject and the possibility of resulting alterations of measured kinetics. An additional critical requirement was to formulate a sufficient amount of [^{11}C]nicotine onto a cigarette such that an appropriate radiolabeled dose could be reproducibly inhaled in a single inhalation and be representative of endogenous nicotine in the tobacco. With the constraints of the 20 min half-life of carbon-11, this required that the nicotine be formulated for application to the tobacco in a minimal volume of solution and the solvent rapidly removed.

Results and discussion

Apparatus design

The apparatus was intended to administer radiolabeled nicotine in tobacco smoke to a volunteer in as similar a manner as possible to smoking a preferred cigarette. Measurements of normal smokers' inhalations indicated a typical inspiratory flow rate of 40 L/min, and a burn rate of 4.5 mm of cigarette per puff. Experiments with a simulated smoking apparatus were then performed (Figure 1, S). The needle valve was adjusted to give

[a]3D Imaging, LLC, Little Rock, AR, USA

[b]Departments of Radiology and Pharmaceutical Sciences, University of Arkansas for Medical Sciences, Little Rock, AR, USA

[c]Department of Chemistry, Case Western Reserve University, Cleveland, OH, USA

*Correspondence to: M. S. Berridge, 3D Imaging LLC, Cyclotron Suite Rm PS010, UAMS Radiology ♯556, 4301 W. Markham St., Little Rock, AR 72205-7199, USA. E-mail: MBerridge@3DImagingLLC.com

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a 40 L/min flow rate. A cigarette reduced flow to 39 L/min. To achieve similar flow resistance from the administration apparatus, minimum lengths of Teflon tubing (ID = 0.1″, 2.5 mm) were used. Luer fitting and stopcock passages were drilled to the maximum possible diameter, similar to the tubing ID of 2.5 mm. The measured flow through the final apparatus configuration with cigarette fragment in place was 38 L/min. Smokers reported the apparatus felt 'similar' to smoking a cigarette. Before use, it was treated with aqueous sodium hydroxide solution (2 N) as a precaution to prevent nicotine trapping, and rinsed with deionized water. Tygon tubing was not used because it was found to retain [^{11}C]nicotine with greater than 95% efficiency.

In simulated smoking of a whole cigarette (Figure 1, S) with [^{11}C]nicotine applied 10 mm from the lit end, a large percentage of nicotine was observed to be released but then to redeposit on the remaining unheated tobacco. Only 10% of the dose was released on average, 36% in the best case. In effect, the distal packed tobacco acted as a gas chromatographic column, retarding the radiolabeled nicotine as it eluted. Therefore, the cigarette was cut before use to leave only 20 mm of tobacco, with the [^{11}C]nicotine applied in the center.

Under simulated smoking conditions with air flow (Figure 1, S) bolus nicotine release occurred when the burn zone came within 3 mm of the nicotine. However, a substantial portion of the nicotine was released gradually and prematurely while heated air passed over it, before the bolus release that occurred when the flame reached a distance of 3 mm (Figure 2, dashed line). This caused the cigarette to deliver an irreproducible and deficient bolus dose. However, under passive 'pre-burning' conditions with no air draw (Figure 1, P) nicotine was released in sidestream smoke only as the burning flame front approached within 1 mm of the nicotine. No nicotine beyond 1 mm from the flame front was released until an inhalation occurred. Therefore, the method was modified to use passive burning to 'pre-burn' the cigarette until the burn zone reached 1–3 mm from the spot of nicotine addition followed by simulated smoking with air flow. This produced a reproducible bolus with no preliminary nicotine release. In some experiments, the nicotine from the burning cigarette was collected from the airstream using a cascade impactor rather than the acid trap and analyzed by HPLC. The particle size distribution was too small for cascade impactor analysis; however, the HPLC analysis showed that nicotine was the chemical form of all radioactivity eluted from the cigarette.

Even with a shortened cigarette, redeposition on the cigarette's filter (Figure 2, solid and dashed lines) similar to that observed on the tobacco of a whole cigarette, prevented a sufficient release of nicotine for a single bolus inhalation. In this experiment, burning the entire 20 mm length of tobacco released only 30% of the applied radiolabeled nicotine. Although not usual among smokers, the filter was then also allowed to burn partially. Even this released only an additional 20% of the radiolabeled nicotine. Therefore, to achieve a bolus administration of smoked [^{11}C]nicotine, the filter was removed. A 20 mm filterless length of a cigarette, with labeled nicotine placed 10 mm from the end and using a passive pre-burn of the first 7–9 mm, gave a consistent release of 70–80% of the administered nicotine in a single simulated puff (Figure 2, dotted line). Finally, to reduce the time and associated radiation exposures required for formulation and administration, the cigarette for PET scan use was further reduced to a 10 mm length of a volunteer's preferred brand. [^{11}C]Nicotine was deposited on the open face of the cigarette, so that it remained 10 mm from the inhalation end, as previously. Deposition on the open face rather than injection into the tobacco packing did not affect release behavior, but it eliminated the pre-burn and reduced the time required for deposition, saving several minutes of radioactive decay and radiation exposure to the operator.

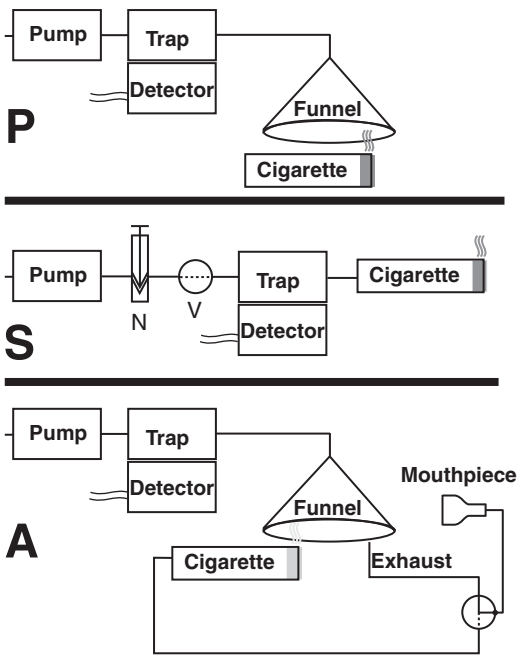

Figure 1. Apparatus used in the experiments. Configurations shown are: P, passive; S, simulated smoking; and A, administration. In configuration S, 'N' denotes the flow control needle valve and 'V' the manual flow cutoff valve.

Release of endogenous nicotine, comparison

When endogenous nicotine release from a whole cigarette was measured with the cigarette filter in place, the release of nicotine did not follow a linear progression (Figure 3, dashed

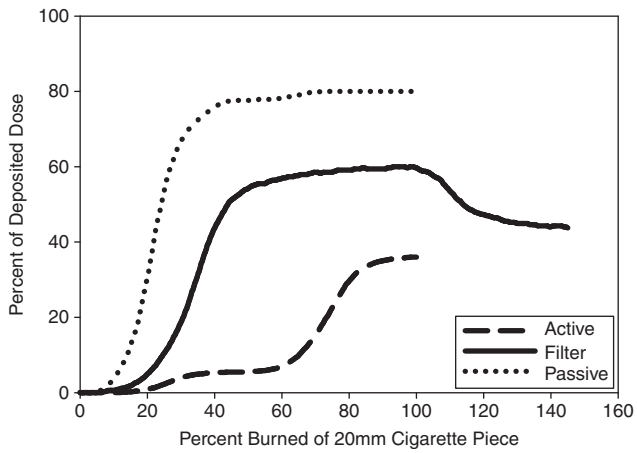

Figure 2. [^{11}C]Nicotine elution from 20 mm cigarette sections as % of dose deposited on tobacco vs cigarette length burned. Dashed line: [^{11}C]nicotine collected with active preburning (continuous air flow) of a filtered cigarette fragment. Solid line: [^{11}C]nicotine accumulated on the section's filter. Values greater than 100% represent time as the filter burned. Dotted line: [^{11}C]nicotine collected with passive preburning of unfiltered fragment (burn distance measured from start of air flow with flame front 2 mm from deposited dose). Time duration of 100% burns were 10–20 s.

line). Similarly to the conclusions of Abel,[28] nicotine release increased as the flame front approached the filter. However, without a filter the nicotine release was linear (Figure 3, solid line). Interesting, although perhaps not surprising, was that total nicotine yield without a filter (1.1 mg) was significantly more than with the filter in place (0.7 mg). To compare radiolabeled and endogenous nicotine, cigarettes were used with filters removed because the method for administration required filter removal (above).

Because endogenous nicotine is evenly distributed on tobacco, it is difficult to compare bolus release of endogenous and radiolabeled nicotine. Therefore, to test whether release of radiolabeled nicotine was similar to that of endogenous nicotine, a more uniform spatial distribution of labeled nicotine was created. Twelve equal nicotine aliquots were spaced at equal intervals along the length of the central axis of a whole cigarette with filter removed. The [^{11}C]nicotine release behaved similarly to the unlabeled endogenous nicotine (Figure 3, dotted and solid lines). This confirmed that the added radiolabeled nicotine was acting as a reasonable tracer for endogenous nicotine from the tobacco, as intended.

Formulation of [^{11}C]nicotine onto cigarettes

If radiolabeled nicotine was applied as the free base dissolved in methylene chloride there was a difference in behavior from the endogenous nicotine. Up to 50% of the [^{11}C]nicotine could be removed in 1–2 min of unheated air flow, while loss of endogenous nicotine under the same conditions was negligible. However, deposition of [^{11}C]nicotine on the tobacco as the hydrochloride salt produced nearly identical elution profiles (Figure 3). To deposit the hydrochloride, an aqueous solution proved necessary. A 1:1 mixture of methanol in water was optimal. Additional methanol reduced solubility. Even at 60% methanol, [^{11}C]nicotine dissolution was only 60–70%. Additional water did not affect recovery, but increased the time required for solvent removal. In 50% aqueous methanol, the entire synthesis yield, typically 7.5 GBq (200 mCi), could be taken up in 60 μL in a syringe for cigarette dosing. To form a nicotine salt, a volatile organic acid might be thought to be superior to hydrochloric acid. Acetic acid was tested, and it did produce at least 90% solubility in methylene chloride and elution behavior like endogenous nicotine. However, an unintended consequence was a distinct and highly unpleasant vinegar taste when smoked, which would affect a smoker's inhalations. This led to preference for the hydrochloride salt. The dosing procedure is illustrated in Figure 4, which shows the cigarette fragment, holder, a part of the smoking apparatus, and the technique for deposition of [^{11}C]nicotine on tobacco.

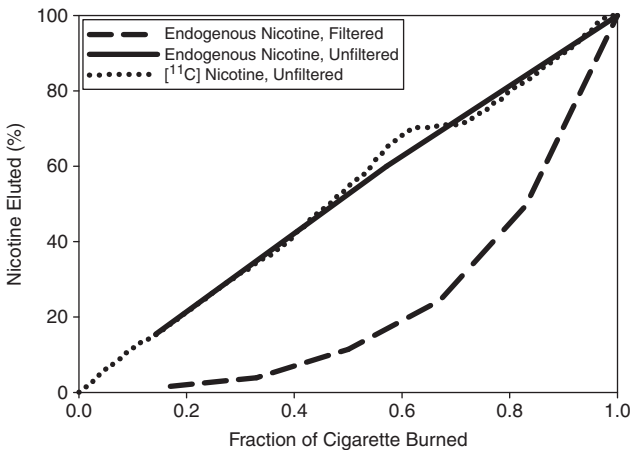

Figure 3. Elution of endogenous (unlabeled nicotine mass) and radiolabeled nicotine from whole cigarettes as a percentage of total nicotine eluted. Dashed line: endogenous nicotine eluted from a filtered cigarette. Solid line: endogenous nicotine eluted from a cigarette with the filter removed. Dotted line: [^{11}C]nicotine eluted from a uniform deposition along the length of the cigarette.

Experimental methods

Reagents and solvents were obtained from Aldrich Chemical Co. and Fisher Scientific and used without further purification unless otherwise noted. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketal. Gas chromatography was performed on a Hewlett-Packard 5890 Series II chromatograph with flame ionization detection (J&W DB-1 column, 110°C, He flow 2 mL/min). Radioactivity was measured with a Beckman 170 radiation detector. Serial dilutions of nicotine in methanol were used to create a standardization curve and calibration factor. The nicotine detection limit was less than 0.01 mg/mL.

[^{11}C]CO_2 was prepared by the ^{14}N(p,α) reaction on 2% O_2 in N_2 target gas. Human subject participation was approved by the Case Western Reserve University IRB, RDRC, and Radiation Safety Committee. Synthesis of [^{11}C]nicotine, via N-methylation by [^{11}C]methyl iodide (lithium aluminum hydride/HI method) of 1 mg (6.2 μmol) racemic nornicotine with 7 μmol NaOH in THF at 85°C, 10 min, followed previously reported and common methods.[27,29] After reaction, 2 μL 6 N HCl (12 μmole) was added to the THF solution and solvent evaporated with 3 mL/min He gas flow under vacuum at 85°C. The resulting residue contained approximately 0.05 μmole labeled nicotine and 6 μmol (1 mg) nornicotine and was used without further purification. Nornicotine

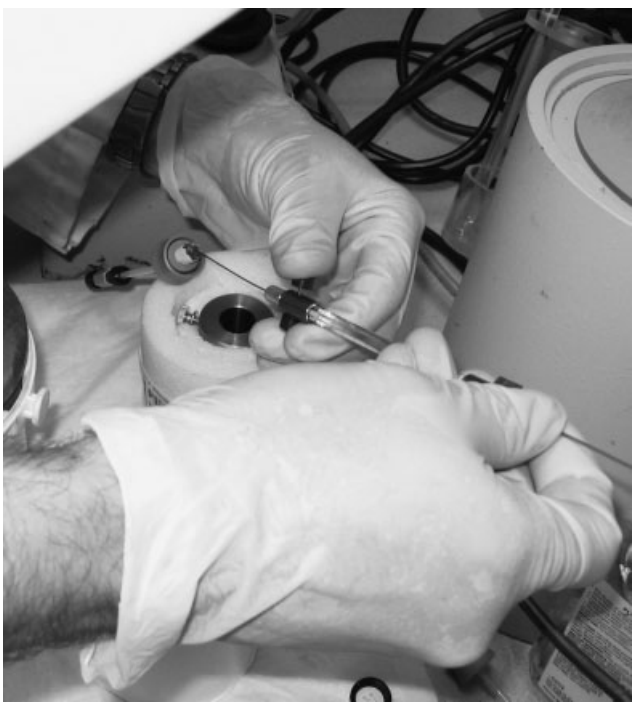

Figure 4. Dosing of a cigarette with [^{11}C]nicotine. The microliter syringe, technique of loading on the cigarette fragment face, the fragment itself and holder, and the tubing attached to the vacuum source for drying, which subsequently is attached to the smoking apparatus, are all shown.

is a normal metabolite of nicotine that is less volatile with lower pharmacologic potency.[30] It is present in micromole quantities in cigarette tobacco and is administered during smoking.[30] Cigarettes typically contain 3–16 mg nicotine and 0.52 mg nornicotine, and deliver much less, with wide variation.[31,32] The radiolabeled product was also typically divided among four cigarettes during experiments. Therefore, the residual nornicotine was not deemed significant for this work and was allowed to remain in the product.

Radiolabeled cigarettes were prepared by dissolution of the [^{11}C]nicotine residue in 30 μL water, with agitation, followed by 30 μL methanol. A 10 mm length cut from a cigarette was used for dosing. Labeled nicotine was added dropwise to the face of the cigarette section using a 10 μL or 100 μL glass syringe, as appropriate, allowing time for absorption and distribution of each drop onto the tobacco. Air was then drawn through it for 10 s to 1 min to evaporate traces of water and methanol so that the cigarette would burn normally. Solvent removal was completed by carrying on the air flow for twice the time required to achieve a dry visual appearance and a lack of methanol odor. Radiolabeled cigarettes for optimization experiments were prepared similarly, but from whole or partial cigarettes as described below.

Apparatus and technique optimization experiments

In Passive burning configuration (Figure 1, P), air was drawn through a funnel placed above a radiolabeled cigarette to collect sidestream smoke on an acidic trap. The acid trap contained 5 mL chromatographic silica gel prepared by wetting with 1 N H_2SO_4, filtering to remove bulk solution, and drying at 100°C. A radiation detector (Beckman model 170, shielded and collimated with lead brick) was positioned near the trap to detect accumulated radiolabeled nicotine. The cigarette was lit with no air flow through the packing while [^{11}C]nicotine release in sidestream smoke was measured. In simulated smoking configuration (Figure 1, S) air was drawn through a radiolabeled cigarette, using a rubber sleeve stopper (Thomas Scientific) around the end of the cigarette to form a tight seal to Teflon tubing. A needle valve (Swagelok) controlled air flow. A manual valve (Hamilton) was used to produce discreet draws, or puffs, which consumed 4.5 mm of cigarette. The acid trap and collimated radiation detector in the smoke path measured nicotine delivered by the smoke. Detectors were similarly used to observe the nicotine on the cigarette, deposition on the cigarette filter, or combinations of these. Cigarettes were radiolabeled at various positions and then burned using various techniques to explore nicotine retention and release.

Three smoker volunteers were tested for smoking techniques, using normal, non-radiolabeled, cigarettes of their choosing. Each was instructed to inhale through a large-bore (low resistance) flowmeter (Gilmont GF-6540-1240) in the manner of smoking. The flow meter and a cigarette of the volunteer's preference were alternated for a series of measurements until the measured smoking inspiratory flow rate became reproducible. The progress of the flame front through a cigarette was also measured as the volunteers smoked their own choice of cigarette at a time of their own choosing.

Apparatus for Nicotine Administration by Smoking

The smoking apparatus for administration (Figure 1, A) was composed of thin-wall (1/8″OD × 1/10″ inch ID, 3 mm × 2.5 mm) Teflon tubing and a manual disposable 3-way luer stopcock. Tubing sections to the cigarette and mouthpiece were 30 cm long, with a 10 cm exhaust section. A 5 cm section led from the vacuum funnel to the acid trap (to collect exhaled nicotine), and an additional 30 cm section to a small mechanical vacuum pump. Tubing and components were pretreated by washing with 2 N NaOH, deionized water, and air drying. The cigarette was positioned below the funnel to collect sidestream smoke. During inhalation, the stopcock was set to connect the cigarette to the mouthpiece. After inhalation, the stopcock was turned to direct exhaled smoke to the exhaust tube at the funnel. All nicotine that was released in sidestream or exhaled smoke was therefore collected on the acid trap for measurement.

Administration of nicotine was done by coordinated smoking using the apparatus. An investigator brought a flame nearly into contact with the face of the cigarette without igniting it. Inhalation through the apparatus drew the flame to the tobacco, simultaneously igniting the cigarette and releasing the nicotine as a bolus. Released nicotine was typically 70–80% of the dose present on the tobacco. Immediately after the single inhalation, the cigarette was doused in a 1.3 × 6 cm test tube containing water. Residual nicotine radioactivity on the cigarette and acid trap was measured in a dose calibrator so that the administered dose could be calculated.

Conclusion

A smoking apparatus and formulation strategy was designed to produce cigarettes radiolabeled with [^{11}C]nicotine. Eighty percent of the formulated nicotine was released by a single simulated inhalation suitable for administration to a human volunteer. Radioactive nicotine elution from cigarettes was similar to elution of endogenous nicotine, and also was transported and trapped in the same manner as the endogenous nicotine. The radiolabeled nicotine therefore behaved as a true tracer for the endogenous nicotine in a cigarette. The resulting device and techniques were then used successfully in a PET investigation of the rate of rise of smoked nicotine in the human brain.

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