Nasal Absorption of Digoxin in Rats

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The nasal administration of digoxin was studied in rats and compared to intravenous, intraduodenal and rectal administration of the drug. The results indicated that the plasma level of digoxin after nasal administration was comparable to the level after intravenous injection. Administration by the intraduodenal and rectal routes resulted in considerably lower plasma levels. These data reveal that digoxin absorption across the nasal membranes is a reasonable approach. In the *in situ* nasal and intestinal perfusion experiments, digoxin disappeared from the perfusate following the apparent first-order kinetics. The nasal and intestinal absorption rate of digoxin was reduced by an increase in the perfusion volume. The plot of absorption rate constant against 1/volume resulted in a straight line, suggesting that digoxin is absorbed from nasal mucosa by a passive diffusion process.

Keywords — digoxin; nasal absorption; bioavailability; rat; *in situ*; *in vivo*

Introduction

Recently, the possibility that the intranasal administration route might be useful for many drugs which are absorbed inefficiently and variably after oral dosage has received a great deal of attention. The absorption from the nasal route has been studied widely in order to improve drug bioavailability. Studies have shown that the nasal administration of propranolol, progesterone, ergotamine and clofilium tosylate were superior to oral administration from the standpoint of bioavailability and were as effective as intravenous administration in several species including human.¹⁻⁶ These drugs undergo extensive degradation due to first-pass hepatic metabolism which can be minimized after nasal administration.

Meanwhile, digoxin is the most widely used cardiac glycoside for the treatment of congestive heart failure and atrial fibrillation. However, digoxin exhibits a narrow therapeutic range and consequently toxic manifestations are frequently encountered during establishment of dosing regimens.⁷⁻⁹ For this reason, the dosages must be carefully adjusted and the levels in plasma or serum are routinely monitored to achieve optimum therapeutic effect while avoiding toxic episodes. Additionally, the reports by Lindenbaum *et al.*¹⁰ and Wagner *et al.*¹¹ showing reduced or delayed absorption of digoxin from different oral

tablet formulations or different lots of the same formulation emphasized the clinical importance of digoxin bioavailability problems. Therefore, it is desirable that a new route of administration of digoxin be developed to obtain high and non-variable bioavailability.

The pharmacokinetics and bioavailabilities of cardiac glycosides given by various routes have been studied previously.¹²⁻¹⁴ However, the nasal administration of cardiac glycosides has not yet been evaluated. It was the object of this study to report the results on the nasal absorption of digoxin in rats.

Materials and Methods

Chemicals — Digoxin was purchased from Aldrich Chemical Co., Inc.; phenobarbital sodium salt, from Fujinaga Seiyaku Co., Ltd.; pentobarbital sodium salt, from Tokyo Kasei Kogyo Co., Ltd.; and Digoxin Fluorescence Polarization Immunoassay Reagents Kit, from Abbott Laboratories, North Chicago, IL. Other chemicals were obtained as commercial reagent grades.

***In Vivo* Absorption Study** — Male Wistar rats, weighing approximately 250 g were used in the present study. They were housed in an air-conditioned room with free access to a commercial chow (MF, Oriental Yeast Co.) and tap water. All rats were fasted for about 20 h prior to administration.

For all the routes of administration, rats were anesthetized by i.p. injection of a mixture of sodium pentobarbital (50 mg/kg) and sodium phenobarbital (100 mg/kg). The surgical procedures and methods of administration for the *in vivo* nasal absorption experiments were carried out as described by Hirai *et al.*¹⁵⁾ Drug solution was introduced into the nasal cavity at a dose of 50 $\mu\text{g}/200 \mu\text{l/kg}$. Bolus i.v. administration of a 50 $\mu\text{g/kg}$ dose was made through a femoral vein. For intraduodenal or intrarectal administration, the abdomen was opened by a midline incision, and the drug solution which was the same as that prepared for nasal administration was injected directly into the duodenum (2 cm from pylorus), or the rectum which was ligated with thread (3 cm above the anus). After the administration, 0.2 ml of blood was taken periodically from the tail vein. The plasma was separated by centrifugation at 3000 rpm.

Absorption Study by *in Situ* Recirculation — The surgical operation for the *in situ* nasal absorption experiments was performed using the same method as described previously.¹⁶⁾ The intestinal and rectal recirculation absorptions were studied by the *in situ* duodenum-ileum loop and rectal loop methods in rats. The digoxin solution prepared by dissolving in 50 mM isotonic phosphate buffer (pH 7.4), was recirculated by a peristaltic pump at a rate of 2.0 ml/min at 37 °C. For the duodenum-ileum loop recirculation, the drug solution was introduced into the intestine at the upper part of the intestinal opening of bile duct and was withdrawn from the ileum close to the caecum. For the rectal loop circulation, the drug solution was introduced into the rectum at the part 5 cm from anus and was withdrawn from the glass tubing attached to the anus with adhesive. Unless otherwise noted, the volume of the perfusion solution was 7 ml and the concentration of digoxin was 5 $\mu\text{g/ml}$. An aliquot of 100 μl was sampled from the perfusion solution at an appropriate time. In the *in situ* nasal recirculation for 120 min, there was little change in the concentration of inulin (1 mg/ml) used as a volume indicator.

From Fick's law of diffusion, the absorption rate constant observed (k_{obs}) was described according to the following equation;¹⁶⁾

$$k_{\text{obs}} = k_a' / v \quad (1)$$

where k_a' represents an intrinsic absorption rate constant; v is a volume of the perfusion solution.

Assay Procedure for Digoxin — Digoxin in the recirculated solution was assayed by high-performance liquid chromatography (HPLC). The perfusion solution was centrifuged at 3000 rpm for 10 min and a sample (15 μl) of the supernatant fluid was injected into the chromatograph.

The conditions of HPLC were as follows: A liquid chromatograph (Tosoh SP-8700) equipped with ultraviolet (UV) detector (Tosoh UV-8 model II) was used. The column, 30 cm $\times$ 4 mm i.d., and the precolumn, 5 cm $\times$ 4 mm i.d., of stainless steel, were packed with TSKgel ODS-120A (ODS, 5 μm , Tosoh Ltd.). The mobile phase for assay consisted of acetonitrile and distilled water (6:11, v/v). The flow rate was 0.7 ml/min. The wavelength for detection was 220 nm.

The concentration of digoxin in plasma was determined by the fluorescence polarization immunoassay method using ABBOTT-TDX (Ab-

Fig. 1. Digoxin Level in Plasma after Intravenous(●), Nasal(○), Intraduodenal(△) and Rectal(▲) Administration of Digoxin (50 $\mu\text{g/kg}$) in Rats

Each point represents the mean $\pm$ S.D. for 4—5 animals.

TABLE I. Pharmacokinetic Parameters of Digoxin after Intravenous, Nasal, Intraduodenal and Rectal Administration of Digoxin (50 $\mu\text{g/kg}$) to Rats

Route	Rate constant			$AUC_{0 \rightarrow \infty}$ ($\mu\text{g} \cdot \text{min} \cdot \text{l}^{-1}$)	Relative bioavailability (%)
	k_a (min^{-1})	$\alpha^{a)}$ ($\times 10^{-3} \text{ min}^{-1}$)	$\beta^{b)}$ ($\times 10^{-3} \text{ min}^{-1}$)		
Intravenous	—	98.5 ± 23.8	4.00 ± 0.95	2158 ± 289	100
Nasal	0.441 ± 0.151	51.0 ± 18.3	4.26 ± 0.90	2097 ± 132	97
Intraduodenal	0.226 ± 0.028	60.7 ± 34.7	3.40 ± 0.76	924 ± 185	43
Rectal	0.143 ± 0.045	63.2 ± 33.0	4.02 ± 1.78	1201 ± 365	56

Each value represents the mean $\pm$ S.D. for 3—5 rats. *a)* Distribution phase rate constant. *b)* Elimination phase rate constant.

bott Laboratories, North Chicago, IL.).

Data Analysis — The data were analyzed using a microcomputer, NEC PC-9801 VM 2. The program for nonlinear least squares regression analysis, MULTI (RUNGE)¹⁷⁾ was used to fit the data to the pharmacokinetic models. The area under the experimental part of the curves ($AUC_{0 \rightarrow t}$) was calculated by the trapezoidal rule. Total area under the blood concentration *versus* time curve ($AUC_{0 \rightarrow \infty}$) was calculated from $AUC_{0 \rightarrow t}$ and $AUC_{t \rightarrow \infty}$ which was obtained from dividing the concentration at the last-measured time by the elimination rate constant.

Results

Nasal, i.v., Duodenal and Rectal Administration of Digoxin

The absorption kinetics of digoxin in the plasma of rats by various administration routes are shown in Fig. 1. Plasma level of digoxin increased rapidly and showed the maximum 6 min after the nasal administration. The peak concentration at the 6 min was $27.94 \pm 2.31 \text{ ng/ml}$. The nasal administration of digoxin resulted in much higher plasma levels of digoxin than the levels obtained by the intraduodenal and rectal administrations. Since the elimination of digoxin from the plasma of rats after i.v., nasal, duo-

Fig. 2. Effect of Various Volumes of Perfusion Solution on *In Situ* Absorption of Digoxin from Nasal Mucosa of Rats

The initial concentration of digoxin in the perfusion solution was 5 $\mu\text{g/ml}$. Each value in parenthesis represents the volume (ml) of perfusion solution. Results are expressed as the mean $\pm$ S.D. for 3 animals.

Fig. 3. A Plot of Nasal Absorption Rate Constant of Digoxin in Rats *versus* Reciprocal Volume of the Perfusion Solution

Each points represents the mean $\pm$ S.D. for 3—4 animals. $r = 0.994$.

denal and rectal administration exhibited biphasic kinetics, the two-compartment open model was adopted in this study. Table I summarizes the pharmacokinetic parameters calculated for the different routes of administration. The absorption rate constant (k_a) of digoxin in the plasma following nasal administration was 0.441 min^{-1} . The k_a of digoxin from rat nasal coincided well with the value from rat lung reported by Lanman *et al.*¹²⁾ In contrast, the absorption rate constant following intraduodenal and rectal administration was 0.226 min^{-1} and 0.143 min^{-1} , respectively. The $AUC_{0 \rightarrow \infty}$ of digoxin following nasal administration was almost equivalent to that after the i.v. dose. The relative bioavailabilities were calculated by comparison of the mean values of $AUC_{0 \rightarrow \infty}$. The relative bioavailabilities after dosing by intraduodenal and rectal routes were only 43% and 56%, respectively, while that by the nasal route was 97%. These results show that digoxin is absorbed much more rapidly and efficiently from the nasal cavity than from the duodenum and rectum.

In Situ Nasal, Intestinal and Rectal Absorption of Digoxin

The effect of varying the volume of the perfusion solution on the apparent rate of absorption of digoxin was studied. Figure 2 shows plots

Fig. 4. Effect of Various Volumes of Perfusion Solution on *in Situ* Intestinal Absorption of Digoxin in Rats

The initial concentration of digoxin in the perfusion solution was $5 \mu\text{g/ml}$. Each value in parenthesis represents the volume (ml) of perfusion solution. Results are expressed as the mean $\pm$ S.D. for 3 animals.

Fig. 5. A Plot of Intestinal Absorption Rate Constant of Digoxin in Rats versus Reciprocal Volume of the Perfusion Solution

Each point represents the mean $\pm$ S.D. for 3 animals. $r = 0.997$.

of the amount of digoxin remaining in the perfusion solution *versus* time using four different volumes (5—20 ml) of the digoxin solution. The absorption was estimated from the drug concentration remaining (unabsorbed) in the perfusion solution at an appropriate time after the start of perfusion, which was expressed as the percentage of initial concentration. The nasal absorption rate of digoxin was decreased with an increase

Fig. 6. *In Situ* Rectal Absorption of Digoxin in Rats

The initial concentration of digoxin in the perfusion solution was $5 \mu\text{g/ml}$. Each point represents the mean $\pm$ S.D. for 3 animals.

●, 7.5 ml ^{a)}; ○, 10.0 ml.

^{a)} Volumes of the perfusion solution.

in the perfusion volume from 5 to 20 ml.

The absorption rate constant observed (k_{obs}) of digoxin was determined from the drug level remaining in the perfusion solution shown in Fig. 2. According to Eq. 1 that described previously, the first-order absorption rate constant was calculated and plotted against $1/\text{volume}$ (Fig. 3). As shown in Fig. 3, the plot of k_{obs} versus $1/\text{volume}$ resulted in a straight line with slope of the intrinsic absorption rate constant (k_a) for digoxin. The k_a calculated for digoxin is $8.23 \times 10^{-3} \text{ min}^{-1} \cdot \text{ml}$.

In comparison with the nasal absorption of digoxin, we studied the intestinal and rectal absorption by an *in situ* perfusion technique. As shown in Fig. 4, the intestinal absorption rate of digoxin was decreased by an increase in the perfusion volume from 15 to 30 ml. The rate of intestinal absorption was much more rapid than that of nasal absorption. A plot of k_{obs} versus $1/\text{volume}$ resulted in a straight line with slope of k_a for digoxin, but did not pass through the point of origin (Fig. 5). The k_a for the intestinal absorption is $63.6 \times 10^{-3} \text{ min}^{-1} \cdot \text{ml}$. In contrast, the absorption of digoxin from rectum was not apparently observed (Fig. 6).

Discussion

It is clear from Fig. 1 and Table I that the plasma level of digoxin after nasal administration is comparable to that by i.v. administration, with the peak plasma level occurring at 6 min. Thus, nasal administration of digoxin results in rapid absorption from the nasal mucosa into the systemic circulation without first-pass metabolism in the liver, in a manner that is highly superior to the intraduodenal and rectal routes and as effective as the i.v. route. The reason why nasal absorption of digoxin is superior to its rectal absorption might be that a part of the digoxin absorbed from the upper part of the rectum could receive first-pass effect in the liver. Another possibility may be related to the previous observation¹⁸⁾ that the nasal membrane is composed of smaller-sized pores but is richer in water channel distribution than the rectal membrane.

In the *in situ* nasal and intestinal absorption experiments, digoxin was shown to be absorbed

through nasal and intestinal mucosa of rats. Digoxin disappeared from the perfusate following the apparent first-order kinetics. The nasal and intestinal absorption rates of digoxin decreased with an increase in the perfusion volume (Figs. 2 and 4), and the plot of k_{obs} against $1/\text{volume}$ resulted in a straight line (Figs. 3 and 5), but the straight line from intestinal absorption not pass through the point of origin. Thus, it seems likely that digoxin may be absorbed from nasal mucosa by a passive diffusion process. The absorption processes of digoxin from intestinal mucosa may include a passive diffusion process.

The *in situ* absorption from the nasal mucosa is much slower than that from the intestine, while the *in vivo* bioavailability after the nasal administration is much higher than that after the intraduodenal administration. The difference in the absorption between *in situ* and *in vivo* studies is probably due to the experimental conditions, e.g. in the *in situ* experiment, the surface area of the rat nasal cavity (the volume of the cavity is estimated to be 0.2–0.3 ml) is extremely small as compared with the volume of the recirculated solution (5–20 ml) whereas the area of the intestinal lumen is very large, and the digoxin concentration of the solution used for the *in vivo* absorption study was about 10 times higher than that used for *in situ* absorption study. On the other hand, in the *in situ* rectal absorption experiment, digoxin is absorbed very poorly from the rectum. However, digoxin is absorbed slightly from rectum *in vivo*. The difference in the absorption between *in situ* and *in vivo* studies is probably due to the gradient of concentration coming from the difference in the digoxin concentration of the solution described above, and to the difference in the relative absorption area (area/volume of solution/min) where the digoxin solution came in contact with.

In conclusion, the administration of digoxin by the nasal route appears to be more effective than the intestinal and rectal applications and would be useful as a simple dosage form in long-term therapy of congestive heart failure and atrial fibrillation.

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