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Subcutaneous nicotine delivery via needle-free jet injection: a porcine model

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Abstract

Subcutaneous delivery of nicotine was performed using a novel electrically-operated needle-free jet injector, and compared to hypodermic needle delivery in a porcine model. Nicotine was delivered as a single, one-milligram dose into the abdominal skin, formulated as a 50 microliter aqueous solution. Plasma levels of nicotine and cotinine, its main metabolite, were then monitored over two hours, following which the injection site was excised for histological examination. No irritation or tissue damage were found at the injection sites, and the jet-injected nicotine exhibited comparable absorption into the systemic circulation to that injected using a conventional needle and syringe. The needle-free jet injection of nicotine is a promising and well tolerated method. The data presented from this porcine model will support a first in human trial towards a new promising nicotine replacement therapy.

Keywords: nicotine, jet injector, subcutaneous, needle-free, smoking cessation

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1. Introduction

Cigarette smoking is among the leading causes of premature death worldwide, and approximately 50 % of smokers will ultimately be killed by their habit [1]. While nicotine is the principal addictive substance in tobacco, it is not itself responsible for the adverse impacts of smoking [2]; as such, nicotine replacement therapy (NRT), in which patients self-administer pure nicotine in lieu of smoking, is seen as a safe and effective method for treatment of tobacco dependence [3]. However, the effectiveness of NRT is not universal, and the ultimate success rate is not high: under 10 % of unassisted quit attempts succeed [3, 4], and NRT is only able to increase this to about 20 %.

One possible reason for the limited success rate associated with NRT is a difference in the rate of nicotine delivery as compared to cigarettes [5, 6]. Smoking delivers nicotine directly to the pulmonary circulation, reaching the brain within seconds, while most NRT is delivered transdermally or across oral mucous membranes, with blood nicotine concentrations rising slowly over a period from 30 minutes to several hours. Smokers suffering from nicotine cravings must therefore suffer a period of discomfort between feeling a craving and receiving an adequate dose of nicotine, discomfort which could be alleviated by resuming smoking rather than waiting for NRT to kick in. As a result, "combination" NRT is often recommended, combining a steady, slow-acting nicotine source, such as a nicotine patch, with a faster-acting nicotine source, such as chewing gum. While commonly available, nicotine gum still offers a relatively slow response, and there has therefore been considerable interest in faster-acting delivery methods [2].

A number of approaches have been proposed to increase the rate of NRT delivery, including higher doses, nasal sprays, and nebulizers [2, 5], but these are still unable to approach the delivery rate of smoking, and/or have side-effects that limit their patient acceptance. E-cigarettes are a promising new tool with near-identical pharmacokinetics to conventional cigarettes, but there remain questions about their safety and social acceptability relative to other

forms of NRT, and there have yet to be sufficient studies to demonstrate their efficacy [7, 8].

One nicotine delivery technique that has seen little exploration to date for NRT is that of subcutaneous injection [9]. This method achieves a comparable delivery rate and maximum plasma nicotine concentration to nicotine cannons or sublingual tablets [2], with fewer side-effects. The limited exploration of this method should perhaps be unsurprising, given the prevalence of needle-phobia and the safety issues associated with hypodermic syringes, but alternative injection mechanisms can bypass these issues. In particular, in this work we propose the use of needle-free jet injection as a technique for subcutaneous nicotine delivery.

Jet injection entails the generation of a small-diameter, high-velocity jet of liquid, capable of penetrating the skin using its own momentum [10, 11]. The depth of delivery can be controlled from intradermal [12] to intramuscular by adjusting the velocity of the jet [13], and injections are generally better-tolerated than standard hypodermic injections [14]. Jet injection also often leads to more rapid drug delivery than needle-based subcutanous injections, suggesting a possible additional benefit for NRT [11, 15].

While jet injection holds promise for nicotine delivery, there are a number of unexplored issues that must be addressed before it can be attempted in humans. One relates to practical considerations: jet injectors increase in size and complexity as the volume of the delivered dose increases [16], making it desirable to minimize the dose volume through the use of a concentrated formulation. However, jet injection is normally done with standard injectable formulations, not highly-concentrated ones; the pharmacokinetics and tissue reaction to jet injection of a highly-concentrated injectate are not well characterized. Specifically for nicotine, previous studies used subcutaneous delivery of dilute (1 mg/mL) formulations [17], and it is not known whether a concentrated formulation is safe for injection, by needle or jet.

Another unexplored issue is the jet injection behavior of a substance with the transport properties of nicotine. Most jet injection efforts have used large-

molecule drugs, such as vaccines, peptide hormones, antibodies, or DNA [12], which disperse slowly through tissues before uptake by the vasculature or contact with immune cells. The main efforts with small-molecule drugs have focused on local anesthetics [10], with some application to sedative drugs and steroids. Of these, sedatives (specifically midazolam) are the only drugs with significant lipophilicity [18] to be jet injected, but they do not approach the ability nicotine has for diffusing through tissue [19]. Thus, while jet injection is well-established as providing faster absorption than needle injection for other drugs, the rapid diffusion of nicotine may overwhelm this behavior.

The objective of this study is to demonstrate, in a porcine model, the properties of nicotine delivery via needle-free jet injection, and to assess the injection site for any evidence of any acute tissue damage or other adverse reaction caused by the injection method and/or concentrated nicotine formulation. Pigs have very similar skin properties to humans [20], and *in vivo* pig skin has greater similarity to living human skin than does frozen or preserved human skin *in vitro* [21]; pigs are thus ideally suited as models for comparing transcutaneous delivery methods. However, pigs have been seldom used in nicotine delivery studies, likely due to the general acceptability of nicotine for human experimentation. Live pigs have also seldom been used for jet injection studies, with most work instead using ex-vivo pig skin without its natural tension. There is thus also a need to understand the suitability of this animal model for in vivo jet injection of nicotine. We examined the systemic levels of nicotine and its major metabolite, cotinine, to establish the rate of nicotine delivery and its relative bioavailability, and performed histological analysis of the injection sites. Based on these results, we seek to demonstrate sufficient safety and efficacy to support a future human study.

2. Materials and Methods

All experimental procedures were approved by the University of Auckland Animal Ethics Committee as protocol #001933, and were conducted in accor-

dance with the New Zealand Animal Welfare Act 1999.

2.1. Injection devices

We have developed a miniaturized, electronically-controllable jet injection apparatus, originally described in [16]. This apparatus uses a fixed, stainless steel ampoule with a capacity of 60 μL and a nozzle diameter of 200 μm, driven by a custom electromagnetic actuator and real-time control system as shown in Fig. 1. This is the first reported controllable jet injector in this capacity range; previous controllable injectors have either been much larger ($> 300\,\mu$L, e.g. [13]) or much smaller ($< 1\,\mu$L, e.g. [22]). Electronic control over the jet injection process allows for improved management of injection depth and reduced audible noise caused by the injection process, by gradually increasing the force applied to the piston at the start of injection.

For this study, the controller was programmed to deliver injections of fixed duration from capacitors charged to a constant voltage. Filling the ampoule was accomplished by drawing fluid in through the nozzle under closed loop position control.

Appropriate injection parameters for this study were determined via post-mortem injections into pig abdominal skin, and confirmed by injecting tissue marking dye into an intact animal immediately post-mortem. The volume of injectate delivered and its repeatability were assessed by ejecting water into pre-weighed centrifuge tubes using these injection parameters, and determining the mass delivered.

Needle injections were performed using a 0.1 mL glass syringe (Hamilton Gastight 1710), fitted with a 25G $\times$ 5/8" beveled needle.

2.2. Chemicals and solvents

Nicotine (98.5 % purity), cotinine ($\geq$ 98 % purity) and 6-aminoquinoline (98 % purity) were obtained from Sigma Aldrich (Auckland, New Zealand). Methanol ($>$ 99 %, HPLC grade) was used in sample preparation. LC-grade water (Millipore, Milli-Q system) and acetonitrile ($>$ 99 %, HPLC grade) were

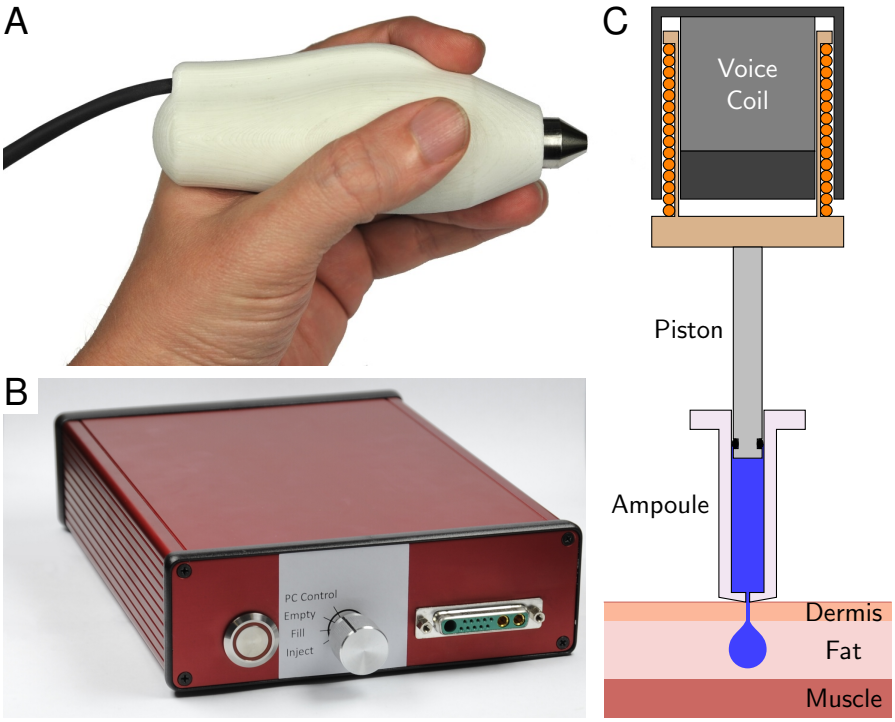

Figure 1: The operating principle of the injection system is shown in (C), with a voice coil motor applying force to a piston, which in turn pressurizes the contents of an ampoule and forces them through a small orifice and into the skin, where they are deposited at the desired depth. The complete jet injector hand-piece (A) has a mass of 178 g, and is connected to its power amplifier (B) via the cable shown. (Part A reproduced from [16].)

used for the mobile phase. In validating the jet injection parameters, green tissue marking dye (Triangle Biomedical Sciences) was used.

2.3. Nicotine injection and sample collection

Female pigs, weighing between 33.8 kg and 39.9 kg (mean 36.5 kg), were anesthetized using Zoletil with isoflurane for the duration of the experiment. Pigs received a single subcutaneous dose of nicotine base (19 mg/mL) in isotonic saline, either by needle free jet injection (1.04 mg, 55 μL) ($n = 4$) or by hypodermic needle (0.95 mg, 50 μL) ($n = 4$). Jet injections were performed using a capacitor voltage of 130 V, and an injection duration of 10 ms. Both types

of injection were delivered into the base of an abdominal skin fold, just lateral of the teats, with the needle or jet injector oriented at a 45° angle to the skin surface. Jet injection was performed with the nozzle pressed firmly against the skin surface. Any fluid remaining on the skin surface following injection was collected with a pre-weighed tissue, and subsequently weighed to determine the volume of fluid.

For each subject, eight arterial blood samples (6 mL) were collected at frequent intervals up to 2 hours following nicotine administration. Sampling times were at time 0 (prior to nicotine administration) and then at 5, 10, 15, 30, 60, 90 and 120 minutes post nicotine injection. Blood samples were collected in EDTA tubes and were placed on ice before they were centrifuged at 3000 g for 10 minutes to separate the plasma and red blood cell fractions. Plasma was stored frozen at −80 °C until analysis.

Following the completion of the experiment, the animals were killed by pentobarbital overdose and histological samples were taken from the injection sites. After collection, the samples were fixed in 10 % buffered formalin, embedded in paraffin and sectioned; sections were stained with hemotoxylin and eosin, then imaged using a digital microscope camera (Leica ICC50 W).

2.4. Nicotine and cotinine determination

For calibration, blank pig plasma samples were spiked with nicotine and cotinine to give working concentrations of 1, 2, 4, 6, 8, 10, and 20 ng/mL. Each working solution (200 μL) was mixed with 6-aminoquinoline internal standard (IS) (20 μL of 200 ng/mL) and then deproteinized with ice-cold methanol containing 0.1 % formic acid (400 μL). Samples were vortex mixed for 2 minutes and then left overnight at −20 °C. Samples were then centrifuged at 10 000 g for 15 minutes and the clear supernatant (450 μL) was removed and evaporated to dryness (SC210A SpeedVac Plus). The dry extract was then reconstituted in acetonitrile:ammonium formate buffer (10:90, v/v; 100 μL) and vortex mixed for 5 minutes. Samples were centrifuged at 10 000 g for 5 minutes before they were injected onto the column.

Plasma samples collected in the experiment were thawed at room temperature, and two aliquots (200 μL) of each sample were prepared in the same method as described above. The duplicate measurements of each sample were averaged to determine the final results.

Plasma concentrations of nicotine and cotinine were determined with an Agilent ChemStation liquid chromatography system coupled with mass spectrometry (LC-MS) using electrospray ionization (ESI). Detection by selective ion monitoring (SIM; positive ion mode) for each mass ion was used: m/z 163.2 (nicotine), 177.2 (cotinine) and 145.1 (IS). These compounds were resolved using a Gemini C18 (4 mm $\times$ 100 mm, 5 μm) column with a guard column (C18, 4.6 mm $\times$ 10 mm, 5 μm) and eluted with a mobile phase of 10 mM ammonium formate buffer, pH 4.5 (solvent A) and acetonitrile (solvent B) with a phase gradient 5 % (B) from 0 to 1 minute, 25 % from 1 to 6 minutes, 80 % from 6 to 7 minutes and 5 % from 8 to 11 minutes. Drying gas flow was 12.0 L/min and nebulizer pressure was 35 psig. The total run time was 11 minutes plus 1 minute post injection time, with a flow rate of 0.5 mL/min. Sample injection volume was 30 μL. Retention times for nicotine, cotinine and IS were 2.1, 6.1 and 5.1 minutes respectively.

Plasma samples collected from each pig prior to nicotine injection were run using this method to ensure absence of nicotine and cotinine prior to nicotine administration. Calibration curves were prepared to assess linearity, precision and accuracy. Linearity was verified for this method by visual inspection and coefficients of determination (R^2) of calibration curves were above 0.99. Precision and accuracy across all concentrations were all within $\pm$15 % of the actual values ($\pm$20 % at the lowest limit of quantification). The minimum detection level for each compound was 1 ng/mL.

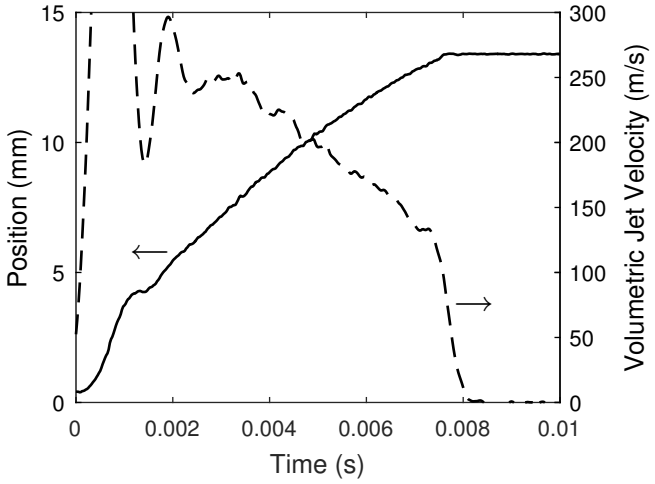

Figure 2: Jet injection trajectory as used on pig #4, with an initial applied voltage of 130 V; the solid line denotes the piston position, while the dashed line indicates the jet velocity estimated from the piston position. Neglecting the initial peak in jet velocity due to mechanical compliance, the average jet velocity was 190 m/s.

3. Results

3.1. Jet injection parameters

Each jet injection device, as defined by the combination of actuator, ampoule, and orifice, can exhibit different drive requirements in order to deliver a particular depth and volume of injection. As such, a series of trial injections were performed on post-mortem pig tissue to empirically determine a set of injection parameters (voltage and time) that provided reliable injections of the target volume to the target depth using our device. Based on observations during these injections, an injection voltage of 130 V was chosen, at which only 8 ms was required to empty the ampoule; an injection duration of 10 ms was chosen to guarantee complete injection. The volume delivered, as measured by repeated ejections into air, was $55.5\,\mu\text{L} \pm 2.1\,\mu\text{L}$ ($n = 6$). This was consistent with the injection waveform when applied to the skin as shown in Fig. 2; the piston swept volume in this case was $57.7\,\mu\text{L}$, with $3.0\,\mu\text{L}$ of this volume corresponding to mechanical compliance in the system.

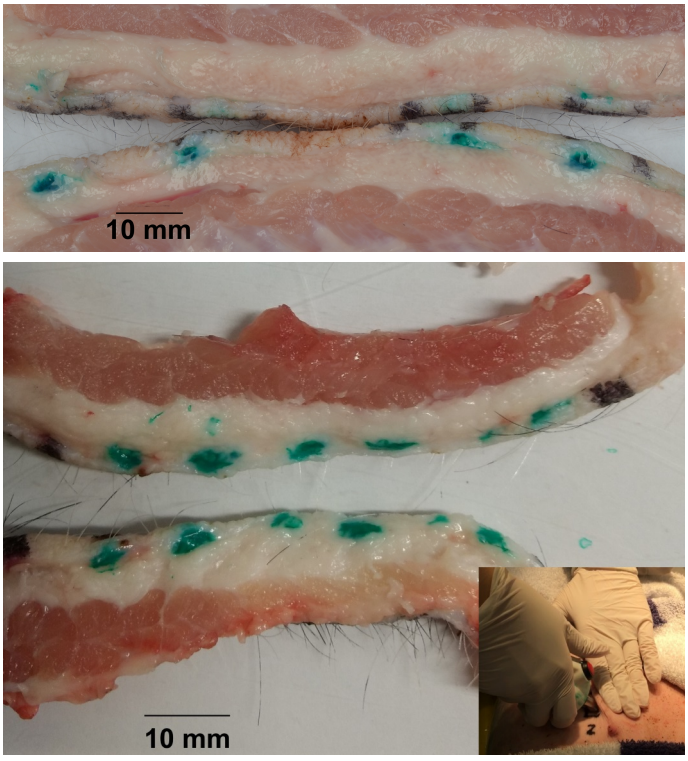

Figure 3: Top: cross-section through four subcutaneous needle intections, showing that the technique employed yields a consistent injection depth at the top of the subcutaneous layer. Bottom: Cross-section through six subcutaneous jet injections of green dye, showing consistent injection depth similar to needle injection and no penetration of muscle tissue. The inset shows the positioning of the skin and jet injector.

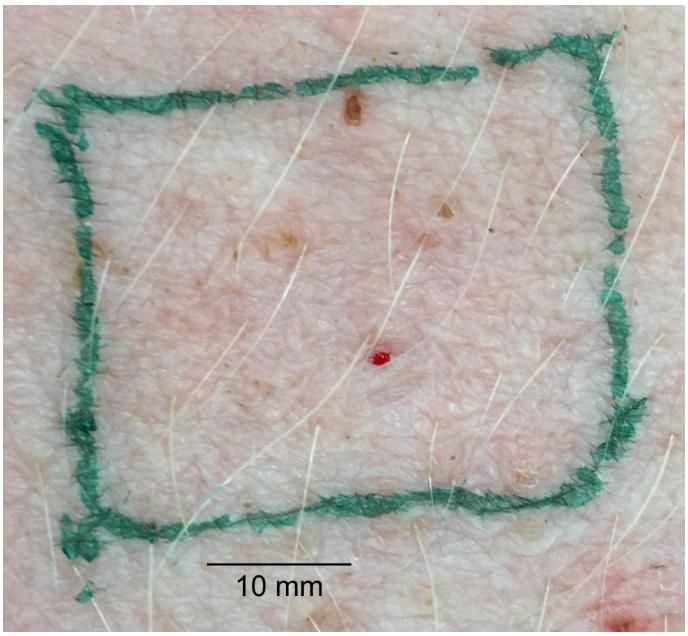

Figure 4: Skin surface following a nicotine jet injection, showing a tiny drop of fluid (3 μL) at the injection site.

During post-mortem tissue injections, with the injector positioned normal to the skin surface, it was observed that the selected injection parameters caused some penetration of the muscle below the subcutaneous fat, despite most of the injection being delivered to the correct depth. To prevent this, we adopted a similar approach as is used in subcutaneous injections via hypodermic syringe: the injector was positioned at a 45° angle to the skin surface, at the base of a fold of skin as shown in Fig. 3. Six injections of tissue marking dye were performed in this manner into an intact pig immediately post-mortem, as well as four injections via hypodermic syringe. Sectioning these injections, as shown in the main portion of the figure, illustrates that the injection depth ranged from 2 mm to 3 mm; all injections remain within the subcutaneous fat, with no muscle penetration.

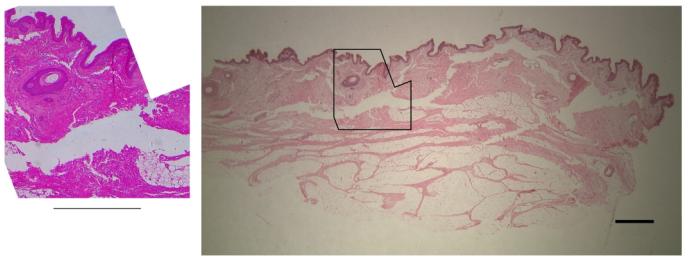

Figure 5: Histological section through a nicotine injection site, showing normal morphology of the skin and subcutaneous fat. (Scale bars are 1 mm.)

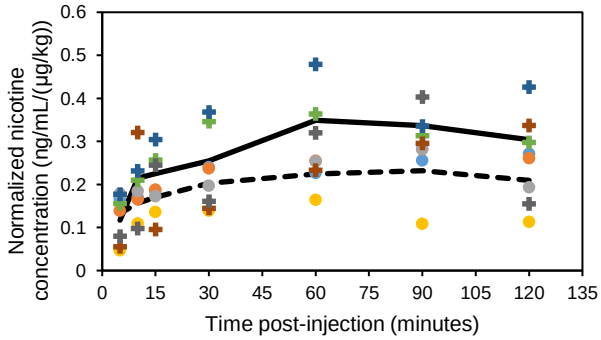

Figure 6: Dose-normalized plasma nicotine concentration is shown for the eight subjects, with needle injections ($n = 4$) shown by circles and jet injections ($n = 4$) by crosses, and each subject shown in the same color. Average nicotine levels for the needle injection (dashed) and jet injection (solid) groups are given by lines.

3.2. Tissue Effects

Fig. 4 shows the typical immediate aftereffects of a jet injection of nicotine. A small amount of fluid appears on the skin at the immediate injection site, in this case about 3 μL, composed of a mixture of injectate, interstitial fluid, and blood. No redness or swelling were observed during the two hours following the injection. Fig. 5 shows a histological section through one of the injection sites; no evidence of tissue damage or disruption is present.

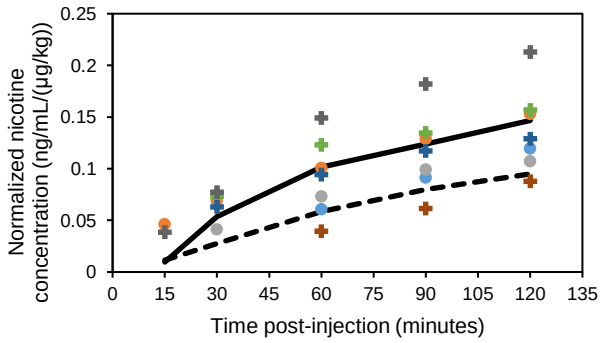

Figure 7: Dose-normalized plasma cotinine concentration is shown for seven of the subjects, with needle injections ($n = 3$) shown by circles and jet injections ($n = 4$) by crosses, and each subject shown in the same color. Measurements below the detection threshold are omitted. Average cotinine levels for the needle injection (dashed) and jet injection (solid) groups are given by lines.

3.3. Pharmacokinetics

Fig. 6 shows the time course of plasma nicotine concentration for the two hours following injection, normalized by dose. There is substantial subject-to-subject variability, though generally the differences between the jet injection and needle groups are not statistically significant. The rise in nicotine concentration is rapid over the first 15 minutes, followed by a slow rise or decline dependent on subject.

Fig. 7 shows the time course of plasma cotinine concentration for these same subjects, normalized by the nicotine dose. (No cotinine data were available for one of the hypodermic needle subjects.) There is once again substantial subject-to-subject variability, but no difference between the needle and jet injection cohorts. In all cases, cotinine levels rise steadily over the course of the monitoring period, but remain low, well below the nicotine concentrations.

4. Discussion

Subcutaneous jet injection effectively delivers nicotine to the general circulation, with comparable absorption as compared to standard needle injection. It appears that the absorption may be faster, but greater statistical power would be required to support any conclusions about the rate of absorption. It is important to note that porcine skin has reduced vascularization as compared to human skin [20], which may accentuate differences in absorption rate caused by jet-injection-enhanced dispersion within the tissue. Subcutaneous nicotine delivered by needle in humans is completely bioavailable [9], and it is unlikely that jet injection causes the absolute bioavailability of nicotine to change. Instead, a reduced peak nicotine concentration may be due to slower release into the circulation compared to the rate of nicotine metabolism. Future studies, with samples collected over a longer time and with a greater number of subjects, would allow exploration of these possibilities.

Regardless of the delivery method, there is considerable variation between animals. In addition, the nicotine profiles are not the same as those seen in humans, for which a shorter time to maximum concentration followed by an elimination half-life of approximately 2 hours would be expected [5]. Instead, the concentration remains steady or slowly increases over time following the initial rise. Likewise, cotinine concentrations rise much more slowly than is typically observed in humans [23].

The most likely explanation for the differences between previously-reported human pharmacokinetics and our observations is differences in liver enzyme activity between pigs and humans [20]. Specifically, the activity of a cytochrome P450, CYP2A, the enzyme subfamily responsible for nicotine metabolism, is lower in pigs than in humans, with significant variability between individuals [24]. Caution should thus be used in interpreting the pharmacokinetics beyond the first 15 minutes post-injection, due to the different elimination kinetics.

For human use, it will be important to avoid injection pain. No information on injection pain is available for unbuffered nicotine base formulations, as were

used in studies on cognitive enhancement (e.g. [25]), while one study instead using a formulation of nicotine tartrate buffered to physiological pH explicitly reported no pain at the injection site [17]. At first glance, the high osmolarity of the solution used in this study (425 mOsm/L) might suggest potential for injection pain [26]; however, any hypertonicity quickly diminishes as nicotine base readily diffuses through cell membranes. While our formulation has a strongly basic pH of 10.6, rapid injection of basic formulations is not associated with significant increases in pain as compared to injection of solutions at physiological pH [27]. The lack of tissue damage, edema, or redness at the injection site seen in this study further suggests that nicotine base may be suitable, particularly given the small injection volume. However, the presence or absence of pain can ultimately only be verified through human trials.

The injection parameters used in this study generate a very high jet velocity, which with larger injection volumes would be sufficient for intramuscular injection [28]. The injection volume itself limits the depth to the subcutaneous region, in this case, which minimizes the effect of the tissue properties on the injection depth. Our approach is consistent with the results in [29], which found the most consistent injections in human volunteers to be those operated at the highest pressures. As such, our injection parameters should be directly translatable to human subjects.

In order to develop jet-injected subcutaneous nicotine into a technique for nicotine replacement therapy, a number of further issues need to be addressed, through clinical study in humans and/or in engineering development. Perhaps most importantly, the pain associated with jet injection of nicotine must be determined through clinical trial and minimized. Injection is generally seen as a more invasive delivery method than the sprays, patches, gums, and aerosols currently used for NRT, despite its avoidance of mucosal side effects (unpleasant taste, burning sensation, etc.), and so it will be important to establish the amount of pain and irritation to ensure it is low enough for patient acceptance. The current stainless-steel ampoule will need to be replaced with a disposable unit for safety. Our injection system, with its bench-top power amplifier, is suit-

able for use in clinical and residential settings, such as rest homes and hospitals, but needs to be further miniaturized to fit into a pocket for general use. Alternative designs for the injector motor (e.g. [30]) should allow an even smaller device to be constructed, though purely mechanical jet injection systems are also an option for pocket-sized use.

Beyond these technical issues, it will also be necessary to establish the larger context for the use of an injectable NRT. In particular, the abuse liability and dependence potential should be investigated, although it is unlikely to be high given the low dependence potential of other NRTs [31]. The system will need to be designed to prevent re-use of ampoules, so as to avoid the potential for infection (or blood-borne illness, if injectors are shared).

5. Conclusions

Nicotine has been delivered via subcutaneous jet injection. A procedure for reliably delivering nicotine to subcutaneous fat via needle-free jet injection was developed and demonstrated in a porcine model. The jet injection delivery method showed a similar pharmacokinetic profile to subcutaneous needle injection, and caused no acute side-effects. To the best of the authors' knowledge, this is the first time this delivery method has been used with nicotine.

Overall, subcutaneous NRT via jet injection is a promising method, and our initial animal study has shown that it provides nicotine with comparable pharmacokinetics to needle injection. The information is now in place to support the safety of trialing this method in humans, to establish its acceptability and subjective performance.

Acknowledgements

The authors would like to thank Linley Nisbet, for assistance in performing the animal manipulations, Mahima Bansal, for assistance in preparing the nicotine formulation, Satya Amirapu, for assistance with histology, and Marewa Glover, for the initial inspiration of this project. This work was financially

supported by the Tobacco Control Research Tūranga, funded by the Health Research Council of New Zealand.

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