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NASAL ABSORPTION OF THE CALCIUM ANTAGONIST NICARDIPINE IN RATS AND RHESUS MONKEYS

G.C. Visor, B. Schuessler, J. Thompson, and T. Ling

Syntex Research
3401 Hillview Avenue
Palo Alto, CA 94304

Abstract: Nicardipine hydrochloride, a highly potent calcium entry channel blocker was administered intranasally to rats and rhesus monkeys. The intranasal absorption of nicardipine in rats was studied with aqueous vehicles containing 0.1 M citrate buffer pH 3.5, 0.01 M acetate/propylene glycol (90:10 W/W) pH = 5.0, and a similar acetate/propylene glycol system containing sodium taurocholate. Peak plasma levels were found to occur 30 minutes after an intranasal dose of 1.0 mg/kg, and the fraction absorbed using each of the vehicles described above was determined to be 0.85, 0.54, and 0.82 that of an equivalent intravenous dose. These initial screening studies were extended into a monkey model system, with a similar group of formulations. The results obtained in the monkey were qualitatively similar to the data generated in the rat model

with regard to rapid attainment of peak plasma levels and subsequent elimination from the plasma. The major difference between the two animal models studied was the significantly lower systemic availability observed in the monkey.

INTRODUCTION

Intranasal delivery has been shown to be an efficient route of administration for drugs that undergo significant first pass (hepatic) elimination (1-4). Nicardipine hydrochloride, dl-2,6-dimethyl-4-(3-nitrophenyl)-1,4 dihydropyridine-3, 5-dicarboxylic acid 3-(2-(N-benzyl-N-methylamino))-ethyl ester hydrochloride is a calcium entry blocking agent that is effective in the treatment of angina and hypertension (5,6). Despite its rapid and complete absorption from the gastrointestinal tract following an oral dose, its systemic availability is low due to a large first pass effect in the liver (7,8). Metabolic transformation products that result demonstrate little cardiovascular activity (9). Previous studies using a rat model, have demonstrated the feasibility of delivering nicardipine to the systemic circulation intranasally (10). The rat model system was developed primarily to screen for physiochemical influences of bioavailability. Results from these studies would be used to develop prototype formulations for bioavailability studies in primates and eventually humans.

This paper describes some further nasal absorption studies conducted with nicardipine in the rat and their extrapolation to primates. The validity of using such model systems to simulate potential human studies will be discussed.

MATERIALS AND METHODS

Chemicals - Nicardipine hydrochloride was obtained from the Institute of Organic Chemistry, Syntex Research. All other chemicals and reagents were reagent grade.

Analytical Instrumentation - An HPLC system with electrochemical detection and a capillary column-GC with electron capture detection were used in the analysis of plasma samples from rats and rhesus monkeys (respectively), as previously described (10,11).

Animal Studies - Male Sprague-Dawley rats weighing 350-500 g were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and kept under a heat lamp during the course of the experiments. As in previous studies, blood was collected from a heparinized cannula implanted in the carotid artery and 3-5 drops were discarded prior to sampling. Blood was collected at 5, 15, 30, 60 and 120 minutes post dosing. No further surgery was performed to circumvent or minimize normal

mucociliary clearance and drainage from the nasal cavity. Dosing was performed (n=3 or 4) using a micropipette to deliver (30-40 µl) the appropriate aqueous solution of nicardipine hydrochloride (1 mg/kg) into the nasal cavity of the animals.

Rhesus Monkeys (Macaca Mulatta) - Animals weighing 6-9 kg were used for these studies, with three monkeys per group. Prior to dosing the monkeys were tranquilized with 5-8 µg/kg i.m. injection of Ketamine HCl. Three milliliters of predose blood was drawn prior to nasal administration to establish a base line plasma level.

Approximately 100 microliters of the nasal formulation was sprayed into each nostril while the head of the animal was held in an upright position. Immediately after dosing the monkey was placed in a supine position for 5 minutes. The animals were then placed in metabolism chairs for 10 hours after nasal administration. Food and water was permitted throughout the study period. The exact amount of dose administered was determined by the difference in the weight of the spray pump prior to and following dosing (3 mg/kg). Three milliliter blood samples were collected via an indwelling catheter in the saphenous vein. Blood was collected at 2, 5, 10, 15 and 30 minutes, and 1, 2, 4, 6, 10 and 24 hours post-dose. Blood samples were centrifuged and the plasma separated and frozen until analysis.

RESULTS AND DISCUSSION

Rat Studies

Average plasma levels achieved in rats after intranasal administration (1 mg/kg) are shown in Fig. 1. A summary of pharmacokinetic parameters and systemic availability are presented in Table I. Peak plasma levels achieved with the three formulations tested were all attained within thirty minutes. Previous studies performed by Higuchi, et al. (7) with oral (solution) doses of nicardipine hydrochloride in rats required 2-5 fold larger doses to provide comparable peak plasma levels and systemic availability.

The fraction absorbed after intranasal administration, as measured by the area under the concentration-time curve, was in the range 0.54-0.85 of that after an equivalent intravenous dose.

The intranasal absorption of nicardipine from each of the vehicle formulations may involve a number of distinct processes. Since each of the vehicles were formulated at pH values (3.5 and 5.0) well below the pKa of nicardipine (pKa 7) and the surface pH of the nasal mucosa 7.4 (12), the possibility of aqueous channels or pores for nicardipine absorption seems plausible. The presence of aqueous channels in the mucous membrane has previously been suggested to play a major role in

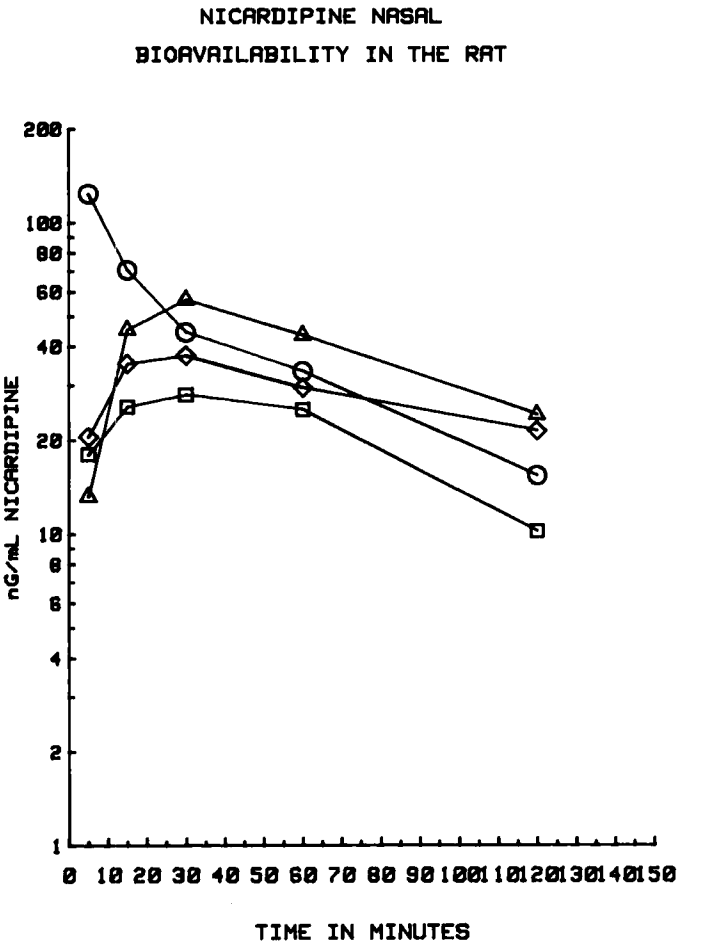

Figure 1. Mean plasma nicardipine levels after intranasal delivery of 0.1M Citrate pH 3.5 (△), 0.01M Acetate propylene glycol (90:10) pH 5.0 (□) and 0.01M Acetate propylene glycol (90:10) pH 5.0 ± 2% Sodium Taurocholate (◇) in rats.

the intranasal absorption of hydrazaline (1), another basic drug. This compound also exhibits good bioavailability at a relatively low pH.

The nasal absorption of nicardipine in the rat was determined to be greatest from a simple citrate buffer vehicle (pH 3.5) and an

Table I - Bioavailability of Nicardipine in Rats Following Intranasal Administration

Solution [a,b] Composition	AUC[c] ng • min/mL	T_{max} (min.)	C_{max} ng/mL	Relative Systemic[d] Availability, %
0.1M Citrate pH = 3.5[a]	4610 ± 748	30	56.5	85
0.01M Acetate pH = 5.0 + 10% Propylene Glycol[b]	2960 ± 442	30	28.0	54
0.01M Acetate pH = 5.0 + 10% Propylene Glycol + 2% Sodium Taurocholate	4480 ± 442	30	37.4	82

[a]All solutions contained 0.25% (w/w) hydroxyethyl cellulose
[b]Solutions contained 1% nicardipine hydrochloride
[c]Values represent mean ± SEM (n = 3 or 4)
[d]Calculated relative to an intravenous dose of 1.0 mg/kg, 5440 ± 245 ng • min/ml.

acetate propylene glycol system containing the absorption promoter sodium taurocholate (Table I). It is interesting however, to note the significant decrease in absorption efficiency observed without this bile salt being present in this drug/vehicle system. Previous studies have suggested that taurocholate/glycocholate absorption promoters affect the mucous membrane as well as inhibit enzymatic degradative process (13).

The role of such surface active agents with nicardipine is not clear, but it may involve a drug-promoter ion pair complex that facilitates diffusion across the membrane.

Studies in Rhesus Monkeys

Intranasal absorbtion studies were extended to the monkey model using a similar set of aqueous based formulations as those previously used in rats.

The plasma level profiles of nicardipine with four nasal solutions is shown in Fig. 2, and the bioavailability summarized in Table II. As is commonly seen with intranasal delivery, absorption is quite rapid, with detectable levels obtained within 2-5 minutes. Also significant is the remarkably consistent level of absorption observed between animals. The bioavailability of 0.3 mg/kg intranasal doses of nicardipine relative to i.v. administration varied between 15% and 27%. This is dramatically lower than that observed in rats. For example a 0.1 M citrate formulation (Formulation A) had a systemic availability of 14% in monkeys vs. 85% in rats. This in part may be explained in terms of the reduced mucociliary clearance and drainage from the nasal cavity in rats which were anesthetized throughout the study. There may also be a dramatic difference in absorption due to the method and technique of intranasal delivery in rats and monkeys. Hence the use of

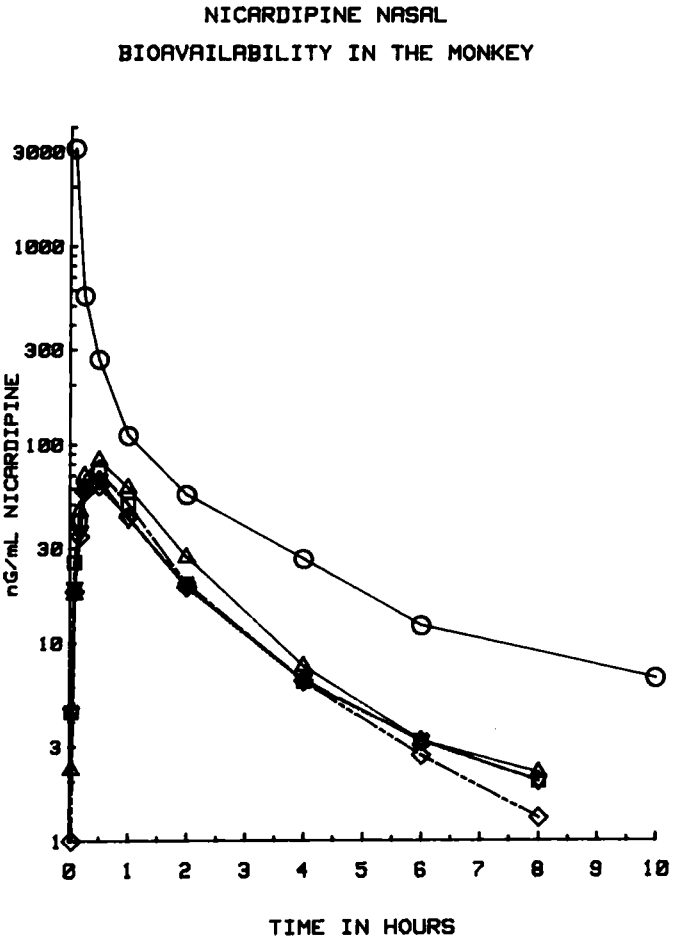

Figure 2. Mean Plasama Concentrations of Nicardipine in Monkeys following Nasal Administration of Nicardipine Hydrochloride in Aqueous Solution. (△—△—△ .01M citrate pH 3.5), (▽—▽—▽ 0.01M acetate, pH5, + 10% propylene glycol), (□--□—□ 01M acetate pH 5, + 10% propylene glycol + 2% sodium glycocholate), (◇-◇-◇ 0.1M citrate pH 3.5), (○—○—○ I.V. administration 0.3 mg/kg).

Table II. Bioavailability of Nicardipine in Rhesus Monkeys following Intranasal Administration

	Aq. Soln. pH 3.5 0.01 M Citrate	Aq. Soln. pH 5.0 + 10% Propylene Glycol	2% NaGlycocholate pH 5.0 + 10% Propylene Glycol	Aq. Soln. pH 3.0 0.1 M Citrate
Tmax (hr)	0.5 ± 0	0.42 ± 0.14	0.42 ± 0.14	0.5 ± 0
Cmax (ng/ml)	84 ± 12	70 ± 25	78 ± 12	62 ± 8
t1/2	2.71 ± 1.25	2.34 ± 0.2	2.55 ± 0.5	2.85 ± 0.64
AUC	166 ± 54	129 ± 29	139 ± 33	127 ± 13
F	27 ± 13	15 ± 5	16 ± 5	14 ± 4
Dose, mg/kg	0.28 ± 0.02	0.30 ± 0.01	0.29 ± 0.01	0.31 ± 0.02

F = fraction of dose absorbed, systemic availability.

$$F = \frac{AUC_{(Nasal)}}{AUC_{(IV)}}$$

drop-wise administration in one model system may not correlate well with spray delivery in another. Thus the extent of drug dispersal and coverage in the nasal cavity as well as resulting clearance or positioning of the animal (supine vs. upright) may greatly affect absorption efficiency (14).

Drug absorption from each of the aqueous vehicles were qualitatively similar to that observed in rats. The citrate buffer system (pH 3.5) again provided the highest plasma levels and drug bioavailability. The systemic availability in monkeys was affected by the ionic strength of the citrate buffer system. The significantly larger Cmax and AUC achieved with 0.01 M vs. 0.1 M citrate indicates that the higher buffer capacity of this vehicle may cause increased clearance from the nasal cavity due to deviation from physiological compatability/isotonicity.

Studies with the acetate/propylene glycol vehicle revealed that the inclusion of the bile salt sodium glycocholate did not enhance the nasal absorption of nicardipine in the monkey.

DISCUSSION

The intranasal delivery of nicardipine appears to be a viable route of administration in the animal models studied. Intranasal absorption of the drug results in significant levels in the systemic circulation of both rats and monkeys. The value of using a rat model to study and simulate the intranasal absorption processes in primates has been qualitatively established.

Drug absorption in these species appears to have a low pH optimum, and this suggests that the primary absorption pathway is paracellular rather than transcellular in nature. Aqueous channels in the mucous membrane therefore probably provide the major route for nicardipine absorption into the systemic circulation.

The apparent enhancement of drug absorption in rats with the bile salt sodium taurocholate however was not observed in monkeys. The significance of this is not clear and will require further studies with other surface active agents/promoters in both animal models.

The large differences in bioavailability observed in rats and monkeys with a similar group of vehicle formulations may be an inherent limitation in the model systems and techniques used. The method of drug administration (drops vs. spray) and physical orientation (supine vs. seated upright) as well as the concious state of the animals (anesthetized vs. sedation) all play major roles in ultimate drug bioavailability. Therefore prediction of systemic availability and estimation of dose requirements between species is difficult and will require continued empirical evaluation. Further complications of this type of interspecies absorption data and its possible value are the dramatic differences in metabolism and elimination.

Extension of these studies to a clinical setting has been facilitated by the background data generated. As with any nonparenteral route of drug administration, the extrapolation of intranasal drug absorption data from animals to humans must be carefully controlled to account for major differences in physiology and metabolic disposition. This should not, however, limit the utility of this route of administration.

REFERENCES

(1) S. Kaneo. Acta Pharm. Suec. 20, 379 (1983).

(2) A. Hussain, S. Hirai, R. Bawarski, J. Pharm. Sci. 68, 1196 (1979).

(3) A. Hussain, R. Kimura, C-H Huang, and T. Kashihara, Int. J. Pharm. 21, 233 (1984).

(4) S. Hirai, T. Ikenaga, and T. Matsuzawa, Diabetes 27, 296 (1978).

(5) M. Jerai, T. Takenaka, H. Maeno, Biochem. Pharmacol. 30, 375 (1981).

(6) T. Takenaka, S. Usuda, T. Nomura, H. Maeno, T. Sado, Arzneim-Forsch 26, 2172 (1976).

(7) S. Higuchi, Y. Shiobarra, Xenobiotica 10, 447 (1980).

(8) S. Higuchi, H. Sarahi, Y. Shiobara, T. Sado, Xenobiotica 7, 469 (1977).

(9) S. Higuchi, Y. Shiobarra, Y., Xenobiotica 10, 889 (1980).

(10) G.C. Visor, E. Bajka, and E. Benjamin, J. Pharm. Sci. 75, 44 (1986).

(11) A.T. Wu, I.J. Massey, and S. Kushinsky, J. Pharm. Sci. 73, 1444 (1984).

(11a) A.T. Wu, I.J. Massey, and S. Kushinsky, (In Press) A Capillary Column Gas Chromatographic Method Using Electron Capture Detection for the Simultaneous Determination of Nicardipine and its Pyrrdine Metabolite II in Plasma.

(12) S. Hirai, Y. Takatsuka, T. Matsuzawa, and M. Huoyuki, Int. J. Pharm. 7, 317 (1981).

(13) S. Hirai, Y. Takatsuka, and M. Huoyuki, Int. J. Pharm. 9, 173 (1981).

(14) J.G. Hardy, S.W. Lee, and C.G. Wilson, J. Pharm. Pharmacol. 37, 294 (1985).