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Comparison of Automated and Manual Oral Dosing on the Absorption, Conversion, and Locomotor-Activating Effects of Nicotine

The accurate assessment of a new drug candidate's pharmacokinetic and pharmacodynamic profile are essential components of the drug development process. Given the importance of these characteristics, knowledge of potential influencing factors such as stress becomes crucial. This study examined the effect of low-stress, automated intragastric dosing compared to higher-stress, manual gavage dosing on the absorption phase of nicotine's pharmacokinetic curve, conversion of nicotine to cotinine, and nicotine-induced locomotor stimulation in Sprague Dawley rats. Nicotine (1 mg/kg) was either automatically infused directly into the stomach or manually dosed using the typical gavage needle and syringe. Immediately following nicotine administration, a rapid blood sampling method (every 5 min) was conducted and motor activity was recorded for 60 min. Each rat received both an automated infusion and a manually-injected dose of nicotine in a cross-over design. The results indicated that nicotine absorption was more rapid when automatically dosed, but this effect did not significantly alter the conversion of nicotine to cotinine. Also, no significant differences in motor activity were found between manual and automated dosing. The results suggest that the method of dosing can influence the pharmacokinetics of a drug and should be considered a factor in study designs.

Accurate evaluation of a new compound's pharmacokinetic (PK) and pharmacodynamic (PD) profile is an essential component of the drug development process (1, 2). Preclinical PK evaluation in animals is necessary to characterize the relationship between drug dose, route of administration, and blood concentrations and establish a dose-time course for the new drug candidate in a complex biological system. In addition, the PD profile of the new compound can be used to determine the relationship between tissue or fluid concentration and the therapeutic efficacy and side effects of the drug. Therefore, an understanding of factors that influence in vivo pharmacokinetics becomes an essential component of the drug development process.

Factors shown to affect the PK profile and behavioral manifestations of a drug inlcude the route of drug administration and dose (3, 4). One factor that is typically overlooked during pharmacokinetic testing and can substantially influence the PK/PD profile of a drug is stress (5). Stress can alter drug bioavailability (6-14), and has also been shown to influence an animal's motor response to an administered drug. For example, exposure to stress may alter nicotine-induced locomotor activity in rats (15-18).

Although the ability of stress to alter drug bioavailability and drug-induced changes in motor activity has been documented, little has been done to understand the interaction between typical laboratory procedures and drug actions in the body. Some standard laboratory stressors include handling, restraint, drug dosing, blood sampling, housing conditions, housing transfer, cage changing, and experimenter presence. These have been shown to cause significant alterations in indicators of stress such as heart rate/blood pressure, glucocorticoids, adrenocorticoids, and body temperature in rats (19-35). Thus, stress unknowingly produced by common laboratory procedures may lead to changes in drug pharmacokinetics and motor activity.

The method of drug administration is a critical factor when evaluating the pharmacokinetics of a drug due to route-dependent changes in the pharmacokinetic profile. However, little research has examined differences between automated and manual dosing on PK and locomotor activity. Oral dosing via gavage is a common route of administration for compounds as a model for drug dosing in humans. But unlike adult humans, the animal may become stressed or injured (31). A typical gavage dosing procedure includes immobilizing the animal while inserting a long, stainless-steel needle down the esophagus to inject the drug. Leakage from the stomach or dosing needle may result in aspiration of the test compound causing stress (31, 35, 36).

Current developments in technology now allow the researcher to dose automatically through use of an automated pre-programmed infusion pump. Our laboratory has found that automated intragastric dosing is less stressful than manual gavage dosing (40). We have observed that dosing via gavage produces a larger increase in stress-related indicators such as circulating norepinephrine and corticosterone concentrations when compared to automated dosing (40). This suggests that use of automated dosing leads to a decreased potential for stress-related effects in drug studies. Hence, oral dosing via manual gavage or automated dosing appears to provide adequate "stressful vs. non-stressful" conditions for evaluation of stress-drug interactions.

Nicotine was chosen as the test compound for this study because it allowed us to examine the interaction between stress and pharmacokinetics and motor activity. The goal of the study was to examine potential changes in nicotine absorption and conversion, and nicotine-induced activation of locomotor activity occurring after exposure to a common laboratory stressor, intragastric drug dosing. These attributes were examined by comparing the effects of low-stress, automated intragastric dosing and higher-stress, manual gavage dosing in rats.

METHOD

Subjects and Drugs

Eight male Sprague Dawley rats (Harlan, Indianapolis, IN) weighing between 300 and 350g were individually housed in shoe-box cages with an independent air supply. Food and water were available *ad libitum* until surgery. Nicotine hydrogen tartrate (N5260, Sigma-Aldrich) was dissolved in distilled water, and drug doses were calculated as nicotine-free base.

All procedures were conducted in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals (Council, 1996).

Experimental Setup

Our experimental setup consisted of the Culex® Automated Pharmacology System (APS). The Culex APS is comprised of several components: a Culex (automated blood sampling), a Honeycomb® fraction collector (sample refrigeration), an Empis® (automated infusion pump), and a Raturn® (behavioral monitoring) with a bowl cage. Operation of the Culex has been described in detail elsewhere (41, 42).

Surgery

The rats were anesthetized with isofluorane and cannulated with an intragastric catheter (CX-8001S; Bioanalytical Systems, Inc., West Lafayette, IN). After a three-day recovery period, the femoral vein was catheterized (CX-2020S; Bioanalytical Systems Inc., West Lafayette, IN). Immediately following surgery, the animals were placed in a bowl cage, connected to the Culex (femoral catheter) and Empis (gastric catheter), and allowed 24 hrs of post-surgical recovery. The APS maintained venous catheter patency throughout the experiment by infusing 20µL of sodium heparinized saline (10 units/mL) every 12 min.

Experimental Procedure

Following surgery, the rats were randomly assigned to two groups: 1) IG-PO: an initial automated intragastric dose followed by a manual dose 24 hrs later, or 2) PO-IG: an initial manual dose followed 24 hrs later by an automated dose. After 24 hrs of post-surgical recovery and habituation to the chamber, baseline motor activity was recorded for a 60 min period Twenty-three hours after conclusion of the baseline activity recording, the rats were dosed IG or PO with 1 mg/kg of nicotine. Immediately following drug dosing, blood samples (250µL/sample) were taken every 5 min for 60 min. Sixty minutes of activity was also recorded immediately following the nicotine infusion. Twenty-four hrs following nicotine administration, the procedures were repeated with the IG group receiving the drug PO, and vice versa in a crossover design. All behavioral testing was conducted between 0900 and 1700 hrs. The two groups were contained in separate testing rooms to reduce experimenter/animal interactions. Hence, the experimenter was not in the room when automated dosing occurred.

Sample Analysis

100µL aliquots of plasma were transferred into a 96-well plate and frozen at -80° C until analysis. Nicotine and cotinine were assayed by liquid chromatography and mass spectrometry (LCMS) using a BASi method.

Data Analysis

Pharmacokinetics and Drug Time Course

The C_{max}, T_{max}, and AUC for drug concentration-time curves for the two experimental conditions were calculated for both nicotine and cotinine using the PK Solutions© (PK Solutions, Eugene, Oregon) software program. Paired sample t-tests were used to determine whether differences existed in these variables between the IG and PO treatments. In addition, the slope (absorption) for the drug concentration-time curves was calculated using Microsoft Excel© and differences between the two treatments were analyzed using a t-test. Time-dependent differences in the drug concentration-time curves between the two treatment groups were analyzed using a 2X13 repeated factor ANOVA with both treatment and time as repeated factors. Newman-Kuels post hoc analyses were used to further delineate any significant differences found through the ANOVA. All analyses were performed using the SigmaStat© statistical software (SigmaStat Software, Inc., Point Richmond, CA).

Behavioral Data

Sixty minutes of behavioral data after nicotine administration were compared to control behavioral activity from the same time period of the preceding day. The behavioral parameters obtained from the Culex APS were clockwise and counterclockwise turn count and duration, and rearing (vertical activity) count and duration. 2x2 repeated measures ANOVA with dosing condition (manual vs. automated) and time (pre- vs. post-drug administration) was used to determine if dosing condition influenced nicotine-induced changes in motor activity. Newman-Kuels post hoc analyses were performed as needed.

RESULTS

Pharmacokinetics

T1 lists the mean and standard error values for both the manual and automated dosing conditions. The average slope values for the IG and PO condition for nicotine were significantly different [$t(7) = 2.70$, $p<0.05$]. The automated dosing condition showed a steeper slope compared to the manual dosing condition. The AUC for the automated dosing condition was significantly greater than the manual dosing condition [$t(7) = 3.21$, $p<0.001$], an expected correlate to an increased absorption rate within the 60 min time course. In addition to AUC, the C_{max} for the automated dosing condition was also significantly greater compared to the manual dosing condition [$t(7) = 2.39$, $p <0.05$]. However, T_{max} was not significantly different between the experimental conditions ($p > 0.05$). This effect appears to be due to most PK curves continuing to rise even at the 60 min time cutoff. Thus, most animals had a T_{max} of 60 min, suggesting a ceiling effect for this parameter.

Nicotine plasma concentrations were greater in the automated dosing condition compared to the manual dosing condition [F (1,81) = 6.14, p<0.05). In general, nicotine concentrations increased over time [F (12,81) = 163.27, p<0.001]. More importantly, between-treatment differences in plasma-nicotine concentrations depended on the time sampled [F (12,81) = 2.42, p<0.01]. As seen in **F1A** and confirmed by post hoc analysis, initial nicotine plasma concentrations did not differ from 0-20 minutes between the two treatments. However, from 25-55 minutes, a higher nicotine plasma concentration was seen when subjects were automatically dosed compared to when manually dosed.

As seen in **F1B**, in contrast to nicotine absorption, none of the dependent variables related to cotinine (slope, C_{max}, T_{max}, AUC) demonstrate a statistically significant difference between the two dosing conditions (p>0.05). Confirming this finding, no significant differences in cotinine plasma concentrations existed at any of the sampled time points (p>0.05).

Locomotor Activity

As seen in **F2A-F**, motor activity increased after nicotine dosing for all behavioral parameters (Left duration: F (1,5) = 27.72, p<0.01; Left count: F (1,5) = 16.98, p<0.01; Right duration: F (1,5) = 10.42, p<0.05; Right count: F (1,5) = 28.50, p<0.01; Rearing duration: F (1,5) = 8.93, p<0.05; Rearing count: F (1,5) = 19.23, p<0.01). However, no significant differences existed for dosing condition or the interaction between dosing condition and time (pre- vs post-drug administration), suggesting that nicotine-induced changes in motor activity were not significantly affected by dosing condition regardless of the time sampled (p>0.05).

DISCUSSION

The results of the current study indicate that a common laboratory procedure, manual gavage dosing, leads to biological changes that alter the absorption of nicotine. Indeed, as seen in **T1**, manual dosing slowed nicotine absorption (slope = 1.67+0.11) compared to automated dosing (slope = 2.34+0.18). In addition, manual dosing produced a lower C_{max} for nicotine compared to automated dosing, likely due to the slower drug absorption. Further evidence of retarded nicotine drug absorption with manual dosing is a significantly lower AUC ($\approx$31%) compared to the automated dosing. As illustrated in G1a, nicotine-plasma concentrations diverged between the two groups with significant differences emerging between the 25-55 min time points, demonstrating that dosing condition influences drug absorption.

T1. Pharmacokinetic parameters of nicotine and cotinine after manual and automated intragastric dosing of 1 mg/kg nicotine.

Parameter	Manual Dosing	Automated Dosing	P value
Nicotine slope	1.67+0.11	2.34+0.18	p<0.05
Nicotine Cmax	95.57 ± 9.75	123.48 ± 6.41	p<0.05
Nicotine Tmax	47.50 ± 4.82	52.50 ± 2.11	n.s.
Nicotine AUC	3254.14 ± 388.77	4714.20 ± 235.09	p<0.001
Cotinine slope	2.00±0.24	2.27±0.17	n.s.
Cotinine Cmax	113.11 ± 16.06	119.60 ± 7.41	n.s.
Cotinine Tmax	56.25 ±1.83	56.86 ± 1.32	n.s.
Cotinine AUC	2794.54 ± 347.87	3365.14 ± 212.19	n.s.

In contrast to nicotine, while plasma cotinine concentrations were higher with the automated dosing regime (G1b), this effect was not significantly different between the two dosing regimes at any time point or for any pharmacokinetic parameter. This implies that stress does not alter the pharmacokinetics of the primary drug's metabolites. Lastly, as seen in G2a-f, nicotine increased locomotor activity in general, but this increase did not differ between the two dosing treatments during the 60 min following drug administration. The similarity in motor activity between the manual and automated dosing treatments suggests that outward behavioral expressions such as motor activity do not directly reflect the differences in plasma drug concentrations in this study. Taken together, these data suggest that manual drug dosing alters absorption of the primary compound, but not conversion to a metabolite, and locomotor activity may not directly reflect plasma concentrations of the drug.

The data from the current experiment are consistent with previous findings suggesting that stress from laboratory procedures leads to changes in drug pharmacokinetics. For example, stress induced by several variables (surgical trauma, loud noise, cold swimming, adjuvant rheumatoid arthritis, etc) has been shown to alter the pharmacokinetics of drugs in rodents and humans (6-14, 43). While stress has been linked to altered drug absorption, the direction of this effect varies greatly between studies. Studies in Wistar rats have shown, after the presentation of a variety of stressors, there is an increase in drug (i.e., antibiotics, propranolol, lidocaine) blood concentrations, regardless of how the drug is administered (6, 10-14). In contrast to the aforementioned studies, Winders et al. (8) found that exposure to stress such as a rubber ligature or a loud noise decreased circulating nicotine, but either increased or had no effect on cotinine concentration in Sprague Dawley rats (8). Similarly, Jamali and Kunz-Dober (9) found that after teeth extraction, serum concentrations of ibuprofen in human patients were significantly reduced as indicated by a reduction in AUC as well as a 2hr prolonged reduction of T_{max}. This finding supports the conclusion that surgical stress has a profound impact on ibuprophen pharmacokinetics. While there are conflicting reports in the literature regarding the effect of stress on drug absorption, the current study is consistent with Winders et al. (8) that stressful procedures reduce circulating nicotine with no effect on cotinine. Thus, it appears that stress adds a level of unpredictability when determining drug absorption and should be a consideration in preclinical drug development work.

The exact mechanism for changes in drug pharmacokinetics due to stress is not well studied. Perhaps the most likely scenarios for altering absorption of an orally-dosed drug due to acute stress are changes in gastrointestinal (GI) function, specifically gastric emptying and alterations in blood flow to the gut. Acute stressors are associated with slowed gastric emptying in both experimental animals and humans, while colonic motility, transit, and defecation are stimulated (44-48). The decrease in gastric emptying and increase in colonic motility appear to be under control of the autonomic nervous system. (For review see Mayer, 2000 [49]). Reductions in gastric motility limit transfer of gastric contents from the stomach to the intestines. In relation to drug absorption, gastric emptying may be the rate-limiting step for orally-dosed nicotine absorption, as the nicotine absorption occurs primarily in the intestines (4). Hence, a stress-induced

decrease in gastric emptying has the potential to slow intragastrically-dosed drugs. In addition, the increase in colonic motility may decrease drug absorption by reducing the amount of time the drug spends in the intestines, and by limiting its opportunity to cross the intestinal membrane.

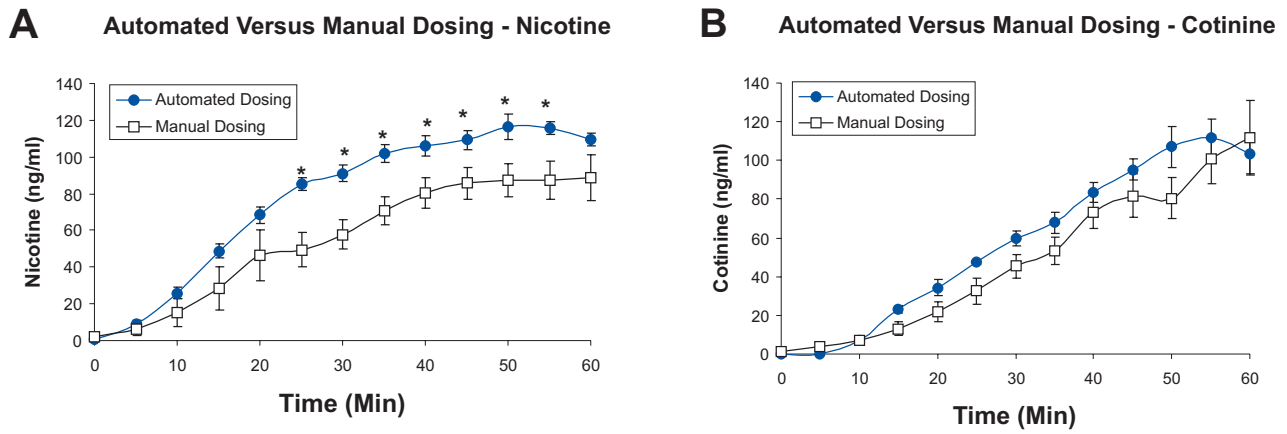
In addition to altering gastric and colonic motility, thoracic sympathetic nervous system activation induces the adrenal medulla to release epinephrine, a vasoconstrictor that decreases blood flow to the gut, making less blood available to carry intragastrically-dosed drugs. This decrease in blood flow within the gut has the potential to decrease circulating drug concentrations, as less blood flow also means less blood to carry drug from the gut to the general circulation.

While stress appears to be the prime explanation of our findings, another possibility is that automated dosing is physically better at delivering the drug to the stomach, allowing a greater amount of drug to be absorbed. However, this possibility appears unlikely, as the difference between manual and automated plasma concentrations did not appear until later time points.

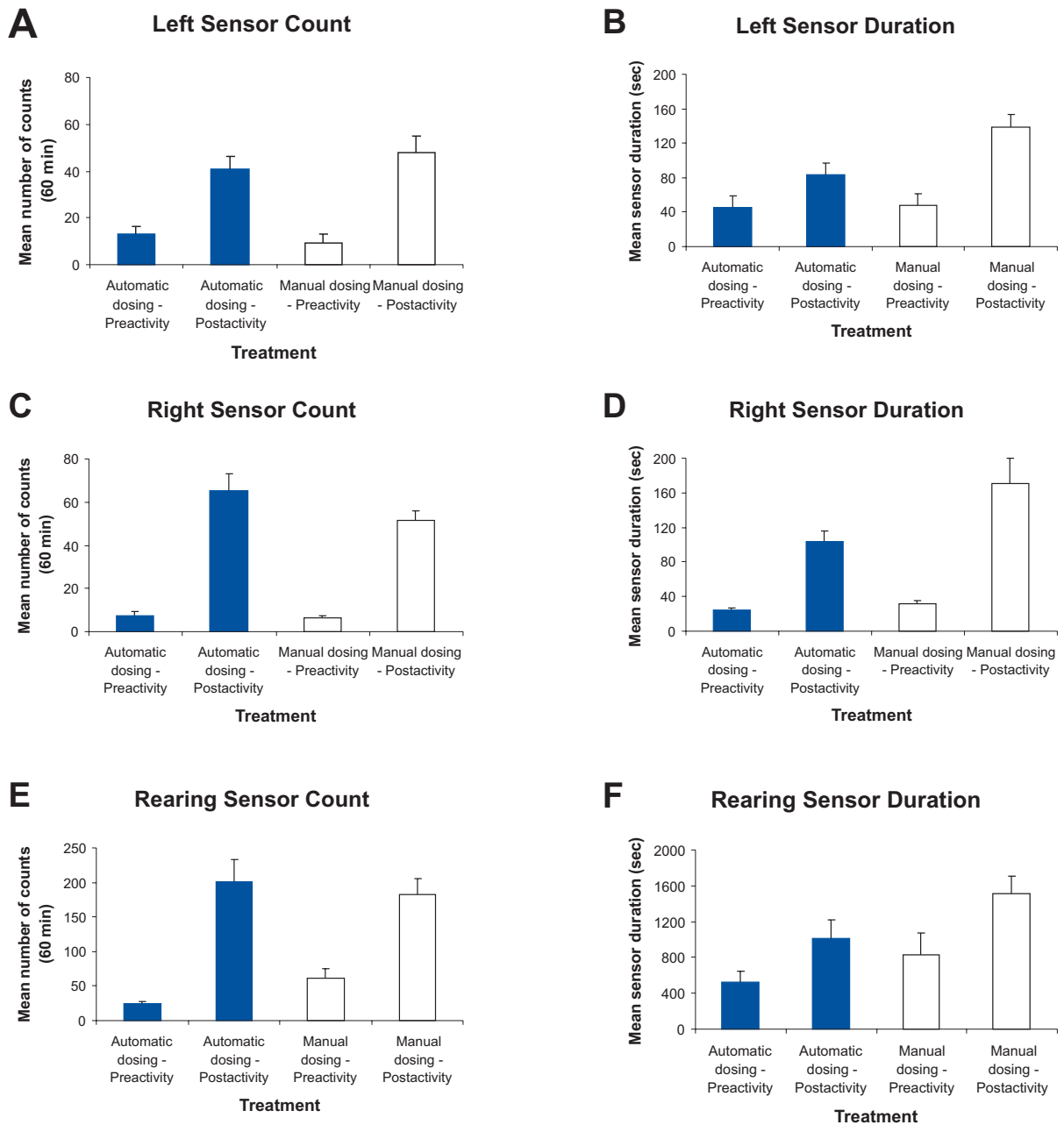
Our findings are also consistent with work demonstrating that acute systemic dosing of nicotine increases locomotor activity in rats (50, 51). Nicotine increased motor activity within 60 min following intragastric dosing of 1 mg/kg nicotine in both the manual and automated dosing conditions. Interestingly, the significantly lower nicotine concentrations in the manually-dosed condition did not lead to a detectable difference in motor activity. Thus, our data suggest that motor activity is not directly reflective of circulating nicotine concentrations. However, the behavioral monitoring system used may not have been sufficiently sensitive to detect existing behavioral differences. In addition, the study may not have had enough power to detect these differences due to a small sample size. Lastly, a possibility is that motor activity is maximized by nicotine concentrations below those found at the 25 min time point. In effect, this possibility would lead to a ceiling effect in nicotine-induced motor activity at plasma concentrations below an approximate 80 ng/mL. Hence, any "extra" circulating nicotine would have no effect on motor activity.

In summary, we found that when animals were automatically dosed, drug absorption of nicotine (1 mg/kg) was enhanced as indicated by a leftward shift in the selected portion of the absorption phase of the PK curve. These data also imply that stress associated with standard laboratory procedures such as manual drug dosing alters drug pharmacokinetics and that stress is an important variable to consider when designing and evaluating pharmacokinetic studies. Motor activity did not appear to be associated with the changes in drug absorption, indicated by a lack of significant differences for all motor-related parameters between the manually and automatically dosed nicotine treatment conditions. Overall, the study implies that stress is an important intervening variable in pharmacokinetic studies and should be considered during the design of such studies.

F1. Effect of automated versus manual dosing on nicotine absorption (A) and conversion (B) to cotinine.



F2. Effect of automated and manual dosed nicotine one locomotor activity indicators.



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