Effects of Concentration and Volume on Nasal Bioavailability and Biological Response to Desmopressin

A. S. HARRIS*x, M. OHLIN*, S. LETHAGEN#, AND I. M. NILSSON#

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Abstract □ The effects of concentration and dose volume on the nasal bioavailability and biological response to desmopressin [DDAVP; 1-(3-mercaptoproprionic acid)-8-D-arginine vasopressin] were investigated in humans. A nasal formulation of 300 μg of desmopressin was administered using a premetered spray device in doses of either 1 × 50-, 2 × 50-, or 1 × 100-μL actuations to both nostrils. Intravenous administration of 0.2 μg/kg was also given as a reference for bioavailability calculations. Plasma levels of desmopressin were measured by radioimmunoassay. The biological response was determined by measuring circulating levels of Factor VIII (F VIII), the antihemophilia factor. Peak plasma levels of desmopressin were greatest after the 2 × 50-μL dose, followed by the 1 × 50- and 1 × 100-μL doses. The bioavailability of desmopressin from the 2 × 50-μL dose was 20%, which was significantly greater than the 11% after the 1 × 50-μL ($p < 0.01$) and 9% after the 1 × 100-μL ($p < 0.001$) doses. The biological response was clearly enhanced after the 2 × 50-μL dose compared with the 1 × 50- and 1 × 100-μL doses. The interindividual response in F VIII levels to nasal desmopressin ranged from 20 (CV) to 30%, which compared favorably with the 36% variation after intravenous administration. This study confirms the premetered spray device as the preferred intranasal drug delivery system, and shows that by optimizing concentration, volume, and technique of administration, a significant enhancement can be obtained in bioavailability and clinical efficacy.

For its ability to deposit well-controlled doses in the nasal cavity which remain there sufficiently long to effect absorption to occur, the premetered spray device is clearly the preferred intranasal drug delivery system.[1] It has been demonstrated in a previous, comparative study that the relative bioavailability and biological response to intranasal desmopressin [DDAVP; 1-(3-mercaptoproprionic acid)-8-D-arginine vasopressin] can be improved by administering the peptide by a nasal spray pump device rather than by nasal drops.[2] The result of this previous study also indicated that bioavailability was a function of the volume and concentration of the nasal spray. For example, we found that a 2 × 50-μL dose gave a biological response superior to that of a 2 × 100-μL dose. However, this study was performed by administering the dose to the same nostril and, therefore, only utilized 50% of the surface area of the nasal mucosa available for absorption.

Although the intranasal spray device may provide a convenient means of administration, few controlled studies have been performed on the selection of the technique for optimum intranasal drug delivery. Against this background, we identified a need for further characterizing the effect of volume and concentration changes of the intranasally applied drug substance on bioavailability and biological response. As it is well characterized, and there exists a reproducible and specific radioimmunoassay and bioassay for the determination of absorption and biological response, respectively, we chose the peptide desmopressin as a model drug. This investigation was part of an aim to improve and optimize the intranasal delivery of peptides.

Experimental Section

Materials—Desmopressin [DDAVP; 1-(3-mercaptopropionic acid)-8-D-arginine vasopressin; Minirin, lot no. 85K24] was obtained from Ferring Pharmaceuticals, Malmö, Sweden. All reagents were analytical grade.

Drug Formulations—The desmopressin (DDAVP) nasal solutions were prepared under aseptic conditions. Nasal formulations were prepared containing either 1.5 or 3.0 mg/mL of desmopressin in 0.9% NaCl (w/v) and 0.5% chlorobutanol (w/v). Desmopressin was also supplied in ampules for intravenous administration at a concentration of 4 μg/mL.

Nasal sprays were supplied as precompression, metered-dose spray pumps (Pfeiffer GmbH, Radolfzell, F.R.G.). Two pumps were tested: one gave a volume of 100 μL, or 150 μg of desmopressin; the other gave a volume of 50 μL, containing either 75 or 150 μg of desmopressin per actuation.

Administration of Desmopressin—Ten healthy male volunteers (aged 24–36 years) were given 300 μg of desmopressin by nasal spray on three occasions and intravenous desmopressin once; drug administrations were separated by an interval of at least one week. None of the subjects had nasal problems and all were free from colds. Each subject received all four treatments which were allocated in a blind, randomized sequence using coded, sealed envelopes. In this way, a total of 40 administrations were made. The study was approved by the hospital ethical committee and radioisotope committee, and each subject gave informed consent prior to entry into the study.

Intravenous desmopressin was administered at a dose of 0.2 μg/kg, diluted in physiological saline to a volume of 10 mL, and injected slowly over a period of 10 min. All nasal solutions were administered with the subjects sitting in an upright position. A standard dose of 300 μg of desmopressin was self-administered in every case in the following way: 1 × 50, 2 × 50, or 1 × 100 μL of spray into each nostril. Prior to administration, each spray device was primed by activating the pump five times. The applicator tip was introduced 5 to 10 mm into the nostril, and each dose was dispensed during normal inhalation, with the contralateral nostril open. Throughout the study, and at each monitoring period, the subjects continued to breath normally but did not blow their noses or sneeze. Food and drink were withheld for the first hour and thereafter were allowed ad libitum.

Blood Collection—Blood samples were collected by venipuncture (21-gauge needles) before and at times 5 and 10 min (iv only), 15, 30, 45, 60, and 90 min, and 2, 4, 6, and 8 h after administration. Blood was collected in a 3.8% trisodium citrate solution (9:1). Platelet-poor plasma was obtained after centrifugation at 2000 × *g* for 10 min at 4 °C, and stored frozen at −70 °C until assayed.

Assay Methods—Plasma desmopressin was assayed using a sensitive and specific radioimmunoassay (RIA). Antiserum to desmopressin was developed as described by Sofroniew et al.[3] Since desmopressin lacks the *N*-terminal amino group it was necessary to use 8-D-arginine-vasopressin (8-D-AVP), which was coupled to thyroglobulin.[4] The monoiodinated derivate of desmopressin was prepared by the chloramine T method.[5] Briefly, 5 μmol of desmopressin (0.25 mg/mL in 0.05 M sodium phosphate buffer, pH 7.5) was added to 1 mCi of [^{125}I]Na (IMS 30, Amersham) and incubated for 60 s. The monoiodinated tracer was immediately isolated by reversed-phase HPLC (27% acetonitrile in 0.1% trifluoroacetic acid), giving a specific activity of at least 1600 mCi/μmol. The RIA contained 100 μL of standard (5.0–640 pg/mL) or sample diluted five times with assay buffer, 100 μL of tracer (~5000 cpm), and 200 μL of antiserum (1:100,000). A 100-μL

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aliquot of normal human serum diluted fivefold was added to the standard and 100 μL of assay buffer was added to the sample tubes. Incubations were carried out for 48 h at 4 °C, followed by separation of bound and free radioactivity by the addition of 1 mL of plasma-coated charcoal. The assay diluent was 0.05 M sodium phosphate buffer, pH 7.5, containing 0.01% sodium acetate and 0.1% human serum albumin. The F VIII coagulant activity (VIII:C) was measured with a chromogenic substrate method (Coatest Factor VIII, KabiVitrum) as described elsewhere.[6] Pooled citrated plasma from 20 normal individuals, which was calibrated against the 13th British Standard Plasma for Factor VIII (85/573), was used as the standard.

Statistical Methods—Unless specified, all results are expressed as mean ± SD. Friedman's two-way analysis of variance by ranks and the Wilcoxon matched-pairs signed rank test were used where appropriate. In addition, t_{max}, C_{max}, and $t_{1/2}$ were calculated for plasma profiles of desmopressin after each method of administration. The AUC was determined, using the logarithmic trapezoidal rule, from the plasma desmopressin concentration versus time curve. The rate of entry of desmopressin to the systemic circulation after intranasal administration was obtained by numerical deconvolution of values using the least squares method.

Results and Discussion

Pharmacokinetics—The plasma profiles of desmopressin after intravenous and intranasal administration are shown in Figure 1. The results show the effect of volume and concentration of the desmopressin nasal spray on plasma levels, with a clear distinction between 1 × 50-, 2 × 50-, and 1 × 100-μL doses. Peak plasma levels were significantly greater after the 2 × 50-μL dose than after the 1 × 50- ($p < 0.05$) and 1 × 100-μL ($p < 0.01$) doses. The pharmacokinetic data (Table I) show a significantly greater AUC after the 2 × 50-μL dose compared with the 1 × 50- ($p < 0.05$) and 1 × 100-μL ($p < 0.01$) doses. The bioavailability of intranasal desmopressin from the 2 × 50-μL dose showed a twofold improvement compared with the 1 × 50- and 1 × 100-μL spray doses. This finding suggests that an optimal dosage is obtained by delivering drug twice into each nostril. Indeed, in an earlier study by Mygind and Vesterhange[7] using a pressurized aerosol system, a better distribution over the nasal mucosa was found when drug was dispensed twice into each nostril compared with single actuations. In our previous study[2] we gave 300 μg of desmopressin intranasally either as 2 × 50- or 2 × 10-μL spray doses into one nostril only. We found AUC values of 3675 ± 2098 and 3556 ± 1848 μg/mL·h, respectively (NS). These values are significantly lower than the AUC value of 5264 ± 2167 μg/mL·h for the 2 × 50-μL dose we found in this study when the drug was administered to both nostrils. Thus, these results indicate the value of delivering drug in divided doses to each nostril. Moreover, the interindividual variation in nasal absorption of the 2 × 50-μL doses was much less than for 1 × 50- and 1 × 100-μL doses. Variation in AUC (CV) was 41% for the 2 × 50-μL doses compared with 81 and 70% for the 1 × 50- and 1 × 100-μL doses, respectively. The corresponding value after intravenous administration was 39% and was not significantly different from the 2 × 50-μL intranasal dose. These results reinforce the value of delivery by double-pump actuation. Figure 2 shows the relative amount of desmopressin remaining to be absorbed as a function of time. This log plot demonstrates that absorption of desmopressin by the nasal route follows first-order kinetics.

No difference was observed between intranasal and intravenous administration in plasma half-life, which ranged between 2.4 and 2.6 h. Time to maximum plasma concentration (t_{max}) for the smaller volume (1 × 50 μL) was significantly faster than for the larger volume doses of 2 × 50 ($p < 0.01$) and 1 × 100 μL ($p < 0.001$). This finding that time-to-peak plasma levels is inversely proportional to dose volume supports the claim by others[8] that systemic clearance of intranasally administered peptides via the nasal cavity may be rate limited by absorption from the nasal mucosa. Figure 3 shows the absorption plots relative to intravenous administration after numerical deconvolution. These data underline

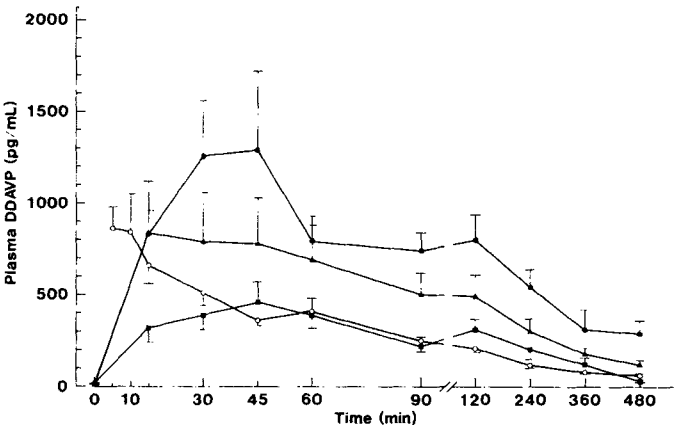

Figure 1—*Plasma levels of desmopressin following intravenous administration of 0.2 μg/kg (○——○) and nasal administration of 300 μg of desmopressin in 1 × 50-μL spray (▲——▲), 2 × 50-μL spray (●——●), and 1 × 100-μL spray (■——■) doses each to 10 volunteers (mean ± SEM).*

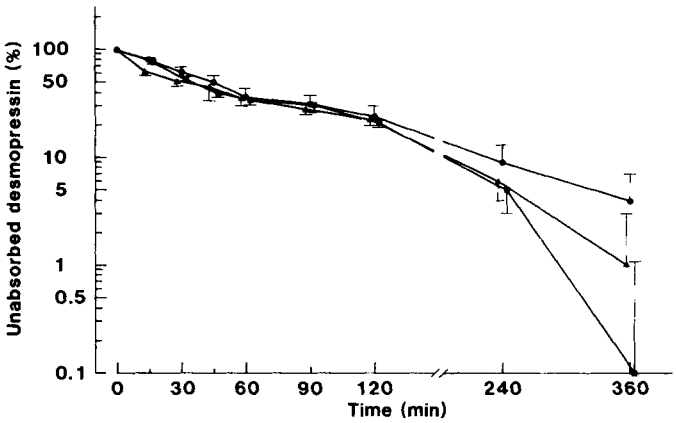

Figure 2—*Plot of desmopressin remaining to be absorbed against time following nasal administration of 300 μg of desmopressin in 1 × 50-μL spray (▲——▲), 2 × 50-μL spray (●——●), and 1 × 100-μL spray (■——■) doses each to 10 volunteers (mean ± SEM).*

Table I—Pharmacokinetic Data after Nasal (300 μg) and Intravenous (0.2 μg/kg) Administration of Desmopressin in Healthy Volunteers[a]

Route	$AUC_{0-\infty}$, pg/mL·h	C_{max}, pg/mL	t_{max}, min	$t_{1/2}$, h	Bioavailability, %
Intravenous	1898 ± 736	—	—	2.4 ± 0.5	—
Spray (1 × 50 μL)	3247 ± 2640	922 ± 874	29 ± 13	2.5 ± 0.5	11 ± 6
Spray (2 × 50 μL)	5264 ± 2167	1796 ± 1406	42 ± 15	2.4 ± 0.5	20 ± 8
Spray (1 × 100 μL)	1934 ± 1350	477 ± 327	54 ± 25	2.6 ± 0.6	9 ± 6

[a] Results are expressed as mean ± SD (n = 10); mean body weight: 76 ± 8 kg.

the speed of intranasal absorption which was clearly faster for the smaller volume (1 × 50 μL). This finding is consistent with a model in which there is competition between rate of entry into the systemic circulation and removal from the site of absorption by mucociliary clearance. In our earlier study of the relationship between nasal clearance and absorption, we found that desmopressin in a larger volume spray or drop solution was cleared faster and was less well absorbed than when given in a smaller volume.[2] These observations raise the possibility that mucus may be a major limiting barrier for nasal uptake of drugs. Work with systems which prolong the time of contact between peptide and the absorptive mucosa may well be worth pursuing as a means of enhancing bioavailability.[9] Our results also indicate that intranasal absorption is more rapid than the oral and subcutaneous routes of administration.[10,11] This is also true for other peptides such as insulin.[12] Indeed, Moses et al.[13] found that in a volume of 75 μL, using an aerosol delivery of intranasal insulin, that peak plasma levels of insulin occurred at 10 to 12 min, with a subsequent nadir in blood glucose at 30 min.

Biological Response—The effect of intranasal and intravenous administration of desmopressin on F VIII:C is shown in Figure 4. The effect of concentration and volume on the biological response to nasal desmopressin produced a similar pattern to the plasma profile. In this way, the 2 × 50-μL dose elicited a significantly greater peak response in F VIII:C (390 ± 78%) than the response after doses of either 1 × 50 μL (309 ± 92%; $p < 0.05$) or 1 × 100 μL (280 ± 70%; $p < 0.01$). Time to maximum activity, again, followed the same pattern as that for plasma desmopressin levels, with an earlier peak at 45 min for the 1 × 50-μL dose in contrast to 60 ($p < 0.05$) and 120 min ($p < 0.001$) for the 2 × 50- and 1 × 100-μL doses, respectively.

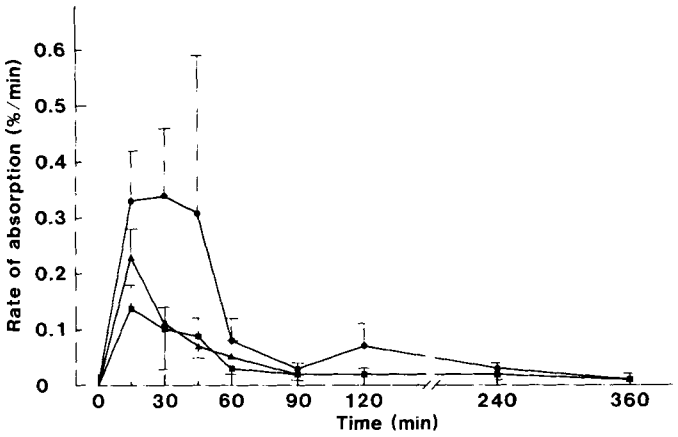

Figure 3—*Deconvolution plot of rate of entry of desmopressin to the systemic circulation relative to intravenous administration after nasal application of 300 μg of desmopressin in 1 × 50-μL spray (▲——▲), 2 × 50-μL spray (●——●), and 1 × 100-μL spray (■——■) doses each to 10 volunteers (mean ± SEM).*

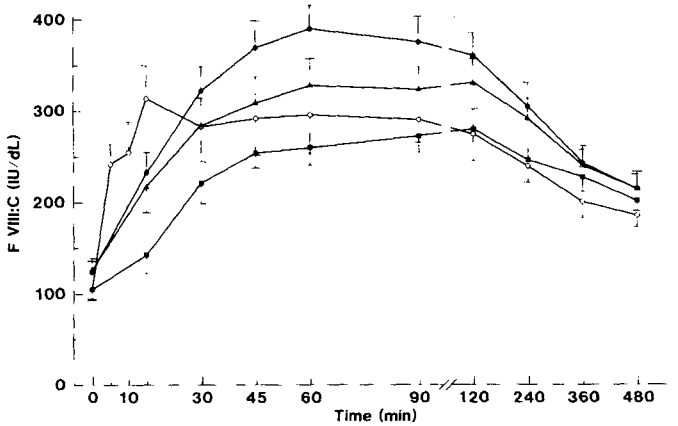

Figure 4—*The effect of intravenous and intranasal desmopressin on F VIII:C levels. Intravenous administration of 0.2 μg/kg (○——○) and nasal administration of 300 μg of desmopressin in 1 × 50-μL spray (▲——▲), 2 × 50-μL spray (●——●), and 1 × 100-μL spray (■——■) doses each to 10 volunteers (mean ± SEM).*

The interindividual response in F VIII:C to nasal desmopressin was remarkably good considering the relatively low bioavailability by this route. Variation in maximum response ranged from 20% (CV) for the 2 × 50-μL dose to 30% for the 1 × 50-μL dose, and compared favorably with the 36% variation after intravenous administration.

These advances in the enhancement of bioavailability, the speed with which maximum activity is attained, and the augmentation of the magnitude of the biological response to nasal desmopressin offer considerable advantages to its therapeutic use. As desmopressin is used today a priori as an important alternative to blood products in the management of bleeding disorders, such as hemophilia A and von Willebrand's disease,[14] it is essential to offer a convenient, noninvasive, and reliable route of administration. Nasal administration by spray has been demonstrated in this study to provide a method of delivery which is equivalent to intravenous administration in both magnitude and reproducibility of the biological response. Indeed, in a recent comparative study, intranasal desmopressin spray was found to be of equal efficacy to intravenous administration in reducing bleeding times in 23 patients with mild to moderate von Willebrand's disease type I.[15] The tolerance to intranasal administration in this study was good. No nasal irritation was reported by any subject. Mild, transient facial flushing, which is associated with the weak vasodilatory action of desmopressin, was observed in 4 of 10 subjects after both intranasal and intravenous administration.

In conclusion, this study shows that by adjusting the volume, concentration, or dosing technique of intranasal desmopressin spray, a significant enhancement can be obtained in its bioavailability, with clinically important improvements in the onset of action and magnitude of the biological response.

References and Notes

1. Su, K. *Pharmacy International* **1986**, *7*, 8–12.
2. Harris, A. S.; Nilsson, I. M.; Wagner, Z. G.-; Alkner, U. *J. Pharm. Sci.* **1986**, *75*, 1085–1088.
3. Sofroniew, M. W.; Madler, M.; Müller, O. H.; Scriba, P. C.; *Fresenius' Z. Anal. Chem.* **1978**, *290*, 163.
4. Lundin, S.; Melin, P.; Vilhardt, H. *Acta Endocrinol. (Copenhagen)* **1985**, *108*, 179–183.
5. Greenwood, F. C.; Hunter, W. M.; Glover, J. S. *Biochem. J.* **1963**, *89*, 114–123.
6. Lethagen, S.; Östergaard, H.; Nilsson, I. M. *Scand. J. Haematol.* **1986**, *37*, 448–453.
7. Mygind, M.; Vesterhange, S. *Rhinology* **1978**, *11*, 79–88.
8. Visor, G. C.; Bajka, E.; Benjamin, E. *J. Pharm. Sci.* **1986**, *75*, 44–46.
9. Illum, L. *Arch. Pharm. Chem.* **1987**, *94*, 127–135.
10. Fjellestad, A.; Tubiana, N.; Harris, A.; Czernichow, P. *Acta Endocrinol. (Copenhagen)* **1987**, *115*, 307–312.
11. Köhler, M.; Hellstern, P.; Miyashita, C.; von Blohn, G.; Wenzel, E. *Thromb. Haemostasis* **1986**, *55(1)*, 108–111.
12. Salzman, R.; Manson, J. E.; Griffing, G. T.; Kimmerle, R.; Ruderman, N.; McCall, A.; Stoltz, E. I.; Mullin, C.; Small, D.; Armstrong, J.; Melby, J. C. *N. Eng. J. Med.* **1985**, *312*, 1078–1084.
13. Moses, A. C.; Gordon, G. S.; Carey, M. C.; Flier, J. S. *Diabetes* **1983**, *32*, 1040–1047.
14. Mannucci, P. M. In *Progress in Hemostasis and Thrombosis*; Coller, B. S., Ed.; Grune & Stratton: Orlando, FL, 1986; Vol. 8, pp 19–45.
15. Lethagen, S.; Harris, A. S.; Nilsson, I. M. *Proceedings of the XIth International Congress on Thrombosis and Haemostasis*, Brussels, 1987.