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Intranasal administration of different liquid formulations of bumetanide to rabbits

H.W. Nielsen [a], E. Bechgaard [b,*], B. Twile [c], E. Didriksen [d], H. Sørensen [d]

[a] *Nycomed Danmark, Langebjerg 1, P.O. Box 88, DK-4000 Roskilde, Denmark*
[b] *Royal Danish School of Pharmacy, Department of Pharmaceutics, Universitetsparken 2, DK-2100 Copenhagen Ø, Denmark*
[c] *Statens Seruminstitut, Artillerivej 5, DK-2300 Copenhagen S, Denmark*
[d] *Leo Pharmaceutical Products, Industriparken 55, DK-2750 Ballerup, Denmark*

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Abstract

The bioavailability of bumetanide in rabbits after intranasal administration of eight formulations intended for use in acute situations has been studied. The vehicles tested were combinations of phosphate buffer, pH 7.4, glycofurol 75, polyethylene glycol 200 and coconut oil. A mixture of 5% glycofurol in polyethylene glycol 200 was administered containing doses of 1 and 8 mg bumetanide respectively. For all other formulations the lower dose level only was studied. The t_{max} obtained ranged from 3 to 10 min. The vehicles resulting in the highest rate of absorption were 60% glycofurol in coconut oil and pure glycofurol. The observed bioavailability for the different formulations ranged from 16 to 37% for the time period 0–120 min. The bioavailability was also calculated omitting the initial peak seen after i.v. injection, which may be undesirable. Using this method bioavailabilities of 33–82% for the time interval 5–120 min was found. The study also demonstrated that the total amount of bumetanide absorbed increased proportionally to the dose administered. The rate of absorption of bumetanide from all formulations tested may be relevant for the treatment of acute oedematous states. The t_{max} obtained after intranasal administration was shorter than reported for other non-parenteral routes of administration.

Keywords: Bioavailability; Nasal; Bumetanide; Cosolvent; Rabbit

1. Introduction

Bumetanide is the most potent drug in the group of diuretics known as loop diuretics, which inhibit reabsorption of salt and water primarily in the ascending part of the loop of Henle. Loop diuretics are the most powerful of all diuretics, capable of causing up to 25% of the sodium in the filtrate to be excreted. Bumetanide is used in the treatment of oedema associated with congestive heart failure, hepatic and renal diseases, acute pulmonary congestion and premenstrual syndrome and in forced diuresis during and after surgery. Bumetanide is typically administered orally or intravenously. Bumetanide tablets are

* Corresponding author. Tel.: +45-35306235; fax: +45-35306030.
E-mail address: eb@dfh.dk (E. Bechgaard).

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primarily used in the long-term treatment of oedema when other diuretics have shown inefficient. The onset of diuresis in patients with oedematous states occurs within 10 and 30 min following injection and oral administration respectively (Ward and Heel, 1984). The time to peak plasma concentration (t_{max}) after oral administration to healthy volunteers range from 0.5 to 2.2 h (Ward and Heel, 1984). As an alternative to the existing administration routes the possibilities of administering bumetanide via the rectal route have been evaluated in rabbits and in humans (Yagi et al., 1993a,b). The studies find a t_{max} of 25–50 min, which may be considered relatively slow for use in crisis situations.

A possible way to accomplish rapid absorption relative to peroral and rectal administration is the use of nasal delivery (Hussain et al., 1980; Bechgaard et al., 1997; Gizurarson et al., 1998). The absorption of drugs in the nasal cavity is facilitated by a relatively large surface area, rich vascularisation and, in effect, only a single layer of cells separating the application site from the blood vessels. In acute situations the rapid onset of action can be of vital importance. Additionally, the absorption of drugs from the nasal cavity should be fast, since the mucociliary transit time is about 12–15 min (Marttin et al., 1998). In a number of situations the use of a nasal spray may be preferred to intravenous or rectal administration for practical reasons. Application is easy, does not require patient cooperation and can be performed by untrained personnel.

The solubility of bumetanide in water is very low (0.1 mg/ml) (Tata et al., 1993). It is therefore not possible to administer bumetanide in pure aqueous solutions, since the volume to be instilled into one nostril should not exceed 200 μl (Behl et al., 1998). By use of the excipients polyethylene glycol 200 and glycofurol it is possible to prepare a clinically relevant formulation for intranasal delivery of bumetanide (Nielsen et al., 1999). The aim of this study was to estimate the bioavailability and rate of absorption in rabbits after intranasal delivery of bumetanide in previously developed vehicles with varying polarity.

2. Materials and methods

2.1. Chemicals

Bumetanide micronised and Burinex® injection 0.5 mg/ml was supplied by Leo Pharmaceutical Products (Ballerup, Denmark). Glycofurolum 75 (GF) was obtained from Hoffmann La-Roche (Basel, Switzerland). Coconut oil (CO) was purchased from Nomeco (Copenhagen, Denmark) and polyethylene glycol 200 (PEG 200) was supplied by Hoechst AG (Frankfurt (M), Germany). The 0.007 M phosphate buffer pH 7.4 (PB) was prepared from analytical grade sodium dihydrogenphosphate monohydrate, disodium hydrogenphosphate dihydrate and purified water. Acetonitrile and methanol were of HPLC-grade and purchased from Merck (Darmstadt, Germany) other chemicals for the HPLC assay were of analytical grade.

2.2. In vivo study

New Zealand white rabbits obtained from Hvidesten (Allerød, Denmark) with a weight range of 2500–3262 g were used in the bioavailability study with a wash-out period of at least 7 days. Eight nasal formulations were tested. Formulations I–IV were tested in a cross-over design in four rabbits (one female, three males). Formulations V–IX were tested in four rabbits (selected from a pool of nine rabbits: one female, eight males) randomly allocated to the formulations. The formulations tested are listed in Table 1. The commercially available injectable of bumetanide, Burinex®, was administered intravenously in the marginal ear vein over a period of 30 s (volume equal to 1 mg bumetanide). All other formulations were prepared immediately before use and given intranasally (i.n.) with an Eppendorf® Multipette. Each rabbit was held in a supine position and received 50 μl in each nostril (volume equal to 1 or 8 mg bumetanide). The rabbits were still held in a supine position 1 min after administration. After each application the actual dose received was assessed by visual inspection of the pipette tip and the rabbit nostril. Only administrations estimated to at least 80% were accepted.

In the cross-over study blood samples were drawn just before administration and at 2, 5, 10, 15, 20, 30, 45, 60, 120, 180 and 240 min after administration of the various formulations from a marginal ear vein. In subsequent experiments blood samples at 180 and 240 min were not withdrawn. Samples of 1.5 ml were collected in uncoated polypropylene centrifuge tubes. Serum was obtained after centrifugation at $5000 \times g$ and 4°C for 10 min, and stored at −20°C until analysis.

2.3. Analysis

The serum samples were analysed by HPLC with fluorescence detection using automated solid phase extraction and injection.

Prior to injection into the HPLC system the serum samples (5–250 μl) were loaded onto a cartridge column with C2 packing material from Analytichem International followed by 50 μl of internal standard solution (4-benzyl-3-*n*-butylamino-5-sulfamoyl-benzoic acid 0.2 μg/ml), 25 μl 40% phosphoric acid and 0.5 ml water. The liquid was forced through the cartridge using nitrogen pressure. The cartridge was the washed with 1 ml of water. The cartridges were processed by an AASP™ autosampler coupled to the HPLC equipment. The cartridges were purged with 125 μl of 10% acetonitrile solution in water before elution with mobile phase (valve reset time: 1.0 min). The absolute recovery from this procedure was about 95%.

The Merck-Hitachi HPLC system consisted of a 655A-12 pump fitted with a Shimadzu RF-551 Fluorescence Detector operating at an excitation wave length of 339 nm and an emission wavelength of 434 nm. Signals were recorded by a model D-2500 Chromato-Integrator. Separation at room temperature was carried out using a stainless steel column, 4×125 mm, packed with LiChrospher® RP-18 (5 μm) from Merck. The mobile phase consisted of a mixture of acetonitrile and 0.01 M NaH_2PO_4, pH 2.5 (40:60) at a flow rate of 2.5 ml/min. A mixture of acetonitrile and water (10:90) was used as AASP purge solvent. The purge volume was 250 μl and the AASP reset time was 1.0 min.

Sample concentrations were calculated on the basis of peak response ratio of bumetanide relative to the internal standard, related to corresponding standard curves of bumetanide.

The limit of quantification was 2 ng/ml and the precision about 4%.

2.4. Calculation

The area under the serum concentration–time curve (AUC) was calculated using the trapezoidal rule (the serum 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 and 5 min. On average $AUC_{0-2\,min}$ accounted for 32% of the $AUC_{0-120\,min}$ (range 28–36%)

Table 1
Compositions of bumetanide formulations administered intravenously (formulation IV) or intranasally (formulations I–III and V–IX)

Formulation		Concentration (mg/g)	Vehicle
I	5%GF/PEG/10	10	5% GF in PEG 200
II	GF/PB	10	60% GF in phosphate buffer, pH 7.4
III	GF/CO	10	60% GF in coconut oil
IV	Burinex®	0.5 mg/ml	Burinex® injectable (phosphate buffer and xylitol)
V	30%GF/PEG	10	30% GF in PEG 200
VI	GF	10	GF
VII	PEG	10	PEG 200
VIII	5%GF/PEG/80	80	5% GF in PEG 200
IX	GF/PEG/PB	10	15% phosphate buffer, pH 7.4 in (5% GF in PEG 200)

Table 2
Pharmacokinetic parameters (dose corrected relative to mean i.v. dose) for single intravenous administration (formulation IV) and intranasal administration of bumetanide[a]

Formulation		C_{max} (ng/ml)	t_{max} (min)	AUC $\times 10^{-3}$ (ng min ml^{-1})	
				0–120 min	5–120 min
I	5%GF/PEG/10	118 ± 34	10 (5–15)	5.10 ± 2.11	4.84 ± 2.11
II	GF/PB	304 ± 77[b]	7 (2–15)	10.09 ± 3.20	9.13 ± 3.10
III	GF/CO	345 ± 169	3 (2–5)[c]	8.58 ± 3.25	7.32 ± 2.63
IV	Burinex®	–	–	27.44 ± 2.92	11.07 ± 0.63
V	30%GF/PEG	132 ± 89	8 (2–15)	5.59 ± 2.99	5.28 ± 3.00
VI	GF	170 ± 66	4 (2–5)[d]	5.30 ± 2.13	4.72 ± 1.92
VII	PEG	95 ± 44	11 (5–15)	4.44 ± 1.13	4.25 ± 1.04
VIII	5%GF/PEG/80	139 ± 37	8 (5–15)	4.27 ± 2.03	3.80 ± 1.92
IX	GF/PEG/PB	173 ± 25	10 (5–15)	6.47 ± 0.85	6.13 ± 0.79

[a] The applied dose for formulation VIII was 8 mg, which was also corrected for. For all other formulations the applied dose was 1 mg. C_{max} and area under the curve (AUC) for the time intervals specified are presented as mean ± S.D. Values for t_{max} are shown as mean, and the range in brackets. For formulation I $n = 8$ and for all other formulations $n = 4$.
[b] Differs significantly ($P < 0.05$) from formulations I, V, VII and IX.
[c] Differs significantly ($P < 0.05$) from formulations VII and IX.
[d] Differs significantly ($P < 0.05$) from formulation IX.

In the cross-over study, serum 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. In the experiments where 8 mg bumetanide was given intranasally the AUC was corrected by a factor F:

$$F = \frac{D_{i.v.}}{(D_{i.n.}/8)}$$

where $D_{i.v.}$ is the mean dose per kg given intravenously and $D_{i.n.}$ is the dose per kg applied intranasally.

The serum concentration measured at 2 min ($C_{2\,min}$) after intranasal delivery was related to the maximum serum concentration (C_{max}) determined. Mean serum concentrations obtained at 5 and 10 min respectively after intranasal application were related to corresponding mean serum concentrations obtained after intravenous administration using the following equation:

$$C_{rel} = C(\text{i.n.})_{t=x}/C(\text{i.v.})_{t=x}$$

where $C(\text{i.n.})_{t=x}$ is the serum concentration at x min after intranasal application and $C(\text{i.v.})_{t=x}$ is the serum concentration at x min after intravenous administration. Finally, AUC for the nasal formulations was related to the mean AUC after intravenous administration and calculated for the time intervals 0–120 and 5–120 min.

2.5. Statistical analyses

Results are presented as the mean ± S.D. and statistical analyses were performed using Student's t-test for paired data.

3. Results and discussion

The study has demonstrated that it is possible to prepare a nasal formulation of bumetanide resulting in fast and pronounced absorption, with a potential for clinical application in acute situations. Mean pharmacokinetic parameters for each formulation are shown in Table 2. The t_{max} of about 3–4 min obtained for formulations III and VI containing 60% GF in coconut oil and 100% GF is significantly shorter ($P < 0.05$), than the t_{max} of up to 10 min found for formulations I, VII

and IX. Previously, equally high rates of absorption have been observed for diazepam administered intranasally to rabbits in vehicles containing GF and PEG (Bechgaard et al., 1997).

As seen from Fig. 1 there is a tendency that increasing amounts of GF in PEG increases the rate of absorption and bioavailability as seen for diazepam (Bechgaard et al., 1991). However, no statistical difference is evident for t_{max} and C_{max} for these formulations. With respect to glycofurol, Fig. 2 and Table 3 indicate that there is an upper limit as the bioavailability of the formulations containing 60% CO or 15%PB tends to be considerably higher (32 and 37% respectively) than for GF alone (20%). As coconut oil and water have different polarities, the results are confusing. Further studies are required to clarify this phenomenon. The aqueous formulation may be preferred due to expected low potential of irritation and improved stability (Nielsen et al., 1999). However, the rabbits appeared to be unaffected by all formulations tested intranasally.

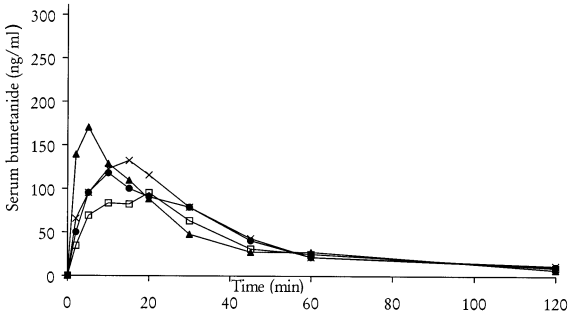

Fig. 1. The influence of increasing amounts of GF in PEG 200 on mean serum concentrations of bumetanide after intranasal administration: (□) 100% PEG 200 ($n=4$), (●) 5% GF in PEG 200 ($n=8$), (×) 30% GF in PEG 200 ($n=4$) and (▲) 100% GF ($n=4$). The presence of S.D. bars for intranasally administered formulations seems confusing, having been omitted for that reason.

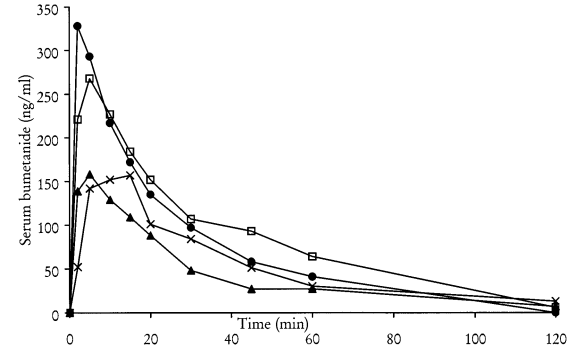

Fig. 2. The influence of vehicle polarity on mean serum concentrations of bumetanide after intranasal administration: (□) 60% GF in phosphate buffer, pH 7.4 ($n=4$), (●) 60% GF in coconut oil ($n=4$), (▲) 100% GF ($n=4$) and (×) 15% phosphate buffer, pH 7.4 in (5% GF in PEG 200) ($n=4$).

As seen from Table 3 the bioavailability from 0 to 120 min ranges from 16 to 37% and from 5 to 120 min the range is 33–82%. The bioavailability for the time interval 5–120 min is calculated because the initial peak ($AUC_{0-5\,min}$) after i.v. injection accounts for as much as 60% of the total area under the curve and a considerable part of this peak may be clinically undesirable due to the risk of side effects. The total period of observation is limited to 120 min as the primary objective was to study the possibilities of acute treatment.

As an attempt to further express the clinical potential in acute situations the C/C_{max} at 2 min is presented as a supplement to t_{max}. $C_{2\,min}/C_{max}$ range from 33 to 100% indicating the possibility of fast onset of action provided that a sufficient dose is given. Finally, $C_{i.n.}$ is also related to $C_{i.v.}$ at 5 and 10 min. At 5 min the range is 4–25% and at 10 min the range is 15–40%. It may be possible to increase the amount of drug absorbed since the bioavailability increases linearily with the dose administered as illustrated in Fig. 3 and Table 3 because the bioavailability after administration of 8 mg bumetanide (formulation VIII) is nearly the same as for 1 mg bumetanide (formulation I). Based on these observations, a human study of nasal bumetanide was performed (Leo Pharmaceutical Products, data on file). It was found that the rate of absorption was higher than for both peroral and rectal administration.

As mentioned earlier, the t_{max} was 0.5–2.2 h after peroral administration to humans. After rectal application to rabbits and humans the t_{max} was in the range 25–50 min. The observed t_{max} (less than 10 min) after intranasal delivery to rabbits indicate a potential advantage of this route of delivery of bumetanide. Intranasal administration of 2 mg bumetanide to healthy humans in a vehicle identical to the vehicle used for

Table 3
Pharmacokinetic parameters (dose corrected relative to mean i.v. dose) for single intranasal administration of bumetanide[a]

Formulation		$C_{2\ min}/C_{max}$ (mean %)	C relative to i.v. at min (mean %)		AUC relative to i.v. in time intervals (mean %)	
			5	10	0–120 min	5–120 min
I	5%GF/PEG/10	50	8[b]	17	19	34
II	GF/PB	82[c]	23	40	37[c]	82[c]
III	GF/CO	100[d]	25	39	32	73
V	30%GF/PEG	51	8	22	21	48
VI	GF	88	13	23	20	43
VII	PEG	40[e]	6	15	16	38
VIII	5%GF/PEG/80	76	12	22	16	34
IX	GF/PEG/PB	33	4	27	24	55

[a] The serum concentration at 2 min is calculated in % of the maximum concentration obtained. Serum concentrations at 5 and 10 min are presented in % of the corresponding value for intravenous administration. Area under the curve (AUC) for the time intervals specified are presented as % of the corresponding data for intravenous administration.
[b] Differs significantly ($P < 0.05$) from formulation III.
[c] Differs significantly ($P < 0.05$) from formulation VII.
[d] Differs significantly ($P < 0.05$) from formulations V, VII and IX.
[e] Differs significantly ($P < 0.05$) from formulation VIII.

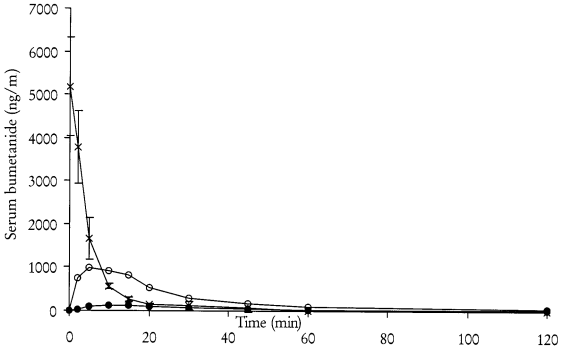

Fig. 3. The influence of dose on mean serum concentrations of bumetanide after intranasal administration: 1 mg bumetanide in 5% GF in PEG 200 (●) ($n = 4$) and 8 mg bumetanide in 5% GF in PEG 200 (○) ($n = 4$). Mean serum concentrations after intravenous administration (×) ($n = 4$) is included for comparison.

formulation IX resulted in a serum concentration at 15 min of about 15% of the serum concentration achieved at the same time after injection. This value is comparable to the relative serum concentrations found in rabbits and within the therapeutic range (Bechgaard et al., 1991).

The data obtained in this study suggest that it may be possible to prepare a clinically relevant nasal formulation of bumetanide containing up to 40% buffer and the cosolvents GF and/or PEG.

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