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Evaluation of the effect of polyethylene glycol 400 on the nasal absorption of nicardipine and verapamil in the rat

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This study has investigated the effect of polyethylene glycol (PEG) 400 on the intranasal absorption and ensuing pharmacokinetics of the calcium entry blockers nicardipine and verapamil in a rat model. To solutions of nicardipine in acetate buffer pH 5.0 and of verapamil in distilled water, PEG 400 was added in concentrations of 0–5%. The nasal bioavailability of nicardipine from plain buffered solution was 44%, and increased steadily to 56–79% in direct proportion to the amount of PEG 400 added. Verapamil, on the other hand, exhibited an intranasal bioavailability of 52% in the absence of PEG 400, and between 61–68% in the presence of increasing concentrations of PEG 400. None of the formulations tested was found to cause adverse effects on the morphology and integrity of the nasal mucosa.

1. Introduction

Nicardipine and verapamil are two chemically dissimilar calcium entry blockers which, by virtue of their ability to modulate the influx of calcium ions across the cell membranes of the arterial smooth muscle and in myocardial cells, are able to cause coronary and peripheral vasodilation with concomitant improvement in oxygen requirements and a reduction in systemic vascular resistance. Hence, these two drugs are found useful to treat various forms of angina pectoris and mild to moderate hypertension [1–3].

Following their oral administration to humans and laboratory animals, nicardipine and verapamil are rapidly and completely absorbed from the gastrointestinal tract [1–8]. However, by this route the bioavailability of nicardipine is only in the range 5–35% [4, 5], and that of verapamil only between 10% and 29% [1–3, 6, 7] because of extensive hepatic first-pass metabolism. An effective way of circumventing this problem while attaining therapeutic drug levels with lower doses is to resort to intravenous dosing but, unfortunately, this route is not suited for self-administration. For this reason, the systemic delivery of nicardipine and verapamil by the sublingual [8–10], buccal [11], transdermal [12] and intranasal [13–16] routes have received attention in recent years as alternatives to parenteral dosing.

Owing to its rich vascularity and extensive absorptive surface, the nasal mucosa is viewed as a potentially viable site for drug administration [17–19]. The fact that the dosing of both hydrophilic and hydrophobic drugs by the intranasal route has lead to plasma drug levels that are comparable to those derived parenterally [19, 20], has stimulated interest on the use of this route as a means of systemically delivering nicardipine and verapamil. Thus, in laboratory animals the nasal bioavailability of nicardipine is reported to range from 14% to 85%, depending on the composition and physical characteristics of the formulations administered and on the animal species used (i.e., rat, rhesus monkey) [13, 14]; and to be about 36% for verapamil in the dog [15]. Furthermore, in man a nasal gel of verapamil, made from a cellulose derivative and a nonionic surfactant, is reported to elicit pharmacodynamic effects of the same magnitude as those derived by the intravenous route, but greater than those by the oral route [16].

The present study was undertaken to (a) establish plasma pharmacokinetic parameters for nicardipine and verapamil after their delivery to rats by the intravenous, intranasal and oral routes of administration, and (b) to ascertain the effect of PEG 400 on the nasal absorption of these drugs. The addition of PEG 400 to an aqueous solution of a calcium channel antagonist was suggested by the results of earlier studies in which enhancing the viscosity of a drug solution with a cellulose derivate [21] or propylene glycol [22] was found to promote drug absorption from the nasal cavity. Furthermore, since PEG molecules of low molecular weight (i.e., 400, 600) are known to enhance drug permeation through epithelial tissues containing aqueous pathways such as the intestinal mucosa [23] and skin [24, 25], it was assumed that they might also aid in the absorption of water-soluble calcium channel antagonists across the aqueous pore-rich nasal mucosa [26–28].

2. Investigations, results and discussion

The plasma drug concentration-time profiles for intravenous and intranasal doses of nicardipine and verapamil over a 3 h period are shown in Figs. 1 and 3, respectively. The effects of different concentrations (0%–5%) of PEG 400 on the respective intranasal absorptions are depicted in Figs. 2 and 4. The pharmacokinetic data calculated from the plasma levels are summarized in Tables 1 and 2. Neither pharmacokinetic nor bioavailability data is reported for the oral doses of nicardipine and verapamil because the plasma levels of these drugs were below the limits of detection of the analytical methods used for their assay.

The *in vivo* animal model used here has already been validated and shown to be appropriate for examining the in-

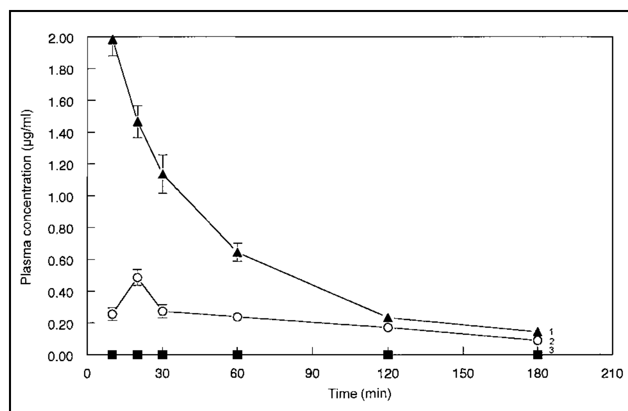

Fig. 1: Temporal changes of plasma nicardipine after delivery by 1 = intravenous; 2 = intranasal and 3 = oral routes. Drug concentrations by the oral route were below the detection limit (0.05 µg/ml). Each point represents the mean value ± S.E.M (n = 5)

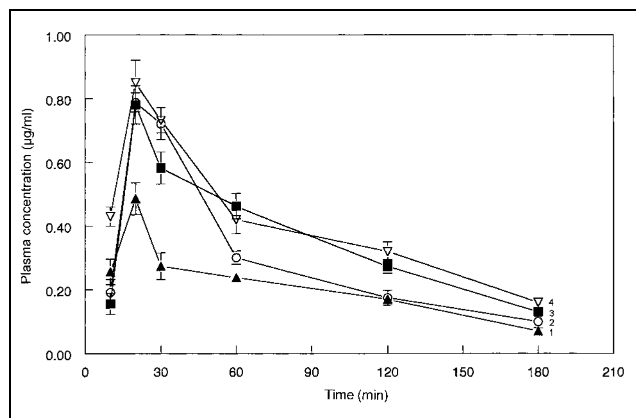

Fig. 2: Temporal changes of plasma nicardipine after intranasal delivery with 1 = 0% PEG 400; 2 = 1% PEG 400; 3 = 2.5% PEG 400 and 4 = 5% PEG 400. Each point represents the mean value ± S.E.M. (n = 5)

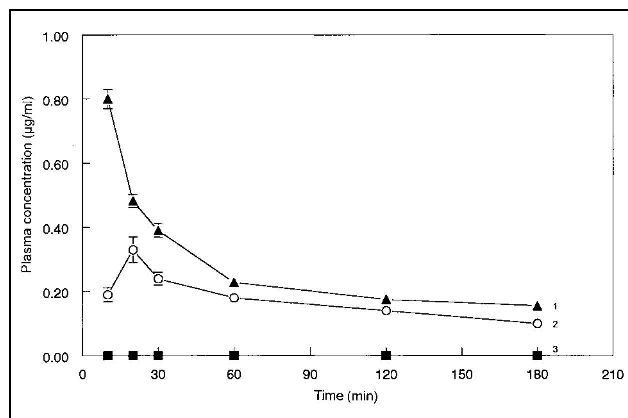

Fig. 3: Temporal changes of plasma verapamil after delivery by 1 = intravenous; 2 = intranasal and 3 = oral routes. Drug concentrations by the oral route were below the detection limit (0.05 μg/ml). Each point represents the mean value ± S.E.M. (n = 5)

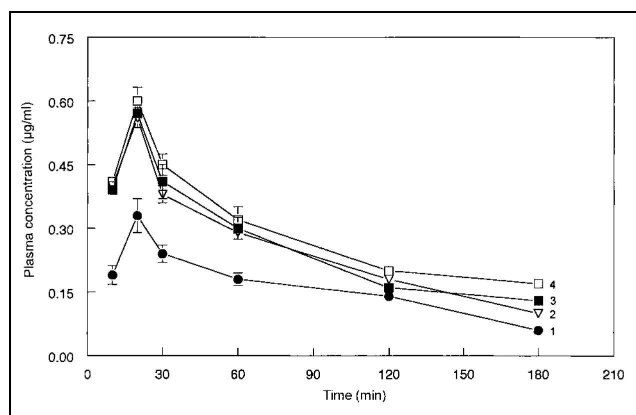

Fig. 4: Temporal changes of plasma verapamil after intranasal delivery with 1 = 0% PEG 400; 2 = 1% PEG 400; 3 = 2.5% PEG 400 and 4 = 5% PEG 400. Each point represents the mean value ± S.E.M. (n = 5)

tranasal absorption of drugs possessing a low oral bioavailability [29]. To more directly correlate the efficiency of absorption from the nasal cavity and gastrointestinal tract with that by intravenous dosing, the test drugs were given in the same doses (i.e., nicardipine, 2 mg/kg; verapamil, 3 mg/kg) by all three routes. The formulation of nicardipine was prepared in the same buffer solution as that reported elsewhere for the intranasal delivery of this drug to rats and rhesus monkeys [13, 14], except that it included PEG 400 in place of propylene glycol and omitted the addition of hydroxypropyl ethylcellulose as a viscosity agent. Verapamil was delivered as an aqueous solution in distilled water by analogy with previous nasal studies on this drug [15] and because of its propensity to precipitate from solutions containing inorganic salts [30].

The intranasal dosing of rats with nicardipine (1.0 mg/kg) has resulted in an absolute bioavailability that exceeded 70% and which varied according to the composition of the formulation used [13]. For example, instilling a solution of nicardipine in 0.1 M citrate buffer pH 3.5 plus hydroxypropyl ethylcellulose onto the nasal mucosy yielded a bioavailability of about 77%, which was reduced to 73% in the absence of the cellulose derivative. On the other hand, the bioavailability was reduced to only about 54% when the strength of the buffer was changed to 0.01 M and the viscosity agent was replaced with propylene glycol; but it rose dramatically to 82% when the glycol-containing formulation also included sodium taurocholate [14]. By repeating the same studies in conscious monkeys, the existence of interspecies differences in the nasal absorption of nicardipine was demonstrated [14]. In this case, spraying the nasal cavity with a solution of nicardipine in 0.01 M citrate buffer pH 3.0 resulted in a bioavailability of about 27%, which became about 14% when nicardipine was dissolved in 0.1 M citrate buffer pH 3.0, and 15% or 16% when formulated in 0.01 M acetate buffer pH 5.0 with or without the addition of propylene glycol, respectively. More recently, a formulation of RS-93522, an analog of nicardipine, in an aqueous solution containing a polyoxyethylated vegetable oil, sorbitol and phosphate buffer was shown to yield a bioavailability of 92% when delivered intranasally [32]. In the present study, the plasma level of nicardipine increased rapidly

Table 1: Pharmacokinetic data of nicardipine (2 mg/kg) administered by the intranasal route with and without PEG 400

Parameters	Amount of PEG 400 added[a, b]			
	0%	1%	2.5%	5%
Intranasal route				
$t_{1/2}$ (min)	85.52 ± 4.53	69.10 ± 4.23*	74.20 ± 5.95	75.76 ± 8.75
t_{max} (min)	20	20	20	20
C_{max} (μg/ml)	0.49 ± 0.05	0.79 ± 0.03***	0.68 ± 0.06**	0.85 ± 0.07***
k_a (min^{-1})	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.02
k_e (min^{-1})	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
AUC (μg · ml/min)	46.50 ± 2.20	59.00 ± 3.30$^{**,+}$	76.00 ± 5.07$^{***,++}$	84.00 ± 5.80$^{***,++}$
F (%)	44	56	72	79
Intravenous route				
AUC (μg · ml/min)	106.16 ± 8.23	–	–	–

[a] Comparison vs. 0% PEG 400 by Student's t-test: * $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
[b] Integroup comparisons by ANOVA and posthoc Student-Neumann-Keuls test: $^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$.

Table 2: Pharmacokinetic data of verapamil (3 mg/kg) administered by the intranasal route with and without PEG 400

Parameters	Amount of PEG 400 added[a, b]			
	0%	1%	2.5%	5%
Intranasal				
$t_{1/2}$ (min)	125.05 ± 8.58	85.80 ± 4.12**	90.72 ± 6.47**	84.82 ± 4.08**
t_{max} (min)	20	20	20	20
C_{max} (μg/ml)	0.33 ± 0.05	0.56 ± 0.02*	0.57 ± 0.01***	0.59 ± 0.03***
k_a (min^{-1})	0.15 ± 0.01	0.12 ± 0.00	0.13 ± 0.01	0.15 ± 0.00
k_e (min^{-1})	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
AUC (μg · ml/min)	46.70 ± 3.45	54.60 ± 4.50	58.00 ± 3.68	61.00 ± 3.63**
F (%)	52	61	64	68
Intravenous				
AUC (μg · ml/min)	90.20 ± 3.65	–	–	–

[a] Comparison vs. 0% PEG 400 by Student's t-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
[b] Integroup comparisons by ANOVA with posthoc Student-Neuman-Keuls test were not significant.

and showed a maximum at about 20 min after nasal administration. By this route, the bioavailability of nicardipine as a solution in 0.01 M acetate buffer pH 5.0 was 44%. Relative to a plain buffered solution, the addition of PEG 400 in concentrations between 1–5% led to a bioavailability that was from 27–79% greater and to a higher C_{max} (0.78–0.85 μg/ml vs. 0.49 μg/ml). The $t_{1/2}$ was shortened by 1% but not by 2.5% or 5% PEG-400. The histopathological examination of sections from four areas of the nasal cavity showed that neither the 0.01 M acetate buffer pH 5.0 nor the same buffer plus 1–5% PEG 400 exerted an altering effect on the nasal morphology (Figs. 5–7). In this regard, columnar epithelial cells remained closely packed and attached to the basement membrane, and the ciliated mucosal surface appeared intact. Furthermore, the results of erythrocyte hemolysis and nasal protein leaching studies on nasal surfaces exposed to drug-containing formulations were not different from those of animals treated with either distilled water or plain buffer of pH 5.0.

The feasibility of delivering verapamil by the nasal route has been previously demonstrated in conscious, tranquilized dogs [14] and human volunteers [15]. In the dog, the intranasal bioavailability of verapamil was shown to be about 3-fold higher (36%) than by the oral route; and would approximate those obtained by the intravenous route if given at twice the oral dose. In the present study, intranasal dosing of an aqueous solution of verapamil produced plasma drug levels that reached a maximum at 20 min postdosing and a bioavailability of 52%. Upon the addition of PEG 400, this bioavailability increased to 61–68% (or 17–30% relative to the drug in plain buffer) in direct proportion to the concentration (1–5%) of PEG 400 added. Since all of these values are at least 44% greater than that reported earlier for a dog model, the concept of an interspecies difference in the nasal absorption of a calcium channel antagonist is confirmed. Relative to the formulation in water alone, the addition of PEG 400 also increased the C_{max} (0.56–0.59 μg/ml vs. 0.33 μg/ml) and shortened the $t_{1/2}$.

A comparison of the nasal absorption of nicardipine with that of verapamil in the rat model used here disclosed several interesting differences. Firstly, verapamil was bet-

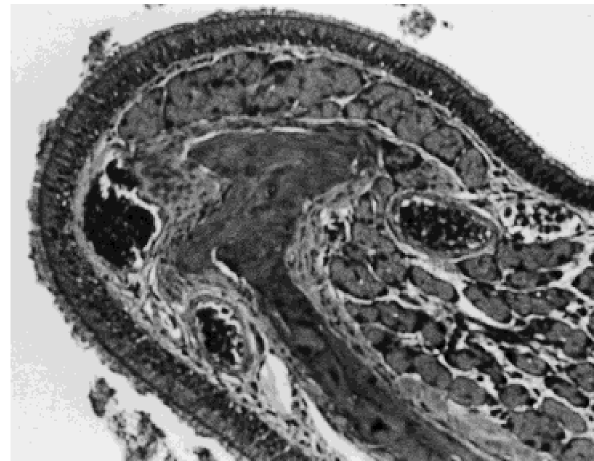

Fig. 6: Nasal mucosa of rat after exposure to 5% PEG 400

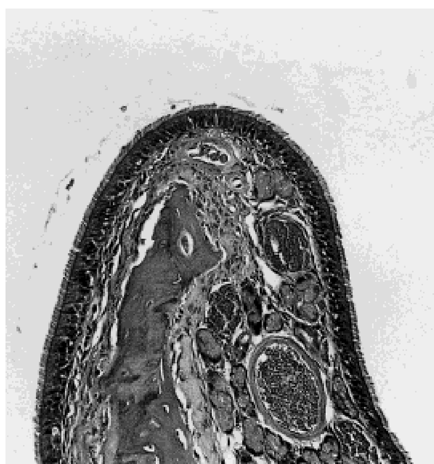

Fig. 5: Nasal mucosa of rat after exposure to acetate buffer pH 5.0

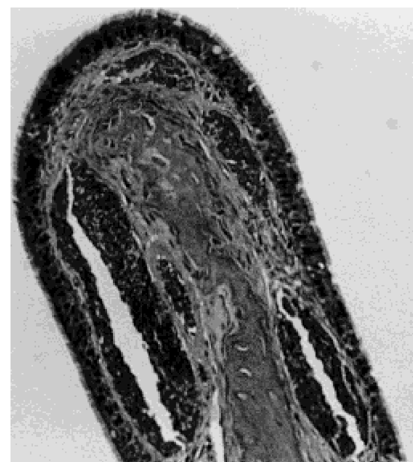

Fig. 7: Nasal mucosa of rat after exposure to 5% PEG 400 in acetate buffer pH 5.0

ter absorbed (about 45% more) than nicardipine from formulations containing no PEG 400; however, nicardipine demonstrated a much higher bioavailability than verapamil upon the addition of PEG 400, particularly at the 2.5% and 5% levels. Secondly increasing the concentration of PEG 400 from 1% to 5% had a lesser effect on the bioavailability of verapamil than on that of nicardipine (i.e., 10% change for verapamil, 41% change for nicardipine). This finding would imply that the effects of a water-soluble, low molecular weight, glycol polymer such as PEG 400 on the nasal permeation of a calcium channel antagonist will vary in magnitude depending not only on the amount of glycol added but also on the particular drug. On this basis, nicardipine appeared to be more sensitive to changes in PEG 400 concentration than verapamil. Whether the noted bioavailability-PEG 400 concentration differences are the result of an increase in viscosity [21, 25], an increase in osmolality [23, 32], a dehydration of the nasal mucosa [24], or a decrease in the thermodynamic activity of the solute [23] by a glycol polymer is not clear at the present time and its explanation will require a more detailed study. On the other hand, the results of the histopathologic study and of the tests for mucosal integrity suggest that disruption of the nasal mucosa by the test formulations does not play a role in the nasal absorption of nicardipine and verapamil.

In summary, the results of the present study indicate that the nasal cavity can serve as a viable and more effective alternative to the oral route for the systemic delivery of the calcium channel antagonists nicardipine and verapamil; and that the nasal bioavailability of these drugs can be enhanced by the addition of low concentrations of PEG 400 to their aqueous solutions.

3. Experimental

3.1. Materials

The sample of nicardipine hydrochloride was a generous gift from Syntex Laboratories, Inc., Palo Alto, CA. Verapamil hydrochloride, PEG 400 and propyl paraben were obtained from Sigma Chemical Co., St. Louis, MO. All solvents for the liquid chromatographic analyses were of HPLC grade and from J. T. Baker Chemical Co., Phillipsburg, NJ. Triethylamine, dibasic sodium phosphate and glacial acetic acid were of AR grade and from J. T. Baker Chemical Co.

3.2. Animals

All experiments were carried out with male Sprague-Dawley rats (Taconic Farms, Germantown, NY), weighing 300–350 g, and acclimated to their surrounding for at least 7 d in a temperature-regulated room and on a 12 h light-dark cycle. During the acclimation period the rats had free access to a commercial rat diet (Purina Rat Chow, Ralston Purina, St. Louis, MO) and tap water. Before each experiment, all rats were fasted overnight for 12 h. Experimental groups consisted of 5 rats each.

3.3. Treatments

Solutions of nicardipine hydrochloride were prepared freshly in 0.01 M acetate buffer pH 5.0, to contain 1 g/dl of the active and 0, 1, 2.5 or 5% (v/v) of PEG 400. Verapamil hydrochloride solutions, containing 1.5 g/dl, were prepared in the same manner but using distilled water as the vehicle. Drug doses were 2 mg/kg for nicardipine hydrochloride and 3 mg/kg for verapamil hydrochloride. After undergoing anesthesia with an intraperitoneal, 40 mg/kg, dose of pentobarbital sodium, the rats were surgically intervened to expose the femoral artery and/or the femoral vein, into which a cannula, made from a piece of polyethylene (PE) 50 tubing and fitted with a three-way stopcock, was inserted. Intravenous injections were made through the indwelling femoral vein cannula, with the volume not exceeding 300 μl/kg. Oral doses were delivered with a 1 ml tuberculin syringe, via a curved oral feeding needle, and in a volume not exceeding 300 μl/kg. The intranasal delivery of the drug solutions, in a 200–300 μl/kg volume, was accomplished as previously described [22] through a blunted piece of PE 20 tubing attached to a 1000 μl microsyringe. The animals remained in the supine position and under a heat lamp for the duration of the experiment.

3.4. Blood collections

Blood samples were withdrawn from each rat at 10, 20, 30, 60, 120 and 180 min postdosing, from an indwelling femoral artery cannula fitted with a three-way stopcock and kept patent with a heparin flush. Blood samples were delivered to ice-cold heparinized polyethylene tubes, and centrifuged at 5000 rpm for 5 min. The clear plasma samples were decanted into clean stoppered polyethylene tubes, and stored at −25 °C pending their analysis for drug content.

3.5. Apparatus

An isocratic liquid chromatograph was used for the assay of nicardipine and verapamil in rat plasma. The chromatographic system consisted of Model 501 pump, Model LC75 spectrophotometric detector (Perkin-Elmer Corporation, Norwalk, CT) and Model HP 3394A recording integrator (Hewlett-Packard Co., Avondale, PA). Samples were introduced using a Model 710B WISP automatic sample injector (Waters Associates, Milford, MA).

3.6. Analysis of drug plasma concentrations

Samples for HPLC analysis were prepared by placing the rat plasma and an equal volume of acetonitrile (verapamil assay) or of internal standard solution (propyl paraben in acetonitrile, 0.25 μg/ml, nicardipine assay) in a 1.5 ml polyethylene microcentrifuge tube with a snap cap, and mixing for 30 s with the aid of a vortex mixer. The resulting suspension was centrifuged at 5000 rpm for 5 min, and 100 μl of the clear supernatant was injected into the liquid chromatograph using an automatic injector. Plasma samples containing nicardipine were analyzed on a Microsorb-MV C18, 25 cm × 4.6 mm i.d., 5 μm, column (Rainin, Woburn, MA), using a mixture of acetonitrile-water-phosphoric acid (60 : 40 : 0.2) at the rate of 1.5 ml/min, and detection at 235 nm and 0.08 AUFS. In the case of verapamil the chromatographic conditions were: Microsorb-MV C18, 15 cm × 4.6 mm i.d., 5 μm, column, eluted with 0.02 M triethylamine/ 0.02 M acetic acid-acetonitrile-methanol (45 : 45 : 10) at 1.5 ml/min, and detection at 235 nm and 0.08 AUFS. All experiments were conducted at ambient temperature.

Both methods were found to be linear over the concentration range 0.1–1 μg/ml of nicardipine hydrochloride (regression line equation $y = 0.527x - 0.006$, $r = 0.998$) or verapamil hydrochloride (regression line equation $y = 218.95x + 5.03$, $r = 0.998$) in spiked drug-free plasma. Recoveries of both analytes were calculated by comparing these calibrations curves, prepared in triplicate, with those prepared in distilled water. In general, recoveries of nicardipine from spike plasma averaged 90% of added; and those of verapamil averaged 95% of added. The concentrations of drug in the plasma samples were calculated on the basis of peak areas (verapamil) or peak area ratios (nicardipine) with reference to calibrations curves prepared in drug-free rat plasma on the day of the analysis.

3.7. Erythrocyte hemolysis

The effects of different concentrations of PEG 400 on erythrocyte integrity was tested using the *in vitro* method of Hirai et al. [28]. For this purpose, a 5 ml aliquot of rat blood, freshly collected from the carotid artery, was freed from fibrin by stirring with a glass rod and separated from the soluble components by centrifugation at 2000 rpm for 5 min. The packed erythrocytes were suspended in 3 ml of physiological saline and centrifuged at 2000 rpm for 5 min. After repeating the washings three additional times, the cells were mixed with sufficient physiological saline to produce 50 ml of cell suspension. Then, 5 ml of each test solution (1% or 5% PEG 400 in physiological saline) and 0.2 ml of erythrocyte suspension were placed in 10 ml capped tubes, mixed by gentle shaking, and incubated at 37 °C for 5 min on a dry heating block. After cooling the contents of each tube in ice-water, and centrifugation at 2000 rpm for 5 min, the absorbances of the clear supernatants were read at 540 nm against a physiological saline blank to determine the extent of hemolysis (in %) relative to the effect produced by an equivalent volume of plain physiological saline, incubated concurrently with the test sample and serving as a control.

3.8. Protein leakage from the nasal mucosa

To determine the effect of different concentrations of PEG 400 on the integrity of the nasal epithelium, a lavage technique was used on rats that had been surgically prepared as follows: After anesthesia with an intraperitoneal 40 mg/kg dose of pentobarbital sodium, an incision was made along the neck, the neck muscles were moved aside, and the exposed trachea was cannulated with a piece of PE 100 tubing to facilitate breathing and minimize the possibility of asphyxiation. A second piece of PE 100 tubing was inserted through the esophagus towards the posterior part of the nasal cavity, and 10 ml of 0.01 M acetate buffer pH 5.0 or an equivalent volume of 1% or 5% PEG 400 in the same buffer, was infused from a 10 ml syringe at the rate of 1 ml/min. The liquid outflowing from the nostrils was collected in a test tube with the aid of a funnel, and used for the assay of total protein using an automated colorimetric method (Kit 1430-10, Abbot Vision Analyzer, Abbott Laboratories, Abbott Park IL).

3.9. Histopathological examination of the nasal mucosa

Drug solutions containing nicardipine (1 g/dl in acetate buffer pH 5.0) or verapamil (1.5 g/dl in distilled water) and either 1% or 5% PEG 400 were separately deposited within the nasal cavities of anesthetized rats as described under 3.3. After a 3 h period, the rats were sacrificed by decapitation, and the lower jaws, brains and excess of soft tissues were removed to free the roof of the oral cavities and adjacent nasal cavities, which were then excised, flushed with 10% buffered formalin, and fixed by immersion in 10% formalin. The nasal cavities were next decalcified by the formic acid-sodium citrate method of Luna [33], and prepared for light microscopic histopathologic examination using the four-section approach of Young [34], with the anterior face being cut first to a thickness of 6 μm. Staining of the tissues in the four sections of nasal cavity was accomplished with a standard hematoxylin and eosin technique. Rats that had received equal volumes of drug free solutions (i.e., physiological saline alone, 0.01 M acetate buffer pH 5.0 alone, distilled water, 1% or 5% PEG 400 in 0.01 M acetate buffer pH 5.0 alone, and 1% or 5% PEG 400 in distilled water) served as the control groups.

3.10. Data analysis

All values are reported as the mean ± SEM for groups of 5 rats each. Pharmacokinetic parameters (i.e. $t_{1/2}$: biological half-life of the drug; t_{max}: time to reach maximum plasma drug concentration; C_{max}: maximum plasma drug concentration; k_a: apparent first-order absorption rate constant, k_e: apparent first-order elimination rate constant; $AUC^{0-\infty}$: area under the drug plasma concentration versus time curve extrapolated to infinity; and F: absolute bioavailability) were calculated using a commercially available computer program (WinNolin, Scientific Consulting, Inc., Apex, NC) and the following equation for the plasma concentration at any time after intranasal administration of the dose of a drug that undergoes first-order absorption [35]:

$$C_p = \frac{Fk_a X_{IN}^0}{V(k_a - k)}(e^{-kt} - e^{-kat})$$

where C_p: plasma drug concentration; X_{IN}^0: the initial drug dose applied to the intranasal site at time zero; k: the overall elimination rate constant; t: any specified time following drug administration; and V: the apparent volume of distribution. A one-compartment pharmacokinetic model was used to analyze the plasma drug concentration-time profile for each rat. Statistical evaluation of intergroup differences was performed using Student's t-test and one-way analysis of variance (ANOVA) with posthoc Student-Neumann-Keuls test. Criterion for significance was $p \leq 0.05$.

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References

1 AHFS Drug Information 91, American Society of Hospital Pharmacists, Inc., pp. 945, 946, Bethesda, MD, 1991
2 Lacy, C.; Armstrong, L. L.; Ingrim, N.; Lance, L. L.: Drug Information Handbook, 4th ed., pp. 849, 850, 1228–1230, Lexi-Comp., Inc., Hudson, OH, 1996
3 Physicians' Desk Reference, 49th ed., pp. 1232, 1233, 2467, Medical Economics Data Production Co., Montvale, NJ, 1995
4 Higuchi, S.; Shiobara, Y.: Xenobiotica **10**, 447 (1980)
5 Graham, D. J. M.; Dow, R. J.; Hall, D. J.; Alexander, O. F.; Mroszczak, E. J.; Freedman, D.: Br. J. Clin. Pharmacol. **20**, 23S (1985)
6 Schomerus, M.; Spiegelhalder, B.; Stieren, B.; Eichelbaum, M.: Cardiovasc. Res. **10**, 605 (1976)
7 Johnston, A.; Burgess, C. D.; Hammer, J. H.: Br. J. Clin. Pharmacol. **12**. 397 (1981)
8 Kates, R.; Keefe, D. L. D.; Schwartz, J.; Harapat, S.; Kirsten, E. B.; Harrison, D. C.: Clin. Pharmacol. Ther. **30**, 44 (1981)
9 Berk, S. I.; Beckman, K.; Hoon, T. J.; Hariman, R. J.; Hu, D.; Siegel, F. P.; Bauman, J. L.: Pharmacotherapy **12**, 33 (1992)
10 John, D. N.; Fort, S.; Lewis, M. J.; Luscombe, D. K.: Br. J. Clin. Pharmacol. **33**, 623 (1992)
11 Asthana, O. P.; Woodcock, B. G.; Wenchel, M.; Fromming, K.-H.; Schwabe, L.; Rietbrock, N.: Arzneim.-Forsch./Drug Res. **34**, 498 (1984)
12 Diez, I.; Colom, H.; Moreno, I.; Obach, R.; Peraire, C.; Domenech, J.: J. Pharm. Sci. **80**, 931 (1991)
13 Visor, G. C.; Bajka, E.; Benjamin, E.: J. Pharm. Sci. **75**, 44 (1986)
14 Visor, G. C.; Schuessler, B.; Thompson, J.; Ling, T.: Drug Dev. Ind. Pharm. **13**, 1329 (1987)
15 Arnold, T. H.; Tackett, R. L.; Vallner, J. J.: Biopharm. Drug Dispos. **6**, 447 (1985)
16 Yazan, Y.; Ozer, A. Y.; Erol, K.: Drug Dev. Ind. Pharm. **22**, 181 (1996)
17 Seki, T.; Sugibayashi, K.; Morimoto, Y.: Chem. Pharm. Bull. **35**, 3054 (1987)
18 Morimoto, Y.; Seki, TR.; Sugibayashi, K.; Juni, K.; Miyazaki, S.: Chem. Pharm. Bull. **36**, 2633 (1998)
19 Duchene, D.; Ponchel, G.: Drug Dev. Ind. Pharm. **19**, 101 (1993)
20 Hussain, A. A.; Hirai, S.; Bawarshi, R.: J. Pharm. Sci. **68**, 1196 (1979)
21 Harris, A. S.; Svensson, E.; Wagner, Z. G.; Lethagen, S.; Nilsson, I. M.: J. Pharm. Sci. **77**, 405 (1988)
22 Hussain, A. A.; Hirai, S.; Bawarshi, R.: J. Pharm. Sci. **69**, 1411 (1980)
23 Riad, L. E.; Sawchuk, R. J.: Pharm. Res. **8**, 491 (1991)
24 Sarpotdar, P. P.; Gaskill, J. L.; Giannini, R. P.: J. Pharm. Sci. **75**, 26 (1986)
25 Hatanaka, T.; Shimoyama, M.; Sugibayashi, K.; Morimoto, Y.: J. Control. Rel. **23**, 247 (1993)
26 Kaneo, Y.: Acta Pharm. Suec. **20**, 379 (1983)
27 Hayashi, M.; Hirasawa, T.; Muraoka, T.; Shiga, M.; Awazu, S.: Chem. Pharm. Bull. **33**, 2149 (1985)
28 Hirai, S.; Yashiki, T.; Mima, M.: Int. J. Pharm. **9**, 173 (1981)
29 Thadikonda, K. P.; Lau-Cam, C. A.; Thadikonda, V. L.; Theofanopoulos, V.: Drug Dev. Ind. Pharm. **21**, 461 (1995)
30 Bar-Or, D.; Kulig, K.; Marx, J. A.; Rosen, P.: Ann. Intern. Med. **97**, 619 (1982)
31 Fu, R. C.-C.; Whatley, J. L.; Fleitman, J. S.: Pharm. Res. **8**, 134 (1991)
32 Ohwaki, T.; Ando, H.; Watanabe, S.; Miyake, Y.: J. Pharm. Sci. **74**, 550 (1985)
33 Luna, L. G. (ed.): Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, 3rd ed., p. 8, McGraw-Hill Book Co., New York, 1968
34 Young, J. T.: Fund. Appl. Toxicol. **1**, 309 (1981)
35 Colaizzi, J. L., in: Chien, Y. W. (ed.), Transnasal Systemic Medication, p. 112. Elsevier Science Publishers, Amsterdam, 1985

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