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Enhanced Analgesic Responses After Preferential Delivery of Morphine and Fentanyl to the Olfactory Epithelium in Rats

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Abstract

Background—Centrally acting opioid analgesics such as morphine and fentanyl are effective, but their efficacy is often limited by a delayed response or side effects resulting from systemic first-pass before reaching the brain and the central nervous system (CNS). It is generally accepted that drugs applied to the nasal cavity can directly access the brain and the CNS, which could provide therapeutic advantages such as rapid onset and lower systemic exposure. The olfactory region of the nasal cavity has been implicated in facilitating this direct nose-to-CNS transfer. If the fraction of opioid administered to the olfactory region could be improved, there could be a larger fraction of drug directly delivered to the CNS, mediating greater therapeutic benefit.

Methods—We have developed a pressurized olfactory delivery (POD) device to consistently and non-invasively deposit a majority of drug on the olfactory region of the nasal cavity in Sprague-Dawley rats. Using the tail-flick latency test and analysis of plasma and CNS tissue drug exposure, we compared distribution and efficacy of the opioids morphine and fentanyl administered to the nasal olfactory region with the POD device or the nasal respiratory region with nose drops or systemically via intraperitoneal (IP) injection.

Results—Compared to nose drop, POD administration of morphine resulted in significantly higher overall therapeutic effect (AUC_{effect}) without a significant increase in plasma drug exposure (AUC_{plasma}). POD delivery of morphine resulted in a nose-to-CNS direct transport percentage of 38–55%. POD delivery of fentanyl led to a faster (5 min vs. 10 min) and more

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DISCLOSURES:

Name: John D. Hoekman, Ph.D.

Contribution: This author helped design the study, conduct the study, analyze the data, and write the manuscript.

Conflicts: John D. Hoekman has equity interest in Impel NeuroPharma. The results in this work, along with others, prompted the founding of a startup company, Impel NeuroPharma to create nasal drug delivery devices to better penetrate the olfactory region of the nasal cavity. In full disclosure of conflict of interest, both authors are inventors on a patent for the POD nasal spray device and both authors also own stock in a startup company, Impel NeuroPharma, Inc. (Seattle, WA) founded to develop olfactory-targeted nasal drug delivery devices.

Attestation: John D. Hoekman has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

Name: Rodney J.Y. Ho, Ph.D.

Contribution: Helped design the study, analyze the data, and write the manuscript.

Conflicts: Rodney J.Y. Ho, Ph.D. has equity interest in Impel NeuroPharma. The results in this work, along with others, prompted the founding of a startup company, Impel NeuroPharma to create nasal drug delivery devices to better penetrate the olfactory region of the nasal cavity. In full disclosure of conflict of interest, both authors are inventors on a patent for the POD nasal spray device and both authors also own stock in a startup company, Impel NeuroPharma, Inc. (Seattle, WA) founded to develop olfactory-targeted nasal drug delivery devices.

Attestation: Rodney J.Y. Ho has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.

intense analgesic effect compared to nasal respiratory administration. Unlike IP injection or nose drop administration, both morphine and fentanyl given by the POD device to olfactory nasal epithelium exhibited clockwise [plasma] versus effect hysteresis after nasal POD administration, consistent with direct nose-to-CNS drug transport mechanism.

Conclusions—Deposition of opioids to the olfactory region within the nasal cavity could have a significant impact on drug distribution and pharmacodynamic effect, and thus should be considered into account in future nasally administered opioid studies.

Introduction

Morphine and fentanyl are widely used opioid analgesics for pain control. Despite the usefulness of these drugs, each has some serious drawbacks. For example, morphine-induced constipation is a serious systemic side effect that affects 80% of patients (1) and can have a negative impact on both the patient's quality of life (2) as well as adherence to their drug regimen (3). A delayed time of onset can also be an issue in outpatient use of opioids in conditions such as breakthrough cancer pain. Oral morphine and buccal fentanyl are commonly prescribed in treatment of breakthrough cancer pain (4) and typically require 22–30 minutes or 5–10 minutes (5–6) respectively to initiate analgesic effect. Directly targeting these drugs to the central nervous system (CNS) could significantly reduce side effects and improve time to analgesic onset with these drugs.

A fraction of intranasally applied drugs may rapidly distribute directly from the nasal cavity to the CNS, effectively bypassing the blood-brain barrier (BBB) resulting in faster onset times and lower systemic concentrations (7). Intranasal morphine instillation in rats has resulted in about 10% direct transport from the nasal cavity to the CNS resulting in significantly larger brain concentrations of morphine in the brain and less systemic exposure compared to IV administration (8). Using this route of delivery with opioid pain medications could improve the time of onset and side effect profile of these drugs.

Despite its potential, direct “nose-to-CNS” distribution is underutilized in clinical settings. This is due to a lack of drug delivery devices to reach the olfactory region. The standard nasal pumps used in clinical trials for intranasal morphine and fentanyl typically deposit less than 5% of the dose in the olfactory region (9). The olfactory epithelium, which is located between the nose and the brain, is only 3–10% of the surface area of the nasal cavity in humans (10–11). Its position in the upper nasal cavity with turbinate restriction makes consistent delivery of drug to this region challenging. The olfactory region in the upper back quantile of the nasal cavity encompasses a narrow, approximately 1–2 mm wide slit. Typical nasal sprays tend to have a wide aerosol plume angle that mainly deposits on the respiratory epithelium (9) located at the lower nasal cavity. Drugs deposited in the lower nasal cavity and respiratory epithelium could potentially penetrate through the trigeminal pathway (12), but evidence for this pathway is more limited.

Therefore, we have developed a pressurized olfactory delivery (POD) device which is able to deposit a majority of drug at the olfactory region of the nasal cavity in rats. The objective of this study was to determine if increasing the fraction of the opioids morphine and fentanyl deposited at the olfactory region of the nasal cavity will lead to a larger fraction of drug being directly transported from the nasal cavity to the CNS. If so, intranasal morphine and fentanyl could be developed with faster onset times and potentially fewer systemic side effects. We determined that preferential nasal olfactory region deposition of morphine and fentanyl produced more rapid analgesic onset and significant direct nose-to-CNS distribution compared to either nasal respiratory deposition or systemic administration in rats.

Materials and Methods

Materials

Morphine sulfate and fentanyl citrate were purchased from Sigma-Aldrich (St. Louis, MO). Beuthanasia-D (Schering-Plough Animal Health Corp, North Chicago, IL) was used for euthanasia at the end of the distribution studies. The drug doses were dissolved in 0.9% saline solution (Hospira, Inc., Lake Forest, IL). Morphine and fentanyl LC/MS standards were purchased from Cerilliant (Round Rock, TX). All other materials used were reagent grade.

Animals

Adult male Sprague-Dawley rats (200–300 g; Harlan, Indianapolis, IN) were housed under a 12-hour light/dark cycle with Harlan Teklad rat chow and water provided ad libitum. Animals were cared for in accordance with institutional guidelines, and all experiments were approved by the University of Washington Animal Care and Use Committee under IACUC #2372-12.

Preparation of drug formulations

Morphine and fentanyl were stored at -20°C as a lyophilized powder. On the day of each experiment, the necessary doses of morphine and fentanyl were solubilized in 0.9% saline solution (Sigma-Aldrich, St. Louis, MO). Each formulation had a pH of 7.0 for each of the doses tested. The nasally administered doses were given in volumes of 7–11 μl and the intraperitoneal (IP) doses were given in volumes of 0.45–0.55 ml depending on the weight of the animal.

Pressurized olfactory delivery (POD) device construction and characterization

The overall construction of the POD nasal aerosol device is diagrammed in Figure 1. A pressurized nitrogen gas supply was connected to a standard two-valve pressure regulator. Plastic tubing with a 200 psi pressure rating connected the pressure regulator to the inflow connection of a pneumatic solenoid (Cramer Decker Industries, Santa Ana, CA). The solenoid was regulated with a foot pedal actuated GraLab 555 digital timer (GraLab Corporation, Centerville, OH). On the outflow connection of the solenoid was a 3-ml cylinder, which made up the device body and fit securely to the solenoid. On the end of the device body was a custom-fit aerosol nozzle with a 0.8-mm outside diameter. The nozzle was fitted with an aerosol insert composed of a small length (2.0 mm) of metal cylinder, which had two spiral fluid passages in which the fluid/gas mixture traveled. This served to mix the nitrogen and liquid drug to create an aerosol output to enhance penetration into the nasal cavity towards the cribriform plate area while minimizing pressure on the nasal epithelia. Finally, a fluorinated ethylene-propylene liner was placed over the outside of the metal tip in order to protect the nasal epithelia from being damaged by the nozzle during use. A fluorescence assay was used to determine what percentage of the total desired volume was dispensed from the device with each actuation. A solution of 50 $\mu\text{g/ml}$ fluorescein was dispensed from the device into a well in a 24-well plate that was prefilled with 1 ml deionized H_2O . After collection of the aerosol, the solution was mixed by pipetting up and down several times. The fluorescence of the solution was measured on a PerkinElmer 1420 multivariable fluorescence plate reader (PerkinElmer, Waltham, MA). The fluorescence signal of the collected volume was compared with the expected signal to calculate the percent of drug expelled from the device with each actuation.

The basic operation of the POD device in rats was as follows: the animal was anesthetized with isoflurane, the dose was loaded into the device and the nozzle was carefully placed 8.0 mm into the rat's nasal cavity and pointed in the direction of the cribriform plate. The foot

pedal switch was then pressed to actuate the solenoid for 0.1 seconds to discharge the aerosol dose.

Nose drop administration targeting the nasal respiratory region

In order to determine any differences in opioid distribution after enhanced olfactory deposition, a simple nose drop method was developed as a control route of administration specifically targeting the nasal respiratory region. The rats were anesthetized with isoflurane and retained in a natural position resting on their stomachs. Drops of 3 μ l were placed near the rat's naris and naturally inhaled into the nasal cavity. This was repeated in opposing naris every 20 seconds until the required dose volume was administered.

Nasal deposition experiments

Deposition was tested using both the POD device and nose drops. Under pentobarbital anesthesia, the rats (N = 6) received 10 μ l dye solution of 0.1% Coomassie Blue with the POD device or nose drop administration. Shortly after administration was complete (< 5 minutes), the animals were overdosed with 250 mg/kg pentobarbital. The nasal cavity was then bisected at the septum, the septum was removed, and the tissues were examined grossly for dye localization. The nasal tissues were then decalcified, sectioned, and stained with hematoxylin and eosin stain according to the method of Young. (13) These sections were then examined under a Zeiss Axiovert 200 fluorescent microscope (Carl Zeiss Inc., Jena, Germany) using the methods of Chandler et al., (14) to examine the epithelial integrity.

Tail-flick analgesia experiments

Each group of animals (N=9) initially went through three days of placebo testing to get a baseline reading for the tail-flick test as well as acclimating the animals to handling. Each animal was exposed to 5% isoflurane in an induction box for 2.5 minutes. The animal was then removed and given a 10 μ l dose of 0.9% saline solution, pH 7.4, via nose drops, POD, or IP injection. Once the placebo dose was administered, the animals were allowed to fully wake from the isoflurane in a padded tray. Then, at 5, 10, 30, 45, 60, 90, and 120 minutes each animal was wrapped gently in a towel, had their tails placed in room temperature water ($18^{\circ}\pm 0.5^{\circ}\text{C}$) for 5 seconds, the tail was quickly dried, and then the distal 3 cm of the tail was placed in $55^{\circ}\pm 0.5^{\circ}\text{C}$ water. The time until tail removal was measured with a digital stopwatch.

After the initial placebo trials, the same procedure was repeated three times, every other day over 5 days, with each rat receiving a single dose of either morphine or fentanyl by nasal spray, nose drops, and IP injection in a randomly chosen order. The cutoff time, at which the tail would be removed from the water to prevent tissue damage, was set at 10 seconds for all tail-flick trials.

Pharmacokinetic experiments

Three days after a group of animals completed the tail-flick studies they were cannulated in preparation for and immediately before a plasma pharmacokinetic experiment. Animals were anesthetized with 2% isoflurane (Novapulus, Lake Forest, IL). Body temperature was maintained at 37°C by a heating pad (Fine Science Tools, Inc., Foster City, CA). For pharmacokinetic experiments, the femoral vein was cannulated for blood draw with PE-0 polyethylene tubing (Becton, Dickinson and Company, Franklin Lakes, NJ) connected to blunt tipped 23-gauge needle (Becton, Dickinson and Company, Franklin Lakes, NJ). The PE-10 tubing was inserted 3 cm into the femoral vein to ensure blood sampling was from the vena cava. Animals were maintained on anesthesia for 30 minutes before drug administration and pharmacokinetic analysis. After the final blood draw, the catheter was

removed from the femoral vein, the proximal end of the femoral vein was tied off with suture string to assure hemostasis, and the incision stitched with suture string (Harvard Apparatus, Holliston, MA).

After a 30-minute resting period under anesthesia after surgery, the animals (N=3) were administered a single dose of either morphine (1.0, 2.5, or 5.0 mg/kg) or fentanyl (7.5, 15.0, or 25.0 µg/kg). Then, at 5, 10, 30, 45, 60, 90, and 120 minutes after drug administration, 300 µl of blood was drawn from the femoral vein catheter. The blood was collected in a 1 ml syringe (Becton, Dickinson and Company, Franklin Lakes, NJ) and transferred to a microcentrifuge tube for blood/plasma separation. The tubes were immediately centrifuged at 8,000g for 5 minutes, and then the plasma was removed and frozen on dry ice. At the end of the experiment 2.0 ml of sterile 0.9% saline solution was injected via the femoral vein catheter to replace the removed blood volume.

CNS tissue distribution experiments

One week after the pharmacokinetic experiments the animals used in the pharmacokinetic studies were used in CNS tissue distribution experiments. The animals (N=6) were briefly anesthetized with 2.5 minutes exposure to 5% isoflurane. A dose of morphine (2.5 mg/kg) or fentanyl (15 µg/kg) was administered via POD, nose drops, or IP injection. At 5 minutes the animals were killed with an overdose of Beuthanasia-D. Immediately after death, the animal was decapitated. A syringe was used to collect 1.0 ml of blood from the trunk. This plasma was processed in the same way as the plasma from the pharmacokinetic experiments, and was used in the brain/plasma concentration ratio data. The base of the skull and the parietal bones were quickly removed. The brain was removed within 2 minutes of death. The brain was dissected into the cortex, diencephalon, brainstem, cerebellum, and olfactory bulbs. The cervical spinal cord was also collected from the body. The olfactory bulbs were the final brain tissues collected.

Analysis of morphine and fentanyl by LC/MS

A fixed volume (20 µl) of either morphine d-6 or fentanyl d-6 (Cerilliant, Palo Alto, CA) was added into each tissue and plasma sample to act as an internal standard. Tissue samples were homogenized in 5–10 times volume of 0.1M borate buffer, pH 8.9 and centrifuged for 10 minutes at 1000g. The tissue supernatant and plasma samples were passed over Certify solid phase extraction cartridges (Varian, Palo Alto, CA) and eluted with methylene chloride: isopropanol: ammonium hydroxide (80:20:2). After elution the samples were evaporated under N₂ gas until dry. The samples were resuspended in 75 µl of mobile phase which consisted of 92% of 0.05% acetic acid and 8% acetonitrile for morphine samples or 40% 10 mM ammonium acetate and 60% acetonitrile for fentanyl samples. An Agilent HPLC/MS series 1100 series B with autosampler (Agilent, Technologies, Inc., Santa Clara, CA) was used for quantification. The injection volume was 5 µl. The morphine samples were passed over a Zorbax SB-C8 column (Agilent, Technologies, Inc., Santa Clara, CA) with a flow rate of 0.25 ml/min. The ionization setting was API-ES in the positive ion mode with a capillary voltage of 1400V.

For both morphine and fentanyl, a standard curve was created on the day of analysis according to the same process described for the samples. Each standard curve was linear with a coefficient of linear regression $R^2 > 0.99$. In addition, two quality control samples with a known amount of drug were processed on the day of analysis in order to ensure day-to-day consistency of the analytical assay.

Data analysis

All tail-flick test values are presented as a percentage of maximal possible effect, which is defined as:

$$\%MPE = \frac{(\text{Post drug latency} - \text{baseline latency})}{(\text{cutoff time} - \text{baseline latency})} \times 100$$

The cutoff time was set at 10 seconds in the tail-flick studies to prevent tissue damage from occurring. AUC values from all experiments were calculated using the trapezoidal rule without extrapolation to infinity. Pharmacokinetic values were obtained from one-compartmental modeling in WinNonlin (Pharsight, Mountain View, CA). Tail-flick data were compared using repeated measures ANOVA. Plasma and tissue concentrations were compared using a one-way ANOVA with a Tukey post-test. $AUC_{\text{effect}}/AUC_{\text{plasma}}$ ratios were calculated from individual animals so they could be statistically compared with a one-way ANOVA. All statistical analyses were performed using Sigma Plot software version 11.0 (Systat Software, Inc., San Jose, CA).

A direct transport percentage (DTP%) to the brain was calculated in order to determine the amount of drug in the brain that was distributed directly from the nasal cavity to the CNS. Because analgesic effect has been shown to correlate well with morphine concentrations in the extracellular brain fluid (15), we used analgesic effect in place of brain concentrations. The DTP% is used to estimate the amount of drug in the brain that cannot be accounted for by systemic distribution. The DTP as defined was calculated as follows (16):

$$DTP\% = \frac{\frac{AUC_{\text{effect(IP)}}}{AUC_{\text{plasma(IP)}}} - \frac{B_X}{AUC_{\text{effect(nasal)}}}}{\frac{B_X}{AUC_{\text{effect(nasal)}}}} \times 100\%$$

We also performed hysteresis analysis to determine the time delay in plasma drug concentration with corresponding analgesic effects observed typically for systemic administration. This analysis would reveal whether the POD device-mediated-enhanced opioid delivery to the CNS impacts the time lag of opioid analgesic effects compared to after oral or IV administration. To do so, plasma opioid concentration for any time point within the time-course of plasma concentration was plotted against the effects observed at that specific time point for each dose of morphine or fentanyl. The time sequences were included as arrows in these plots for rats treated with morphine or fentanyl. These plots were used as a mean to evaluate consequence of direct delivery of opioid by POD device to CNS delivery as drug traveling from the plasma to the CNS would give rise to a faster onset in plasma drug concentration before or at the same time as rise in analgesic effect.

Results

Evaluation of drug administration methods

We first evaluated the dose residuals and nasal deposition of the POD device. Using a fluorescence assay to measure residuals, the POD device dispensed a 10- μ l dose with $99.3 \pm 2.6\%$ accuracy. Using Coomassie Blue dye, the deposition within the rat's nasal cavity was determined after delivery with either the POD device or our nose drop method. When using 10 μ l of dye, the POD administration resulted in deposition on the olfactory epithelium area of the nasal cavity with none or very little observed on the respiratory region, trachea, or esophagus. Administering the dye by our nose drop method resulted in deposition primarily on the respiratory epithelium. No noticeable dye staining was apparent in the olfactory

region, or in the trachea or esophagus. In the nasal epithelial histopathology assessment of membrane integrity, there was no noticeable nasal damage observed in the POD-administered naris compared to the controlled naris.

Time course of drug concentrations and analgesic effects

Morphine—As shown in Figure 2A, IP administration of 5.0 mg/kg morphine in rats resulted in significantly higher plasma concentrations compared to those treated with an equivalent dose using nose drops or the POD device. The plasma morphine concentrations after IP administration were significantly higher ($p < 0.05$) over the first 30 minutes with a $T_{\max(\text{plasma})}$ at 20.0 minutes with a $C_{\max}$ of 597.6 ng/ml (Table 1). The plasma morphine concentrations at 10 minutes were roughly 2.6-fold higher in IP-treated rats than those treated with either type of nasal administration. The only point with a significant difference between the POD device and nose drop-treated rats ($p < 0.05$) was at 5 minutes, when the plasma concentration in the POD device group was nearly 2-fold higher than those in nose drops. Both the POD spray and the nose drops resulted in a $T_{\max(\text{plasma})}$ of approximately 30 minutes and $C_{\max}$ values of 314.5 and 297.4 ng/ml respectively (Figure 2). Although there was a significant trend of higher plasma concentrations after IP administration in the first 45–60 minutes, there were no statistical differences among the three test groups in overall plasma morphine exposure (AUC_{0-120}) for animals treated with the two low doses 1 or 2.5 mg/kg (Table 2). At 5.0 mg/kg morphine, AUC_{0-120} for rats treated with IP was significantly higher compared to those treated with either type of intranasal administration (Table 2).

The tail-flick analgesic test revealed that at 5.0 mg/kg morphine, rats treated with the POD device as compared with the nose drops, produced significantly higher analgesic effect ($p < 0.05$) over the first 60 minutes (Figure 2C). In addition, POD device treatment resulted in a significantly higher analgesic effect ($p < 0.05$) than IP injection at the 5 minute time point. The animals treated with morphine in the POD device exhibited $T_{\max(\text{effect})}$ at 10 minutes and remained at approximately the same level over the following 50 minutes. The overall effects, AUC_{0-120} for animals treated with morphine in either POD device and IP injection, were significantly greater ($p < 0.05$) than those treated with nose drop (Table 2). There was no significant difference in analgesic AUC between rats treated with the POD device or IP administration after 5.0 mg/kg morphine.

Fentanyl—We next tested the effects of the POD device in delivery of a more hydrophobic opioid, fentanyl. The plasma concentrations of 15 $\mu\text{g/kg}$ fentanyl were significantly higher ($p < 0.05$) after both POD and nose drop administration than after IP administration over the course of 120 minutes (Figure 2B). Although the 7.5 $\mu\text{g/kg}$ and 25 $\mu\text{g/kg}$ doses led to similar relative plasma profiles as the 15 $\mu\text{g/kg}$ dose, they did not lead to significant plasma concentration differences between routes of administration. In rats treated with 15 $\mu\text{g/kg}$ fentanyl with either the POD device or by IP injection, the $T_{\max(\text{plasma})}$ was very rapid, 1.70 and 1.46 minutes, respectively (Table 3). The plasma concentrations for rats treated with fentanyl nose drops resulted in a $T_{\max(\text{plasma})}$ at 6.50 minutes with high variation, but all animals had the same experimental $T_{\max(\text{plasma})}$ and overall shape of plasma fentanyl curve. This rapid onset and decline was also observed after the 7.5 $\mu\text{g/kg}$ and 25 $\mu\text{g/kg}$ doses after POD administration. At the 5-minute time point, the plasma concentrations were 5 and 6.7 times higher for the nose drop and POD device groups compared to the IP injection.

POD administration of 15 $\mu\text{g/kg}$ fentanyl to the olfactory region of the nasal cavity resulted in a much greater analgesic effect (Figure 2D) at 5 minutes compared to nose drops or IP administration ($p < 0.05$ vs. nose drops and $p < 0.01$ vs. IP). It should be noted that at the 5 minute time point more than half of the POD-treated rats did not pull their tails from the

water bath before the ten second maximum cutoff time (to prevent tissue damage). Therefore, the analgesic effect from the POD spray at 5 minutes was underestimated. After 5 minutes however, the analgesic effect after POD spray decreased quickly. After this initial time point there was no significant difference among the analgesic effects from three routes of administration. At the 5 and 10 minute time points, animals treated with fentanyl nose drops also had a significantly higher analgesic effect ($p < 0.05$) than those treated by IP injection. The overall fentanyl effects, AUC_{0-120} for POD device treatment, were significantly less ($p < 0.05$) than either nose drop or IP treatment (Table 4).

Comparison of plasma drug exposure and overall analgesic effects

POD administration of morphine resulted in a significantly lower plasma AUC after a 5.0 mg/kg dose and a nonsignificantly lower plasma AUC at 2.5 mg/kg (Table 2). POD administration resulted in nonsignificantly higher plasma AUC at 1.0 mg/kg dose. In addition, POD administration resulted in a significantly higher effect AUC compared to nose drops at 1.0 and 5.0 mg/kg doses and a significantly higher effect AUC compared to IP administration after a 1.0 mg/kg dose. POD administration also resulted in 1.64, 1.61, and 2.24 fold increases in $AUC_{effect}/AUC_{plasma}$ ratio compared to IP after 1.0, 2.5, and 5.0 mg/kg doses administration at each dose tested (Table 2). There was no significant difference in this AUC ratio between IP administration and nose drop administration. The DTP% after POD administration was estimated to be 38.5%, 38.1%, and 55.0% after 1.0, 2.5, and 5.0 mg/kg doses respectively. The DTP% after nose drop administration was estimated to be 0%, 10.9%, and 14.8% after 1.0, 2.5, and 5.0 mg/kg doses respectively.

For fentanyl, since neither nose drop nor POD administration led to significantly higher $AUC_{effect}/AUC_{plasma}$ ratios compared to systemic IP administration, all of the opioid drug causing analgesic effect could be accounted for from blood distribution and thus, a DTP% was not calculated. In addition, there were no significant differences between the plasma normalized brain concentrations after nose drops, POD spray, or IP administration (Figure 7). In both the forebrain and midbrain-cerebellum-brainstem-spinal cord (MCS) the POD spray resulted in a nonsignificant trend of higher blood-to-brain ratios at 5 minutes after administration of 15 μ g/kg fentanyl.

Hysteresis analysis to evaluate first passage of drug from nose-to-CNS

To evaluate plasma drug concentration-effect response in relationship to time-course plasma drug concentrations, we performed hysteresis analysis by plotting the plasma drug concentration versus analgesic effects according to the time sequence for rats treated with morphine or fentanyl. These plots are useful in determining any direct to CNS delivery because drug traveling from the plasma to the CNS should result in an increase in plasma concentration before or at the same time as an increase in analgesic effect. Any direct nose-to-CNS distribution should result in an increase in analgesic effect preceding an increase in plasma concentrations. As shown in Figure 3, several differences among rats treated with nose drops, POD device, or IP injection become apparent. The point on each graph at (0,0) represents the baseline tail-flick test before dosing. It is semiartificial and was primarily added for easier visualization of the data sequence. After a 2.5 mg/kg morphine dose (Figure 3A–C) both nose drop and IP administration resulted in a counterclockwise hysteresis, whereas the POD administration resulted in a clockwise hysteresis. On the other hand, rats treated with 15 μ g/kg fentanyl nose drops exhibited no discernable hysteresis (except the baseline point). Those treated with the POD device exhibited a clockwise hysteresis while the IP group showed a counterclockwise hysteresis (Figure 3D–F). All three fentanyl effect-concentration curves were plotted on the same axis scale so the plot for IP administration appears much smaller due to low plasma fentanyl concentration and analgesic effect.

Drug concentration in the CNS tissues

CNS tissue concentrations were determined as a secondary measure of CNS drug distribution in addition to analgesic effect. The upper cervical spinal cord was processed with other parts of the brain. The brain concentrations of morphine at 5 minutes after POD administration of 2.5 mg/kg to the olfactory region of the nasal cavity were higher than either nose drop administration to the respiratory region of the nasal cavity or IP injection (Figure 4). Although after POD device treatment, the olfactory bulb morphine concentration was much higher, 7.60- or 39.58-times that of nose drop or IP administration, the results did not reach significance. However, POD device-treated animals exhibited higher morphine brain (excluding the olfactory bulbs and including the upper cervical spinal cord) concentrations at 5 minutes ($p < 0.05$). Administration with the POD device to the nasal olfactory region resulted in 1.68-times the brain concentration after nose drops and 3.38-times the brain concentration after IP administration. There was no significant difference between brain concentrations at 5 minutes after nose drop or IP administration.

Similar differences were observed when the brain tissue morphine concentration was normalized by plasma morphine concentration (Figure 5). Again, the POD device provided a trend toward larger blood normalized olfactory bulbs morphine concentrations (but the data were not statistically significant). In the rest of the brain, the POD device provided significantly higher ($p < 0.05$ vs. nose drops and $p < 0.01$ vs. IP) blood normalized, brain morphine concentrations. The blood normalized brain morphine concentrations at 5 minutes for POD device-treated animals were 2.27 and 6.48 times the concentrations, compared to those treated with nose drop and IP injection.

Nasal delivery to the olfactory region 5 minutes after a dose of 15 $\mu\text{g/kg}$ fentanyl with the POD device resulted in significantly higher ($p < 0.05$) concentrations in the brain than after either nose drop administration to the respiratory region or IP injection (Figure 6). In this study, the forebrain included the olfactory bulbs. At 5 minutes, the POD nasal device with fentanyl resulted in 1.63-fold increased concentration compared to nose drop application and a 5.61-fold increase compared to IP administration. The results in the MCS were similar. POD spray application resulted in 1.56 and 5.32 times the fentanyl concentration in the MCS when compared with nose drops administration or IP injection respectively. In addition, animals treated with the POD device exhibited significantly larger ($p < 0.05$) plasma concentrations at 5 minutes after 15 $\mu\text{g/kg}$ fentanyl administration. The POD device resulted 1.28 times higher plasma fentanyl concentrations compared with nose drops and a 4.5 times higher concentration than IP administration.

Discussion

We have developed drug administration techniques in rats to specifically deposit drug at the nasal olfactory region (POD) or the nasal respiratory region (nose drops). Using morphine and fentanyl, which exhibit significantly different hydrophobicity and ability to penetrate the BBB, we found that there are significant differences in distribution between drug delivery to the nasal respiratory epithelium compared to the nasal olfactory epithelium for both fentanyl and morphine. In this study we used the analgesic effect, as determined by the tail-flick latency test, as a measure of the opioid drug distribution to the CNS. Pharmacokinetic studies have determined the use of effect to be a suitable surrogate for extracellular brain concentrations (17–18). The correlations between plasma pharmacokinetics and effect allow a mechanism to determine any direct nose-to-CNS distribution after olfactory nasal administration. Delivering a more hydrophilic morphine to the olfactory region of the nasal cavity with the POD device resulted in increased direct transport from the nasal cavity to the CNS, increasing the analgesic effect while maintaining low plasma values. On the other hand, POD-administered fentanyl deposition on the olfactory region resulted in a very strong

and rapid analgesic effect that could be related to the first pass of drug to the brain that readily cleared into the blood. As a result, the overall time-course of plasma drug concentration did not appear to differ significantly from that of nose-drop nasal administration (Figure 2).

The morphine data demonstrate that in addition to morphine uptake into the blood from the nasal olfactory region, which was similar to uptake at the respiratory region, there was further increased uptake into the CNS after deposition at the olfactory region of the nasal cavity. This additional uptake into the CNS observed after olfactory delivery was rapid. This direct transfer is most likely due to passive diffusion along the fluid-filled channels that connect the olfactory region of the nasal cavity with the subarachnoid space surrounding the olfactory bulb (19). At the initial time point measured, POD administration did not result in higher plasma concentration, but did result in significantly higher analgesic effect compared to the other two routes of administration. The data were plotted as plasma concentration versus effect hysteresis curves in order to more easily visualize the relationship among the changes in plasma levels and brain levels to distinguish differences in distribution between the three routes of delivery.

This rapid distribution into the CNS after POD administration is seen in the plasma concentration versus analgesic effect curves, where POD administration of morphine resulted in a clockwise hysteresis curve compared to the other two routes which displayed a counterclockwise hysteresis. This counterclockwise hysteresis could be explained by either acute tolerance to morphine or to the pain test, or it could be due to direct and rapid distribution to the brain from the nasal cavity (20). The clockwise hysteresis observed after POD administration of morphine does not appear to be due to sensitization (21), which would be observed as a decreased effect at the later time points, and is instead likely to be primarily due to a rapid increase in effect before the increase in plasma concentration. Thus, this clockwise hysteresis after administration with the POD device is more likely due to direct transport from the nasal cavity to the CNS, such as that observed by Westin et al. (8,22).

This conclusion is supported by the brain-to-plasma ratios observed 5 minutes after administration with the POD device, which were significantly higher than after nose drop or IP administration. An increased brain-to-plasma concentration ratio at this early time point indicates that the drug distributed into the brain through an alternate non-plasma route such as direct nose-to-CNS distribution. This increase in the brain/plasma ratios, especially at the earliest time points was also observed by Westin et al. (8) where brain-to-plasma AUC ratios of 0.5 were observed in the first 10 minutes after nasal administration. Morphine was visually observed with autoradiography to distribute from the nasal cavity to the lamina propria surrounding the olfactory bulb. In our study, a large difference was also observed in the olfactory bulb morphine concentrations; however, these differences were not significant, most likely due to the method of collection of the olfactory bulb and the small sample size (N=3).

Westin et al. calculated the percentage of drug delivered directly to the brain, or DTP%, over the course of 240 minutes. They reported that from 0–60 minutes and 0–240 minutes the DTP% morphine were 48% and 10% respectively. In this study, we found that the DTP% after nose drops was between 0–10% depending on the dose; however, this may not be significant as the $AUC_{\text{effect}}/AUC_{\text{plasma}}$ ratios were not significant, while after POD administration, the DTP% was between 38 and 55%. It is difficult to directly compare our DTP% values to Westin et al. because they used brain concentrations while we used analgesic effect. They also did not calculate a 0–120 min DTP%. However, it is clear from our study that POD delivery to the olfactory region resulted in a higher DTP% compared to nose drop administration to the respiratory epithelium.

In contrast to the enhanced CNS distribution observed after POD administration of morphine to the nasal olfactory region, nose drop distribution to the nasal respiratory region displayed little to no evidence of direct transport from the nasal cavity to the brain. The $AUC_{\text{effect}}/AUC_{\text{plasma}}$ after respiratory epithelium and IP administration were not significantly different across the doses tested and resulted in a counterclockwise hysteresis typical of systemic administration. IP administration was chosen as a systemic method of administration because the plasma time course profile of morphine is similar after nasal administration and IP administration (15), thus the AUCs can be directly compared. These data indicate that morphine applied to the respiratory epithelium distributed primarily into the capillaries of the nasal cavity where it was distributed to the CNS across the BBB.

Administration of lipophilic fentanyl ($\log P = 3.9$) to the olfactory region resulted in a different pattern of distribution compared with hydrophilic morphine ($\log P = 0.8$). Immediately after POD administration of fentanyl, the plasma and analgesic levels were very high and then both decreased dramatically. In fact, POD-administered doses of 7.5 and 15.0 $\mu\text{g/kg}$ led to significantly lower AUC_{effect} compared with nose drop administration. The hysteresis curve after POD administration of a 15.0 $\mu\text{g/kg}$ dose indicated slight hysteresis but only at the initial 5 minute point, and this seems to have been due to the dramatic decrease in analgesic effect with the slight decrease in plasma effect. Although this could indicate some direct transport of fentanyl from the olfactory region to the CNS, this conclusion is not supported by the measured brain tissue concentrations. The forebrain and the MCS contained higher fentanyl concentrations 5 minutes after POD administration to the olfactory region. However, when normalized by plasma, there was no statistical difference among any of the routes of administration. In addition, both forms of nasal administration resulted in lower $AUC_{\text{effect}}/AUC_{\text{plasma}}$ ratios compared with IP delivery, indicating that all three routes of administration resulted in CNS distribution from the bloodstream.

Our data suggests that POD administration of fentanyl to the olfactory region of the nasal cavity primarily leads to uptake into the capillaries of the olfactory epithelium followed by distribution to the CNS across the BBB. Fentanyl is a very lipophilic compound, with a nasal bioavailability of 71% (23). Thus, fentanyl applied to either the respiratory or olfactory epithelium would likely be rapidly absorbed into the bloodstream leaving little drug to distribute directly to the CNS. The rapid redistribution of fentanyl would likely make any direct nose-to-CNS delivery difficult to detect. Several other studies of nasally administered lipophilic drugs such as estradiol (24) and lidocaine (25) have also been unable to observe any direct distribution from the nasal cavity to the CNS. Interestingly, we observed an increased level of analgesia and brain concentrations 5 minutes after POD administration to the olfactory epithelium compared with nose drop delivery to the respiratory region of the nasal cavity. One possible explanation is that the higher density of blood vessels in the lamina propria of the olfactory region (26) could lead to a greater rate of initial fentanyl uptake into the blood.

There were significant differences in distribution after deposition on the nasal olfactory or nasal respiratory epithelium with both drugs. Interestingly, the plasma drug concentrations and effect profiles of both fentanyl and morphine after nose drop administration to the respiratory epithelium were the most similar to values obtained from human nasal administration of these opioid drugs (27–28), with morphine having T_{max} plasma values between 10 and 30 minutes, and fentanyl having T_{max} values between 5 and 10 minutes. This is not surprising considering that most of these trials use standard nasal pumps, which primarily deposit drug on the respiratory epithelium (29). Thus, low volume (<15 μl) nose drop administration using our methods in rats may be an appropriate model for nasal administration in humans with a standard nasal pump. In contrast, POD distribution to the

olfactory region in rats could be representative of localized olfactory delivery within the nasal cavity for humans.

POD administration of morphine resulted in an increased analgesic effect compared to respiratory epithelial deposition, and lower plasma values compared to systemic administration. Thus, administering morphine to the olfactory region of the nasal cavity could be an effective, noninvasive method of opioid administration, which could decrease systemic side effects such as opioid-induced constipation. Olfactory delivery of fentanyl resulted in a very high immediate analgesic effect because of rapid distribution into the bloodstream. This could be of clinical value to reduce the onset time and dose required for outpatient fentanyl use.

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Figure 1. Schematic presentation of the pressurized olfactory delivery (POD) device

The pressurized nitrogen is controlled by a pneumatic solenoid which releases the gas in increments of 0.1 seconds. The gas enters the nozzle outlet and mixes with the liquid dose, which flows through the outlet producing a narrow flow with rotational velocity.

Figure 2. Effects of olfactory epithelium pressurized olfactory delivery (POD) administered 5.0 mg/kg morphine (A, C) and 15 µg/kg fentanyl (B, D) on plasma and analgesic time course

Intraperitoneal (IP) morphine (**A**) led to significantly higher plasma concentration at time points up to 30 minutes, while no difference was seen between plasma levels of POD and nose drop-administered morphine (N=3). In contrast, the POD-administered morphine induced greater analgesic effect (**C**) than either nose drops or IP at 5 minutes, and greater than nose drops at all time points up to 60 minutes (N=9). POD-administered 15 µg/kg fentanyl produced similar plasma levels (**B**) compared to nose drop administration, while IP administration led to significantly lower plasma concentrations (N=3). The analgesic effect of POD-administered fentanyl (**D**), led to a very strong and significantly higher analgesic effect only at the earliest time point (5 min) (N=9). (* = $P < 0.05$ compared to nose drops; † = $P < 0.05$ compared to IP; ‡ = $P < 0.05$ compared to POD)

Figure 3. Plasma concentration vs. analgesic effect plots after 2.5 mg/kg morphine and 15 μ g/kg fentanyl

The morphine hysteresis plots (**A–C**) show a clear distinction between routes of delivery. After nose drop and intraperitoneal (IP) administration, there is indication of counterclockwise hysteresis; while after olfactory epithelium pressurized olfactory delivery (POD) administration (**B**), there is indication of a clockwise hysteresis within the first 60 minutes. The fentanyl panels (**D–F**) also show a distinction between delivery methods. While nose drops and IP (**D, F**) show no clear hysteresis, after POD administration (**E**) there is indication of a clockwise hysteresis at the initial time points (plasma data, N=3; analgesic data, N=9).

Figure 4. Brain concentrations of morphine 5 minutes after a 2.5 mg/kg dose (N=6)

Olfactory bulb morphine concentrations were nonsignificantly higher after pressurized olfactory delivery (POD) administration. In the remainder of the brain and upper cervical spinal cord, morphine concentrations were higher after delivery to the nasal olfactory region with olfactory epithelium administration (POD) than either nose drop delivery to the nasal respiratory region via nose drops or systemic administration via intraperitoneal (IP) injection. (* = $P < 0.05$ compared to nose drops; † = $P < 0.05$ compared to IP)

Figure 5. Plasma normalized morphine brain concentrations 5 minutes after a 2.5 mg/kg dose (N=6)

The plasma normalized concentrations of morphine in the brain were nonsignificantly higher in the olfactory bulbs, but significantly higher in the rest of the brain and spinal cord after nasal olfactory region administration (pressurized olfactory delivery (POD)) than when delivered to the nasal respiratory region via nose drops the nasal cavity or systemically via intraperitoneal injection (IP). (* = $P < 0.05$ compared to nose drops; † = $P < 0.01$ compared to IP)

Figure 6. Pressurized olfactory delivery (POD) administration leads to increased fentanyl brain concentrations 5 minutes after a 15 µg/kg dose (N=6)

Fentanyl concentrations in the forebrain (including olfactory bulbs) and the midbrain, cerebellum and upper cervical spinal cord (MCS) were significantly higher when delivered to the nasal olfactory region of the nasal cavity via (POD) spray than when delivered to the nasal respiratory region via nose drops or systemically via intraperitoneal (IP) injection. (* = $P < 0.05$ compared to nose drops; † = $P < 0.05$ compared to IP)

Figure 7. Plasma normalized fentanyl brain concentrations 5 minutes after a 15 μ g/kg fentanyl dose (N=6)

The blood normalized concentrations of fentanyl in the brain were not significantly different after any of the three routes of administration.

Table 1

Effects of olfactory-administered morphine on dose-dependent pharmacokinetics and therapeutic effects in rats in comparison to other modes of administration^a.

Dose (mg/kg)	C _{max} (ng/ml)	T _{max} (plasma) (minutes)	E _{max} (%MPE)	T _{max} (effect) (minutes)
1	Drop	9.8	10.9	10
1	POD	105.2	11.8	17.1
1	IP	88.9	9.4	9.8
2.5	Drop	205.4	18.2	18.6
2.5	POD	219.7	17.8	30.6
2.5	IP	271.9	11.8	23.3
5	Drop	297.4	31.8	24.1
5	POD	314.5	30.9	41.8
5	IP	597.6	20	42.8

^aMorphine was delivered to rats with the pressurized olfactory delivery (POD) device targeting the olfactory region of the nasal cavity, nose drops (Drop) targeting the respiratory region of the nasal cavity, or systemically via intraperitoneal injection (IP). Time course plasma drug concentration and analgesic effects were monitored over 120 min. The pharmacokinetic values were estimated based on one compartment analysis of time-course plasma drug concentrations with WinNonlin. The effect data were analyzed based on E_{max} model to estimate maximum effect as %MPE and time-to-maximum effects or T_{max}(effect).

Table 2

Effects of enhanced olfactory deposition on morphine plasma exposure and analgesic effects in comparison with nose drop or systemic administration^a.

Dose (mg/kg)	AUC _{effect} ^b (%MPE* min)	AUC _{plasma} ^c (ng*min/ml)	AUC _{effect} AUC _{plasma}	DTP% ^d
1	Drop	731	0.097	-25.2
1	POD	1220* [†]	0.161* [†]	38.5
1	IP	687	0.098	NA
2.5	Drop	1466	0.108	10.9
2.5	POD	1899	0.143 [†]	38.1
2.5	IP	1287	0.087	NA
5	Drop	1516 [†]	0.069	14.8
5	POD	3565*	0.132* [†]	55
5	IP	2789*	0.059	NA

^aRats were administered morphine with either the pressurized olfactory device (POD), nose drop (Drop), or systemically [via intraperitoneal injection (IP)] and time course plasma drug concentration and analgesic effects were monitored over 120 min.

^bThe total analgesic effects over 120 min were expressed as AUC_{effect} in percent maximal possible effect (% MPE)*min.

^cThe total plasma exposure is expressed as AUC_{plasma} (in ng*min/ml).

^dThe direct transport percentage (DTP%) to the brain was calculated based on the amount of drug in the brain that could not be accounted for by systemic distribution as described in Materials and Methods.

* P < 0.05 compared to nose drops;

[†] = P < 0.05 compared to IP.

Effects of fentanyl administered to the olfactory region on dose-dependent pharmacokinetics and therapeutic effects in rats in comparison to other modes of administration^a.

Table 3

Dose (µg/kg)	C _{max} (ng/ml)	T _{max(plasma)} (minutes)	E _{max} (%MPE)	T _{max(effect)} (minutes)
7.5	Drop	3.16	26.4	10
7.5	POD	5.92	25.7	5
7.5	IP	0.93	25.8	10
15	Drop	5.91	38.2	5
15	POD	8.15	73.8	5
15	IP	1.06	24.7	10
25	Drop	6.74	59.2	10
25	POD	9.6	93	5
25	IP	6.56	26.7	10

^aFentanyl was delivered to rats with the pressurized olfactory delivery (POD) device targeting the olfactory region of the nasal cavity, nose drops (Drop) targeting the respiratory region of the nasal cavity, or systemically via intraperitoneal injection (IP). Time course plasma drug concentration and analgesic effects were monitored over 120 min. The pharmacokinetic values were estimated based on one compartment analysis of time-course plasma drug concentrations with WinNonlin. The effect data were analyzed based on E_{max} model to estimate maximum effect as %MPE and time-to-maximum effects or T_{max(effect)}.

Table 4

Effects of enhanced olfactory administration on fentanyl plasma exposure and analgesic effects in comparison with nose drop or systemic administration^a.

Dose (µg/kg)		AUC _{effect} ^b (%MPE* min)	AUC _{plasma} ^c (ng/ml* min)	$\frac{\text{AUC}_{\text{effect}}}{\text{AUC}_{\text{plasma}}}$
7.5	drop	691.2	135	4.89
7.5	POD	258.9*	202 [†]	1.56
7.5	IP	689.8	50	11.38
15	drop	800.5	293 [†]	3.93
15	POD	221.5* [†]	285 [†]	1.37
15	IP	657.7	46*	20.85
25	drop	2080.2 [†]	508	4.76
25	POD	2392.3 [†]	367	6.80
25	IP	868.6*	148	10.41

^aRats were administered fentanyl with either the pressurized olfactory device (POD), nose drop (Drop), or systemically [via intraperitoneal injection (IP)] and time course plasma drug concentration and analgesic effects were monitored over 120 min.

^bThe total analgesic effects over 120 min was expressed as AUC_{effect} in % MPE*min.

^cThe total plasma exposure is expressed as AUC_{plasma} (in ng*min/ml).

* P < 0.05 compared to nose drops;

[†] = P < 0.05 compared to IP.