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Intranasal absorption of different aqueous formulations of angiopeptin: in vivo bioavailability study

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Abstract

The bioavailability of angiopeptin in rabbits after intranasal administration of three aqueous formulations has been studied. In each case a total amount of 750 μg angiopeptin was administered. A simple aqueous solution and an aqueous solution supplemented with 5% glycofurol 75 (GF) resulted in bioavailabilities of 133%, whereas the bioavailability of angiopeptin was 53%, when 1% sodium glycocholate (GC) was added to the aqueous solution. The observed reduction in bioavailability from this formulation may be due to precipitation of angiopeptin in the nasal mucus layer, as the formulation containing GC tended to precipitate. The main indication of angiopeptin is inhibition of restenose of cononary arteries after angioplasty or heart transplantation. The pharmacokinetics of angiopeptin could be described by a two-compartment model, and the plasma half-life was about 1.5 h. Addition of GF and GC resulted in faster absorption, the t_{max} being 16 and 14 min, respectively, as compared with 31 min for the simple aqueous solution. The faster absorption with GF and GC is not considered of therapeutic importance, but rapid absorption may give rise to more reproducible dosing in the clinical situation. C_{max} was about 2.8 ng/ml for all three formulations.

Keywords: Bioavailability, nasal; Angiopeptin; Peptide; Sodium glycocholate; Glycofurol 75; Rabbit

1. Introduction

Currently, most peptides are still administered parenterally, however, a few smaller peptides are administered via alternative routes, e.g., vasopressin, desmopressin, oxytocin, and buserelin, which are administered intranasally. Using the nasal route, it is possible to avoid some of the disadvantages associated with enteral administration of peptides, as the nasal mucosa seems to have less proteolytic activity than the gastrointestinal tract (Zhou and Li Wan Po, 1990), first-pass hepatic metabolism is avoided and absorption into the systemic circulation can occur rapidly.

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Drugs with a molecular mass less than about 1000 Da have shown acceptable nasal bioavailabilities, when administered without absorption enhancers (McMartin et al., 1987). The molecular mass limit can be extended when an absorption enhancer is coadministered, i.e., peptides consisting of 10 or less amino acids are expected to have a potential for nasal administration.

The nasal permeability and stability of angiopeptin, a synthetic octapeptide (molecular mass 1156 Da), have been studied in vitro (Jørgensen and Bechgaard, 1993). The peptide was remarkably stable against rabbit nasal enzymes and human nasal wash, and showed a reasonable apparent permeability coefficient ($P_{app} = 9.1 \times 10^{-7}$ cm/s). It was concluded that angiopeptin, due to the above-mentioned results relative to the therapeutic treatment regiment, was a potential candidate for intranasal administration.

The objective of this study has been to evaluate the nasal bioavailability of angiopeptin, when administered from a simple aqueous solution and in combination with 1% sodium glycocholate (GC), which in vitro increased the P_{app} value 2.7-fold (Jørgensen and Bechgaard, 1993), and 5% glycofurol 75 (GF). Coadministration of 5% GF did not increase the P_{app} value in vitro, but has shown promising results when administered nasally together with insulin (Bechgaard et al., 1991). Studies in the Ussing chamber (Bechgaard et al., 1993) have indicated that the in vitro method may not always be able to identify absorption enhancers. GF is normally used as a cosolvent for parenteral solutions of lipophilic drugs, and knowledge about the local nasal toxicity of GF is limited. A mixture of tetraethylene glycol and 5% GF has been tested in healthy volunteers and found to be acceptable for administration of essential biologically active substances to be used occasionally. In addition, the local toxicity of 30% GF in PEG-200 has been studied in rabbits. It was concluded that only mild reversible toxicological effects occurred (Bechgaard et al., 1991). On the basis of the above-mentioned observations, the formulation of angiopeptin and 5% GF was also chosen to be examined in this study.

2. Materials and methods

2.1. Chemicals

Angiopeptin was kindly provided by the Henri Beaufour Institute U.S.A., Inc. Sodium glycocholate approx. 99% was purchased from Sigma Chemicals (St. Louis, MO, U.S.A.) and glycofurol 75 was obtained from Roche (Basle, Switzerland). Sodium chloride solution 0.9%, sterile, was obtained from Nycomed DAK, Denmark. Distilled water was used for all nasal formulations.

2.2. Preparations

All preparations were made by dilution of a freshly prepared stock solution of angiopeptin in distilled water (15 mg/ml). The i.v. preparation was diluted (1 + 19) with isotonic sodium chloride to a final concentration of 750 μg/ml, whereas the i.n. preparations were diluted (1 + 1) with distilled water, distilled water containing GC, or distilled water containing GF to a final concentration of 7.5 mg/ml. The final enhancer concentrations were 1% w/v GC and 5% v/v GF. The preparation containing GC was heated for approx. 1.5 h at 37°C (water bath) to ensure dissolution.

2.3. In vivo study

New Zealand White rabbits, four of each sex, obtained from Hvidesten (Allerød, Denmark) with a mean weight of about 2.4 kg on arrival, were used in a cross-over design with a wash-out period of at least 5 days. 1 ml of the angiopeptin solution was administered intravenously in the marginal ear vein over a period of 30 s. All nasal preparations were administered with the rabbit in a supine position, the rabbit being kept in this position for 1 min after administration. Nasal solution (50 μl) was administered with an Eppendorf® Multipipette into each nostril.

Blood samples of 1.7 ml withdrawn from the marginal ear vein were collected in lithium-heparin-coated centrifuge tubes just before administration and 2, 5, 10, 20, 30, 45, 60, 120, 180,

240, 300 and 360 min after administration of angiopeptin. Plasma was obtained after centrifugation at 5000 $\times g$ and 4°C for 10 min, and stored at −20°C until analysis.

2.4. Analysis

The plasma concentration of angiopeptin was determined by radioimmunoassay (RIA) at Georgetown University Medical Center. Concentrations between 100 and 2500 pg/ml were determined from a calibration curve, whereas lower concentrations were evaluated by extrapolation of the calibration curve.

2.5. Calculation

The area under the curve (AUC) was calculated using the trapezoidal rule (the plasma concentrations being corrected with respect to body weight). The AUC from 0 to 2 min for i.v. administration was determined by extrapolation of the zero value by using linear regression analysis on the concentrations at 2, 5 and 10 min. On average $AUC_{0-2\ min}$ accounted for 1% of the $AUC_{0-360\ min}$ (range 0.6–1.7%).

Plasma concentrations were corrected for differences in body weight during the test period by a factor f:

$$f = W/W_{mean}$$

where W is the body weight of the individual rabbit and W_{mean} denotes the average body weight of the rabbits.

The plasma half-life of angiopeptin is estimated from the mean concentrations by use of the following equations:

$$k_e = \frac{A + B}{\frac{A}{\alpha} + \frac{B}{\beta}}$$

and

$$t_{1/2} = (\ln 2)/k_e$$

where k_e is the rate constant for elinimantion, and $t_{1/2}$ represents the plasma half-life. A, B, α and β are determined from a semilogarithmic plot of plasma concentration vs time. Plotting the deviation of the early points from the regression line on the same coordinates reveals the fast phase (α-phase; 0–45 min). α and β denote the rate constants (slopes) of the α- and β-phase (1–6 h), respectively, and A and B are the point of intersection with the y-axis of the α- and β-phase, respectively.

2.6. Statistical analyses

Results are expressed as the mean ± standard deviation (S.D.). The results from rabbit I have been excluded from the calculations as the $AUC_{0-360\ min}$ differs from the mean by more than double the S.D.

Statistical analyses were performed using Student's t-test for paired data.

3. Results and discussion

As seen from Fig. 1, the pharmacokinetics of angiopeptin can be described by a two-compartment model. The plasma half-life has been estimated as 1.5 h from the mean concentrations after i.v. administration. The observed pharmacokinetics are consistent with previous determinations made in rats, dogs and humans (Internal report (1989) from Ipsen Biotech, France).

The intervariation in plasma concentration after i.v. administration is very small when rabbit no. I is excluded. Since the $AUC_{0-360\ min}$ for rabbit no. I after i.v. administration was only about 25% of the mean $AUC_{0-360\ min}$ (Table 1), the results (both i.v. and i.n.) from this rabbit were excluded from all other calculations. The excessively low i.v. result may be due not only to biological variation but also faulty administration.

As seen from Fig. 1b and Table 2 the bioavailabilities of the simple aqueous nasal solution and the aqueous solution containing 5% GF are about 130%. There is even a statistically significant difference at the 5% significance level when comparing the $AUC_{0-360\ min}$ of the nasal formulations with that after i.v. administration. The observed bioavailabilities are remarkable relative to other results on peptides administered nasally. Based on data from different references, Su (1991) has

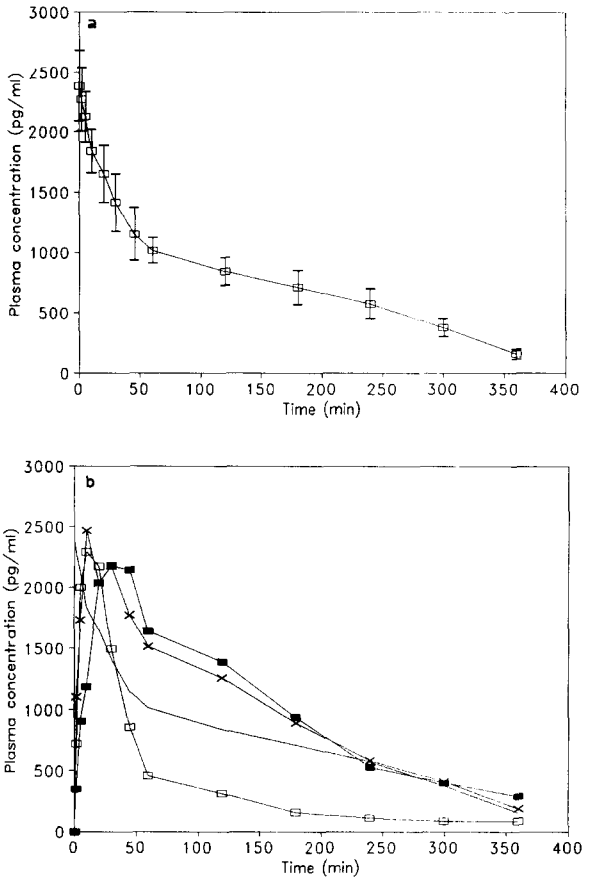

Fig. 1. Mean plasma concentration-time profiles of angiopeptin after (a) intravenous administration and (b) intranasal administration in the presence of various enhancers: (■) control, (×) 5% glycofurol 75 and (□) 1% sodium glycocholate. The presence of S.D. in (b) seems confusing, having been omitted for that reason, and the i.v. curve is represented in (b) by the line without symbols ($n = 7$).

determined the relative absorption of nasally administered peptides to be between 2 and 90% with an average of about 30%; none of the results were from rabbits but from rats, dogs, monkeys and humans. Drugs of molecular mass comparable to that of angiopeptin (1156 Da), e.g., oxytocin, vasopressin analogs and LHRH agonists and antagonists, have relative absorptions between 2 and 40%, i.e., much lower than the observed bioavailability of angiopeptin. One explanation for the high bioavailability of angiopeptin could be the considerable stability towards peptidases prior to absorption. In vitro studies have shown that angiopeptin is completely stable against enzymatic degradation in rabbit nasal tissue homogenate and only negligible degradation, probably hydrolysis, is observed (Jørgensen and Bechgaard, 1993). In addition, the lipophilicity of angiopeptin is similar to that of steroids, which may be favourable for the absorption process.

Table 1
Area under the curve (AUC) from 0 to 360 min for the individual rabbits after i.v. administration and i.n. administration of an aqueous solution (i.n.) and an aqueous solution containg 1% sodium glycocholate (GC) or 5% glycofurol 75 (GF)

Rabbit	$AUC_{0-360\,min}$ (ng min ml^{-1})			
	i.v.	i.n.	+1% GC	+5% GF
I	61.6	318.8	76.7	155.9
II	224.0	341.4	115.4	471.1
III	265.6	432.6	207.8	450.7
IV	268.9	319.7	190.9	371.9
V	286.9	431.7	90.0	308.2
VI	307.3	342.6	112.3	259.1
VII	292.0	346.3	168.9	374.2
VIII	276.9	309.5	109.5	250.7
Mean [a]	274.5 [b]	360.5	142.1 [c]	355.1
S.D. [a]	26.5	50.7	46.2	87.1

[a] Results from rabbit I were excluded from the calculations as the i.v. result differed from the mean by more than 2×S.D.
[b] Differs significantly ($p < 0.05$) from the intranasal formulations.
[c] Differs significantly ($p < 0.001$) from the other two intranasal formulations.

Table 2
Peak plasma concentration (C_{max}; weight corrected), time to peak (t_{max}) and bioavailability of intranasal angiopeptin formulations after administration of 750 μg angiopeptin

Formulation (enhancer)	C_{max} (ng/ml)	t_{max} (min)	Bioavailability (%)
–	2.96±0.65	31±10 [a]	133±22
1% sodium glycocholate	2.69±0.64	14± 5	53±18 [b]
5% glycofurol	2.78±0.48	16±10	133±46

Results are expressed as mean±S.D.($n = 7$).
[a] Differs significantly ($p < 0.05$) from formulation II and III.
[b] Differs significantly ($p < 0.001$) from formulation I and III.

Addition of 1% GC to the nasal solution of angiopeptin resulted in a lower bioavailability (about 53%; Table 2) than for the solution without additives. This effect of GC contrasts with observations in the Ussing chamber with angiopeptin (Jørgensen and Bechgaard, 1993), where 1% GC enhanced the apparent permeability coefficient by a factor of 2.7, and the effect of GC on the bioavailability of other drugs (e.g., Duchateau et al., 1986; Chan et al., 1988). This effect of GC on the bioavailability of angiopeptin may be due to, e.g., precipitation of angiopeptin in the mucus layer as heating to 37°C was necessary to dissolve the precipitate formed after mixing the drug solution and the GC solution during preparation.

As seen from Fig. 1b and Table 2, no difference in C_{max} is evident for the three different nasal formulations, whereas addition of 1% GC or 5% GF resulted in statistically significant ($p < 0.05$) faster absorption (t_{max}). This observation indicates that GC and GF have a positive effect with respect to the rate of availability. It also supports the assumption that further studies with optimization of the GC formulation, e.g., lowering of the angiopeptin concentration, may increase the extent of availability. The more rapid absorption observed with GF and GC is not considered to be of therapeutic importance, but may give rise to more reliable dosing in the clinical situation, due to the fast ciliary clearance from the nasal cavity of unabsorbed substance (Illum, 1987).

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