Pharmacokinetic and Pharmacodynamic Studies in Rats Using a New Method of Automated Blood Sampling

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A well-characterized prescription medication was used to demonstrate the utility of an automated blood sampling device for basic research in pharmacokinetics and pharmacodynamics. Both the parent drug and one of its major metabolites were monitored in plasma samples and urine collected simultaneously from the same animal. Animal activity measurements during the sampling period were used to evaluate behavioral changes after administration of the drug, and correlated both with observations by the investigators and drug concentrations in plasma.

High-throughput screening and combinatorial chemistry have greatly expanded the collection of compounds from which to pick future drug candidates. New in vitro techniques have improved our ability to narrow the pool of potential candidates. For example, microsomal metabolism data can help select a suitable animal model by studying the interspecies differences in liver enzymes. Although these in vitro techniques have reduced the number of animal studies, in vivo studies still contribute greatly to understanding the pharmacology, toxicology and efficacy of drugs in development. Obtaining ADME (absorption, distribution, metabolism and elimination) parameters early in the drug discovery process is important to avoid the failure of these drug candidates in clinical trials. Combining in vitro data with in vivo data provides the most complete picture of the behavior of the new drug candidate.

The pharmacological effect of a drug directly correlates with the concentration of the drug at the target site, which is related to the drug concentration in blood/plasma. Therefore, the knowledge of effective blood/plasma concentration of the drug in animals can serve as a useful guide in human clinical trials. Rodents are typically used for obtaining preliminary pharmacokinetic data.

Automation of in vivo techniques has not kept pace with in vitro techniques. Serial blood sampling from small animals such as rats for a long period of time (24 hours) can be a logistical challenge.

Here we describe the use of the Culex ABS, an automated system for blood sampling, to obtain all the ADME information from a *single* animal. This system is capable of collecting all biological materials including blood, urine and feces without manual labor. The system also monitors animal activity during the experiment, providing an look at possible CNS activity earlier in the drug screening process. The pharmacokinetics of a known, antipsychotic drug were studied to demonstrate the utility of this device.

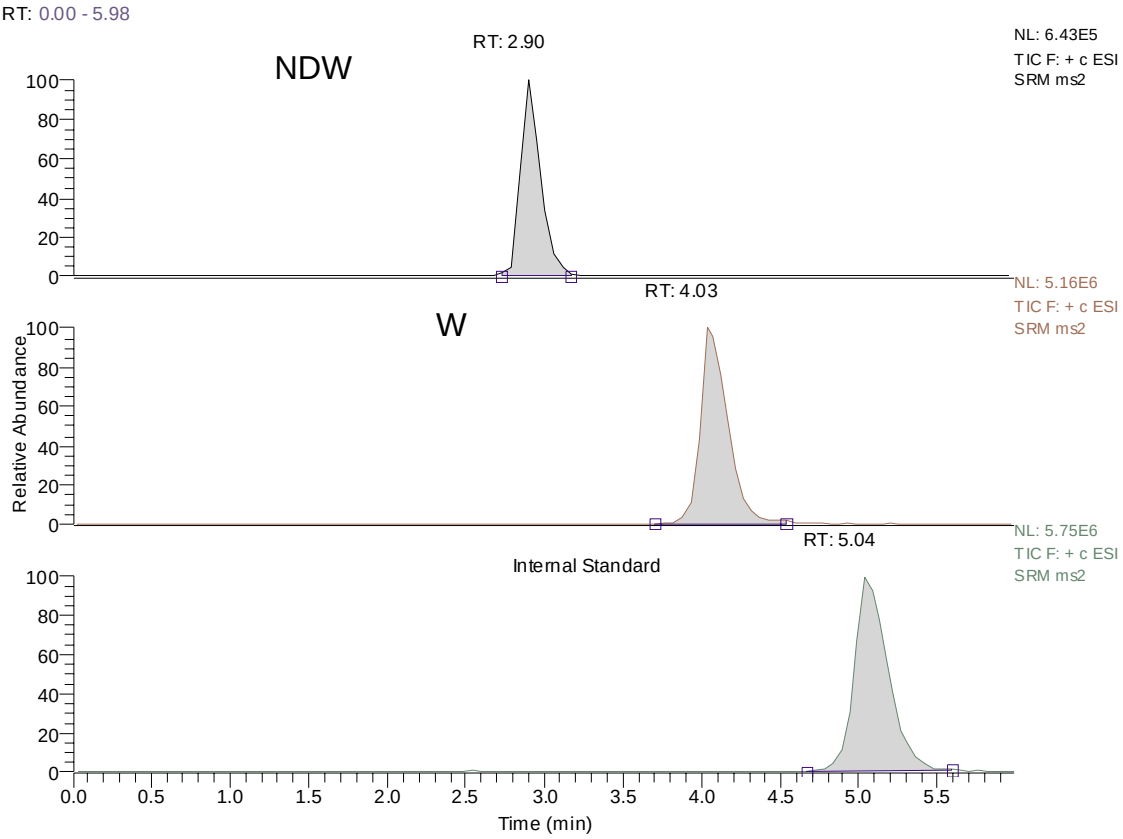
Both the features and operation of the Culex ABS are described fully in other articles in this issue (1, 2).The automated system managing four awake and freely-moving rats is shown in ***F1***. Rats are cannulated in

F1

The Culex ABS system (foreground) was used to automatically collect whole blood, urine and feces from a single rat over the course of several days. Extracts of the plasma and urine were subsequently analyzed by LC/MS/MS. Raw animal activity data were exported to a Microsoft Excel file and charted to examine behavioral anomalies.

F2

MS chromatogram of a blood sample obtained 72.5 minutes after administration of an oral dose of 4 mg/kg of W. NDW is the primary metabolite.



the jugular vein for blood sampling and the femoral vein for drug administration. Each animal is then lightly tethered to a movement-responsive arm within the animal containment system. Connections are then made between the jugular catheter and the Culex sterile tubing set, or the femoral catheter and the syringe pump delivering drug. This arrangement accommodates the fluid lines required for blood collection and drug infusion without entanglement (2). The timing of blood sample collection, and the volume sampled are independently programmed from the software.

The blood samples in this study were diluted with an equal volume of heparinized saline (10 units/mL). A volume of heparinized saline equal to the blood volume removed was then returned to the animal to maintain fluid balance and clear the intravenous catheter. Samples were collected at a variable frequency.

The purpose of this study was to demonstrate the utility of an automated blood sampling system rather than to add to the DMPK literature about any specific pharmaceutical product. A well-characterized, antipsychotic drug was selected that was known to bind to $\propto_1$, dopamine, histamine H_1, muscarinic, and serotonin type 2 (5-HT_2) receptors. When administered orally, the drug is well absorbed. Protein binding is about 93%, primarily to albumin and $\propto_1$-acid glycoprotein. There is extensive first-pass metabolism with about 40% of the dose being metabolized before reaching the systemic circulation. The drug (referred to as W in this paper) is primarily metabolized by glucuronidation and cytochrome P450 (CYP) oxidation. The major circulating metabolites are a 10-N-glucuronide and 4'-N-desmethyl compound. Drug W and its N-desmethyl metabolite (NDW in this paper) were assayed by LC/MS/MS using an internal standard provided by the manufacturer. Blood and urine samples were collected from the rat at pre-determined time intervals for the PK study.

Experimental

Analytical Technique

W, its metabolite NDW and the internal standard were all obtained from the manufacturer. A Finnigan LCQDeca ion trap mass spectrometer with electrospray ionization was used for the assays.

Pharmacokinetic Studies

The jugular vein of a Sprague-Dawley male rat was cannulated and a catheter was implanted for blood collection. During the 24-hour post-surgery recovery period in the Culex ABS, the catheter patency was maintained automatically using the Tend protocol which frequently flushed the catheter with sterile saline. The animal was then dosed by oral gavage or IV infusion with 0.5 mL of an 8 mg/mL solution of W which was dissolved in 0.1 M HCl and then adjusted to pH 6 with 0.1N NaOH. The first blood sample was collected at 2.5 minutes and after that sampling was continued for 2 hours at 10 minute intervals, 2 hours at 30

minute intervals, and then at 1 hour intervals for up to 8 hours. A 24-hour sample was taken the next day. The samples were diluted two-fold with an equal volume of saline during the collection. Total sample volume was 150 µL (75 µL whole blood + 75 µL saline).

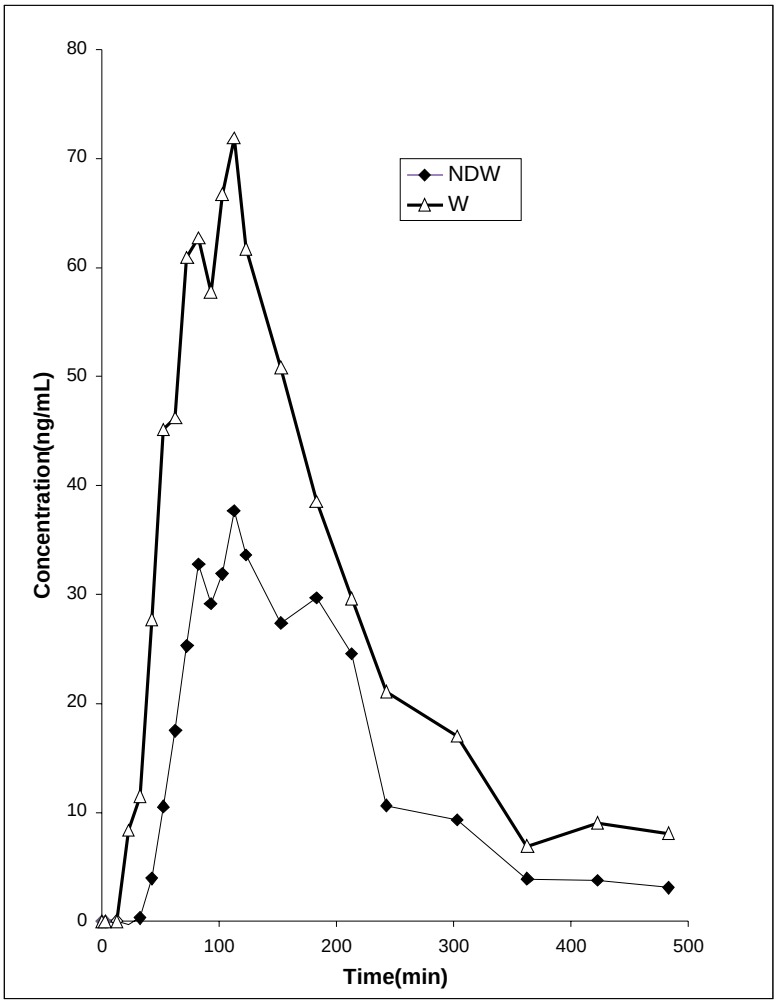
Sample Extraction

Blood samples were centrifuged and plasma was separated. After adding 5 µL of a 1 µg/mL solution of the internal standard to a 100 µL plasma sample (50 ng/mL), 400 µL of 2%NH_4OH in ethyl acetate was added to precipitate protein. The sample was then vortexed for 2 minutes and centrifuged for 10 minutes. The supernatant (400 µL) was dried under N_2 and reconstituted in 100 µL of 0.1M ammonium acetate/ACN (50/50 v/v). Samples were assayed by LC/MS.

Solid phase extraction was used for the urine samples. Standards were prepared by spiking blank urine. Internal standard was added to a 500 µL urine sample (50 ng/mL internal standard). Bond-Elut C_{18} cartridges (100 mg/1 mL) from Varian were used for the extraction.

F3

Plasma concentration vs. time profiles for both W and NDW after an oral dose of 8 mg/kg (n = 1).

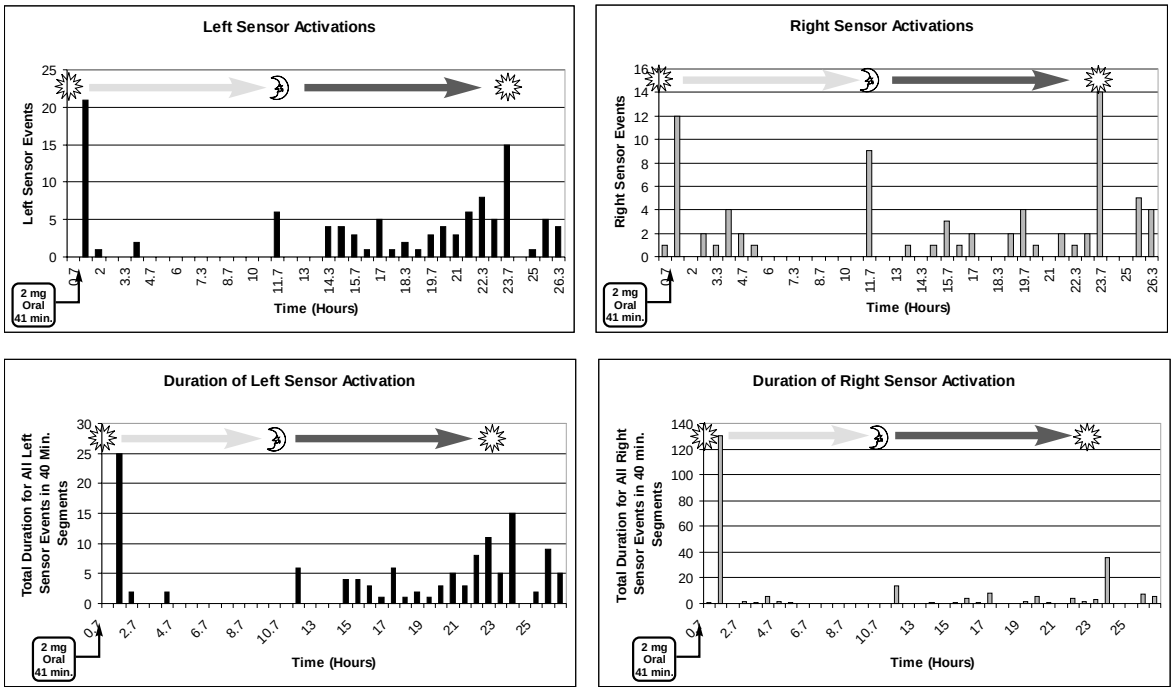


F4

Animal behavior was monitored after oral administration of 2 mg of W, for a period of more than 27 hours. Each bar represents sensor data within a 40 minute segment, starting with the initiation of behavioral monitoring 41 minutes prior to dosing.

All four data sets show an increase in activity shortly after drug administration, followed by a quiescent period which matched observations by the researcher. Another layer of information is obtained by examining the differences between sensor activations and total sensor durations (see text discussion).

The sun/moon symbols and the light/dark arrows indicate the time of day when these activity measurements were made.



Cartridges were preconditioned by washing with 2 mL methanol, followed by 2 mL of 50 mM phosphate buffer at pH 6.0. The samples were applied and washed with 2.0 mL of cartridge washing buffer (30% methanol in Fisher pH 10 buffer). After drying the cartridges, the samples were eluted with 500 µL of 2% NH_4OH in ethyl acetate solution. Eluates were dried under N_2 and reconstituted in 100 µL of 0.1M ammonium acetate - ACN (50/50 v/v).

Chromatography conditions

The mobile phase consisted of 40% 0.1M ammonium acetate/50% ACN and 10% MeOH. A YMC basic column (150 X 4.6 mm, 5 µm) was used for the isocratic separation at 0.9 mL/min at ambient temperature. Samples (10 µL) were injected manually.

Animal Activity Monitoring

Animal behavior was monitored after oral administration of 2 mg of W. Software on the Culex ABS recorded the number of times each sensor was activated, and how long each sensor remained engaged, for a period of more than 27 hours. Sensor activation data was then exported to a Microsoft Excel file and grouped into 40 minute segments, starting with the initiation of behaviorial monitoring 41 minutes prior to dosing. The 40 minute data segments were plotted as bars on the graphs representing either sensor activations or the sensor durations, (***F4***). Left sensor activation represented clockwise rotation of the animal within the Culex cage, while right sensor activation represented counterclockwise rotation. Normal behavior for rats in our colony is not illustrated in this article, but they typically generate approximately 0-5 or more sensor events per hour during the day, and up to 10 times that rate at night.

Results and Discussion

After oral administration of W to the rat, there was a noticeable difference in behavior, compared to normal activity seen among rats in our laboratory. The animal was observed to be very active shortly after receiving the dose and then became immobile, showing neither activity nor interest in food or water for several hours. This same pattern was recorded by the animal activity software.

Somnolence is reported to be a common side effect of W. The animal activity was monitored 41 minutes prior to dosing and then throughout the course of blood sampling by the Culex ABS. After 12 hours, when the drug concentration in the blood was much lower, the animal began to resume activity. Urine collection during the blood sampling experiment showed that the animal didn't produce sufficient urine for a collection during the first 24 hours, paralleling the lack of activity and cessation of food and water consumption.

Another layer of information was obtained by examining the differences between sensor activations and total sensor durations. Looking at sensor activations alone, the animal activated the clockwise (left) sensor more than the counterclockwise (right) sensor during the initial burst of activity. Looking at the duration of the sensors however, we find that the animal spent considerably more time traveling in a counterclockwise direction during this same time. In other words, it didn't trigger the right sensor as often but it spent far more time traveling in the counterclockwise direction. Later, as the concentration of drug diminished, the animal spent more total time turning in a clockwise direction.

F3 shows the plasma concentration-time curves for W and NDW after an oral dose of 8 mg/kg. The non-compartmental pharmacokinetic parameters are listed (***T1***).

Plasma concentration-time profiles were obtained after administering an IV dose of W (***F5***). The rat was given another IV dose 24 hours after the first dose to study the patency of the jugular catheter. Both IV profiles for W were similar. There is an observable increase in the amount of NDW after the second

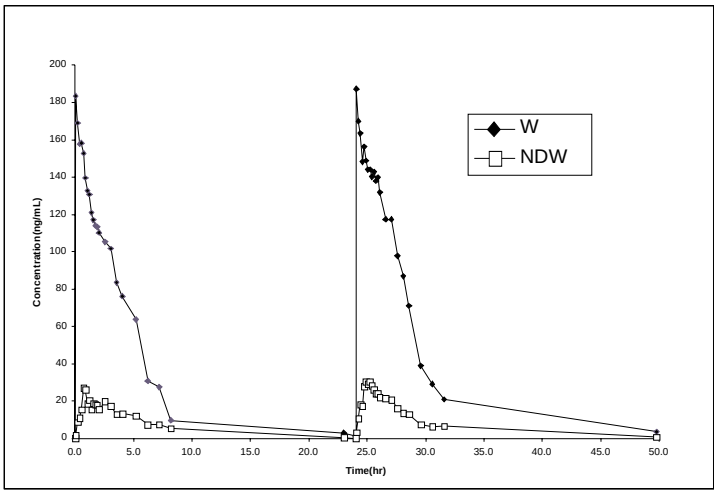
T1

Pharmacokinetic parameters for drug W and metabolite N-desmethyl W (NDW).

Parameter	W	NDW
C_{max} (ng mL^{-1})	75.92	47.68
t_{max} (hr)	1.71	1.71
$t_{½}$ (hr)	9.44	3.09
AUC (0-8hr)(ng h mL^{-1})	300.22	88.18

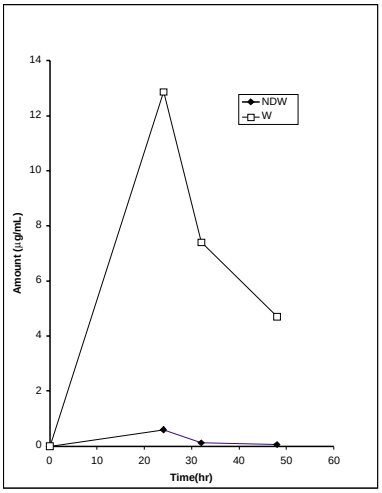
F5

Plasma concentration vs. time profiles for both W and NDW after two consecutive IV doses of 8 mg/kg of W.



F6

Excretion profiles of W and NDW in rat urine.



dose possibly due to the induction of metabolizing enzymes.

Conclusion

In these preliminary studies, we have demonstrated successfully the utility of this new automated blood sampling device, and the concept of simultaneously collecting urine and animal activity data. The blood sampling frequency and volume could be programmed and samples drawn as long as the catheter remained patent in the animal. Catheters remained in place throughout the experiment and blood was withdrawn as programmed. A smaller than expected volume of urine was collected because of a reduction in urinary output which seemed to correlate with the animal's behavior. Animal behavior was apparently affected by the dose of drug given, and this also affected the consumption of food and water.

We demonstrated the use of the same animal for two consecutive IV doses within 48 hours. Urine samples were also collected for 48 hours. This device kept both blood and urine samples cold at less than 4°C until use.

We found that this automated system also allowed a rat to be implanted with one or more microdialysis probes so that dialysates could be collected simultaneously. However, the use of brain microdialysis probes was incompatible with the use of the metabolic cage. Once the animal resumed an interest in food, the skull-mounted probe came into contact with the edge of the food bin. Another style of cage was used for brain microdialysis studies which prevented contact of the probe with the cage wall. However, this second cage did not permit the collection of urine and feces.

In the future, we plan to use the Culex system to study the pharmacodynamics and the blood-brain-barrier distribution of drugs such as W. The neurotransmitters, serotonin and dopamine which are released upon the action of W-class drugs can then be followed by simultaneous brain microdialysis sampling and blood sampling.

We have blinded the example used in this publication because the data presented here are only intended to be a representation and are not validated or archival due to the small number of animals studied. Our company regularly works with proprietary drug candidates under contract to pharmaceutical firms.

References

1. *S. Peters, J. Hampsch, M. Cregor, G. Gunaratna and C. Kissinger, Current Separations 18:4 (2000)*
2. *C. Bohs, M. Cregor, G. Gunaratna and C. Kissinger, Current Separations 18:4 (2000)*