The Influence of Blood Sampling Site in Nasal Drug Delivery Studies in Rats[1]

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INTRODUCTION

In vivo animal models provide tools for drug delivery research. A major part of using an *in vivo* animal model involves specimen collection for evaluation of drug delivery parameters. Blood is the most common specimen used for analysis and many techniques for obtaining blood specimens from rats have been described (1–10). Piercing of the retroorbital plexus has gained wide popularity since 1913 when Pettit originally proposed the use of the "sinus caverneaux" for blood collection in the mouse, rat, and guinea pig (10). The technique is useful for multiple or serial procedures requiring relatively small quantities of blood. It is a reliable, convenient, and safe method for collecting blood from small laboratory animals. This method has been widely used in drug delivery research including nasal delivery of growth hormone in rats (11) and substance P in rats (12).

This paper reports the influence of blood sampling site on the mean AUC values of three ^{14}C-labeled drugs after nasal administration. During nasal absorption studies with dextromethorphan HBr (DM) in rats utilizing the retroorbital bleeding method described previously (11,12), it was discovered that the mean AUC value of this drug was much higher compared to blood samples collected by the carotid artery cannulation method. In order to confirm these findings, two other drugs, dextrorphan HCl (DX) and clonazepam (CZP), were administered to rats both nasally and intravenously. Blood specimens were collected by retroorbital bleeding and carotid artery cannulation for both routes of administration.

MATERIALS AND METHODS

Chemicals

Dextromethorphan HBr, dextrorphan HCl, ^{14}C-labeled drugs, dextromethorphan HBr (sp act, 160 μCi/mg), dextrorphan HCl (sp act, 217 μCi/mg), and clonazepam (sp act, 29.3 μCi/mg or 160 μCi/mg) were obtained from Hoffmann-La Roche, Inc., Nutley, NJ.

Animal Protocol

Male Sprague Dawley rats 9–15 weeks of age were obtained from Charles River Laboratories, Wilmington, MA.

Drug Solutions

For each drug, an aliquot of the labeled drug solution in ethanol or acetone was dried under nitrogen. Unlabeled drug solutions of dextromethorphan hydrobromide, 15 mg/mL, and dextrorphan hydrochloride, 10 mg/mL, were prepared in 0.9% saline. For clonazepam no unlabeled drug solution was made. The corresponding unlabeled drug solution was then added to the vial containing the labeled drug residue and the contents were sonicated. For clonazepam, propylene glycol was added to the corresponding vial and the contents were sonicated and used for administration directly. The mixture of unlabeled and labeled drug (about 9:1 for DM and about 12:1 for DX) was then used for administration.

Animal Studies

The animals were anesthetized with ketamine HCl, 90 mg/kg, and xylazine, 10 mg/kg, intramuscularly prior to drug administration. For iv administration, 0.2 mL of the drug solution was administered by jugular vein cannulation. For nasal administration, the anesthetized animals were laid on their backs. A volume of 5 μL was administered into each nostril using a positive displacement micropipet. In some experiments, 10 μL was administered only in one nostril. Blood specimens were collected into heparinized tubes (Microtainer) by retroorbital bleeding in one set of experiments and by carotid artery cannulation in another set of experiments at specified time intervals.

Table I. Summary of Areas Under the Curves After Intravenous and Nasal Administration of ^{14}C-Dextromethorphan HBr and ^{14}C-Dextrorphan HCl in Rats[a]

Route administered; collection method	AUC (0–2 hr), (μg/mL) · hr	
	Dextromethorphan HBr	Dextrorphan HCl
IV		
Arterial cannulation	0.0723 ± 0.0112	0.0892 ± 0.00912
Retroorbital	0.0760 ± 0.0113	0.126 ± 0.0215
Nasal		
Arterial cannulation	0.0472 ± 0.0147	0.0460 ± 0.0115
Retroorbital	0.131 ± 0.0396	0.177 ± 0.0451

[a] AUC data are expressed as mean ± SD. Dextromethorphan HBr: animal weights (mean ± SD)—nasal, 432 ± 24.3 g; IV, 423 ± 37.1 g; dose—0.167 mg/rat; n = 3 to 4 rats per group. Dextrorphan HCl: animal weights (mean ± SD)—nasal, 313 ± 19.9 g; IV, 377 ± 4.93 g; dose—0.108 mg/rat; n = 3 to 4 rats per group. Blood samples were collected at 0, 2, 5, 15, 30, 60, 90, and 120 min.

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Table II. Summary of Areas Under the Curves After Intravenous and Nasal Administration of ^{14}C-Clonazepam in Rats[a]

Route administered; collection method	Dose (mg/rat)	AUC (0–5 hr), (μg/mL) · hr
IV		
Arterial cannulation	0.0267	0.0847 ± 0.0274
Retroorbital	0.00555	0.0198 ± 0.00533
Nasal		
Arterial cannulation	0.0261	0.0419 ± 0.00581[b]
Retroorbital	0.00563	0.0221 ± 0.00428[b]
One nostril; retroorbital (contralateral side)	0.00565	0.0250 ± 0.00692

[a] AUC data are expressed as mean ± SD. Animal weights (mean ± SD): nasal, 475 ± 37.1 g; IV, 519 ± 44.5 g; n = 3 rats per group. Blood samples were collected at 0, 2, 5, 15, 30, 60, 120, 180, and 300 min.

[b] To correct for the difference in doses, percentage bioavailability was used. The bioavailability of clonazepam by nasal administration was 50.6 and 110% for arterial cannulation and retroorbital bleeding, respectively.

The amount of radioactive DM, DX and CZP administered was 2.5, 2, and 1 μCi/animal, respectively.

Sample Analysis

Plasma samples (0.1 to 0.2 mL) were pipetted into 10 mL of the liquid scintillation cocktail (Ecoscint), mixed well, and assayed for ^{14}C content in a liquid scintillation counter (Beckman LS 9800, Beckman Instruments, Inc., Fullerton, CA).

Calculation

The areas under the plasma level time curves (AUC) up to the last data point at 2 or 5 hr were determined using the Lagran program (13).

RESULTS AND DISCUSSION

The mean AUC values obtained after intravenous administration of DM and DX are comparable when blood was sampled by either the retroorbital bleeding or carotid artery cannulation method (Table I). However, the mean AUC values after nasal administration for DM and DX are about three and four times higher, respectively, when blood was collected by retroorbital bleeding compared to blood sampled by carotid artery cannulation (Table I).

For iv administration of CZP, the mean AUC for a dose of 26.7 μg per animal was 0.0847 [(μg/mL) · hr] when blood was collected by carotid artery cannulation (Table II). The mean AUC for a dose of 5.55 μg per animal by the iv route was 0.0198 [(μg/mL · hr)] when blood was collected by retroorbital bleeding (Table II). The mean AUC values from these two doses are dose proportional, indicating no difference in the plasma levels when the drug is administered iv and the blood specimens are collected by either method. However, when the drug was administered nasally, blood collected via the retroorbital bleeding method provided a bioavailability of 110% compared to 50.6% when the blood was collected by carotid artery cannulation (Table II).

In another study, CZP was administered in one nostril and the blood was collected from the orbital sinus on the contralateral side. This procedure provided a comparable mean AUC as the two nostrils administration (Table II). The mean AUC was not affected by the administration of the drug into one or two nostrils. This may be because the nasal vein is equally accessible from either eye.

Dependence of blood or plasma drug concentrations on sampling site in both humans and animals has been well recognized in the past. An excellent review attesting to the implications of sampling site on blood or plasma concentra-

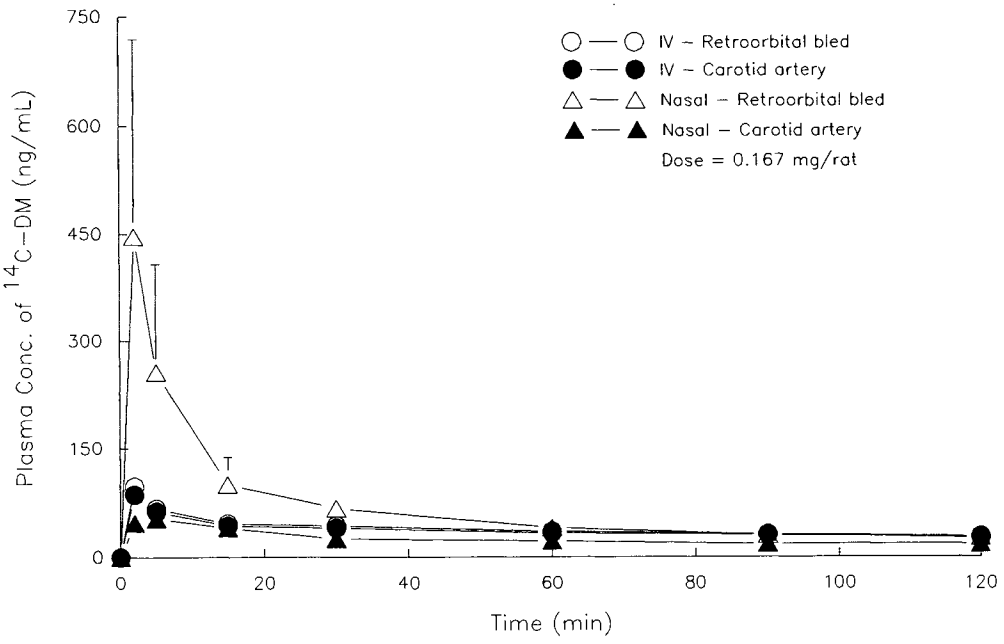

Fig. 1. Levels in plasma after IV and nasal administration of ^{14}C-dextromethorphan HBr in rats.

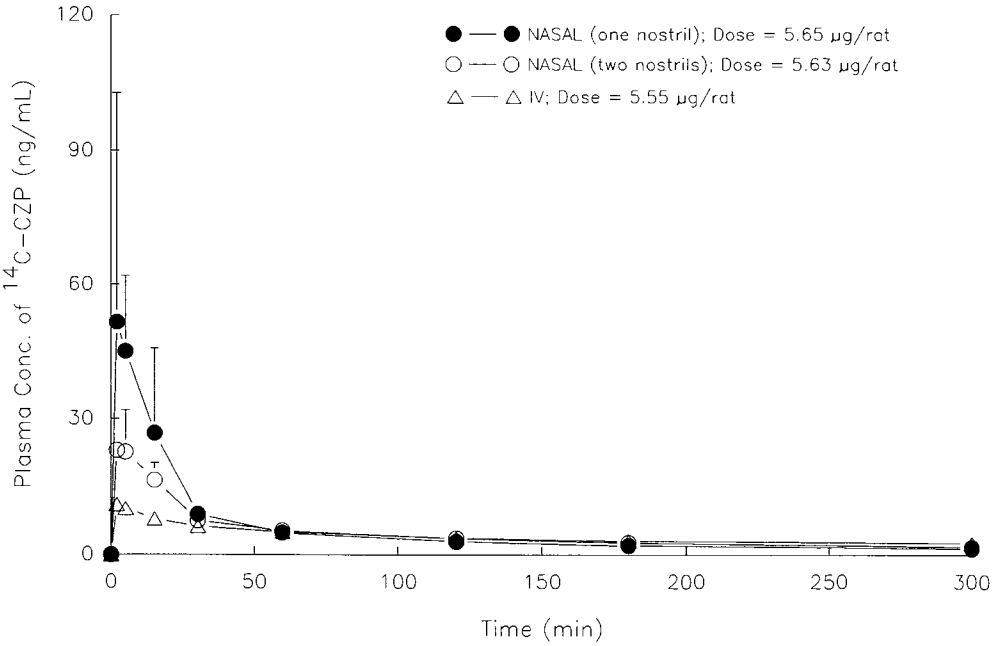

Fig. 2. Levels in plasma after IV and nasal administration of ^{14}C-clonazepam in rats and blood collection by retroorbital bleeding.

tions of drugs has been provided by Chiou (14). A review of the rat anatomy (15) shows that the nasal vein joins the supraorbital vein and the frontal vein. This junction is where the blood is collected when the orbital sinuses are punctured. Thus, sampling from the orbital sinus after nasal drug administration results in higher initial drug levels as compared to blood sampled via the carotid artery cannulation method (Figs. 1 and 2). The higher mean AUC observed is mainly attributed to the higher initial drug peak.

As evidenced from the literature, retroorbital bleeding after nasal drug administration has been utilized by investigators as recently as 1986 (12) and 1988 (11). In this paper, it is shown in rats that the use of an inappropriate sampling site can result in an incorrect estimate of the mean AUC of drugs administered via the nasal route. After nasal administration, blood sampled via the retroorbital sinus resulted in about three or four times higher mean AUC compared to blood sampled by carotid artery cannulation. Based on results presented in this paper, the retroorbital bleeding method should be used with caution in rats for evaluating nasal drug absorption studies.

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