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The influence of tonicity and viscosity on the intranasal absorption of salmon calcitonin in rabbits

Ramneik Dua, Hossein Zia, Thomas Needham *

Drug Development and Research Laboratory, University of Rhode Island, Kingston, RI 02881, USA

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Abstract

As with any drug delivery system, a clear understanding of the physicochemical and formulation factors is necessary for the rational design of a dosage form. Formulation factors need to be carefully assessed to identify those which may influence pharmacological or physiological response and thus assure optimum therapeutic activity. On the basis of physicochemical and biopharmaceutical studies a nasal peptide formulation when administered with an appropriate delivery device may be designed to provide optimal nasal activity. In the present study an attempt was made to investigate the effect of tonicity and viscosity on the intranasal absorption of salmon calcitonin (sCT). Formulations were designed as nasal sprays with viscosity at 1 and 76 cps, using 0% w/w and 1% w/w methylcellulose as the viscosity enhancing agent; and with a tonicity of 100, 300 or 600 mOsms, using sodium chloride as the tonicity adjusting agent. The low viscosity formulations were delivered using a metered nasal spray pump and the high viscosity formulations were administered using a prototype device, the nasal micron spray pump (NMSP), to facilitate a uniform distribution of the spray into the nasal cavity. Serum levels of sCT were determined in healthy male New Zealand rabbits after intranasal administration of 2000 I.U. of sCT in 200 μl. The pharmacodynamic effect of salmon calcitonin of lowering blood calcium levels was measured using a visible spectrophotometric technique. Deviation from isotonicity increased the bioavailability by 4–5 times. Variation in the viscosity did not influence the bioavailability of salmon calcitonin. Response surface methodology and the canonical analysis parameters were applied to predict the optimum formulations. © 1997 Elsevier Science B.V.

Keywords: Calcitonin; Intranasal; Tonicity; Viscosity; Methylcellulose; Nasal spray; Osteoporosis; Spray pumps

1. Introduction

Nasal absorption of many polypeptides such as oxytocin (Muller and Osler, 1967), synthetic lysine vasopressin (Morimoto et al., 1985), synthetic

* Corresponding author.

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LH-RH and its analogues (London et al., 1973), insulin (Nagai et al., 1984; Longnecker et al., 1987; Illum et al., 1989; Dondeti et al., 1997), enkephalins analogs (Su et al., 1985), growth hormone releasing factor (Pontiroli et al., 1989) have been reported. The permeability of the nasal mucosa to peptides seems to decrease with increasing molecular weight. For peptides with a molecular weight of more than 1000 D, bioavailabilities are in the range of 1–3% (Lee et al., 1988, Zia et al., 1993). The nasal absorption efficiency of peptides and proteins is also influenced by the overall design of the dosage form as well as the devices used for administration. Some of the dosage forms which have been investigated are solutions (Hussain et al., 1979) gels (Morimoto et al., 1991), powders (Keenan and Chamberlain, 1969) and microspheres (Illum, 1980). Various nasal dosage forms have been used for the administration of insulin (Salzman et al., 1985, Dondeti et al., 1997) human growth hormone (Vance et al., 1986.), oxytocin (Hendricks and Gabel, 1960), and desmopressin (Harris et al., 1989). The earliest and the most classical dosage form for nasal administration was the solution. However solutions have often been shown to provide lower bioavailability because they are more readily cleared from the nasal cavity, particularly when administered as drops. There is little information on how the formulation variables of a nasal spray such as buffers, ionic strength, viscosity, charge, pH, osmolarity, type and concentration of preservatives could further influence the absorption of peptides and proteins.

Injectable formulations of salmon calcitonin, a polypeptide hormone, have been used with some success in the management of metabolic bone disorders such as osteoporosis and Paget's disease but this form of administration is inconvenient and has been poorly tolerated by the patients. The development of intranasal preparations of salmon calcitonin provide a more convenient means of administering the drug (Clissod et al., 1991). Intranasal spray preparations containing salmon calcitonin have been shown to be effective in metabolic bone disease (Kurose et al., 1987). Salmon calcitonin in a spray form is now registered in some European countries, and has now been recently approved in the US. Unfortunately the nasal bioavailability of sCT is significantly lower than that realized by injection. The available data indicates a reasonable absorption but there is very little detail addressing the effects of formulation parameters, such as pH, buffers, osmolarity, viscosity, etc., on the bioavailability of the drug (Hanson et al., 1986, Chien et al., 1989).

To optimize drug activity, the formulation variables which influence drug formulation (in combination with delivery devices) must be identified and modified to realize significant drug activity. The objective of this project was to evaluate the effect of the formulation variables, viscosity and tonocity on the intranasal absorption of salmon calcitonin.

2. Experimental

2.1. Materials

Salmon calcitonin (sp. activity 5384 I.U./mg) was a gift from Armour Pharmaceuticals, Kankanee, IL). RIA Kits were procured from Diagnostics Systems Laboratories (Webster, TX). Methylcellulose used was Methocel A15C (Dow, Midland, MI). Acetic acid and sodium acetate, were purchased from Fisher (Fair Lawn, NJ). The anesthetics, acepromazine maleate and ketamine hydrochloride (Aveco, Fort Dodge, IA), DMA Calcium Plus Reagent® (DMA, Houston, TX), Chlorbutanol (Sigma), Catheters and Serum separators (Baxter Healthcare, Deerfield, IL), Heparin Sodium injection, USP (Elkins–Sinn, Cherry Hill, NJ) were purchased and used as received.

A standard 50 μl metered nasal spray pump was chosen to deliver the low viscosity formulations (1 cps). Due to the failure of the commercially available nasal spray pump to deliver high viscosity solutions (76 cps) as a dispersed spray, a prototype device, the nasal micron spray pump (NMSP), capable of spraying 35 μl per actuation was employed (Jager–Waldau, 1992) to deliver those formulations. Both pumps were provided by Pfeiffer (Radolfzell, Germany).

2.2. Methods

2.2.1. Preparation of formulations

Formulations were prepared with two concentrations of methylcellulose (0% to 1% w/w) in 0.03 M sodium acetate–acetic acid buffer at pH 4 and to provide a viscosity of 1 and 76 cps. A pH of 4 and a concentration of 10 I.U./ml were selected for the formulation as salmon calcitonin has maximum stability between pH 3 and 5. The formulations were refrigerated as salmon calcitonin has been reported to be most stable when stored at 2–4°C. All the formulations were prepared on the day of administration to the animals.

2.2.2. Measurement of tonicity

Formulations of various tonicity (100, 300, 600 mOsm) (and viscosity) were prepared. Tonicities were adjusted using a A-010 Microdiagnostics Osmometer.

2.2.3. Administration of nasal formulations to the rabbits

A standard metered manual spray pump was chosen to deliver the low viscosity formulations (1 cps). A metered 50 μl nasal spray pump based on a ball and spring mechanism alone with a two piece flanged actuator using a spray insert of 35° spray angle was used. The total volume of 200 μl to contain the 2000 I.U. was instilled by spraying twice into each nostril. The nasal micron spray pump was employed to deliver formulations of higher viscosity (76 cps). The volume delivered was 35 μl at one time, and a total volume of 210 μl dose was administered by spraying three times into each nostril.

2.2.4. In-vivo study

The New Zealand white rabbit was selected as an animal model because it provides a well controlled animal model for screening the nasal absorption potential of sprayed drug formulations. The blood volume of the rabbit is sufficiently large to permit frequent blood samples and allow a full characterization of the absorption profile of the drug (Gizurason, 1990). Rabbits were obtained from Milbrook farms (Amherst, MA) with a mean weight of about 3 kg. The study protocol was reviewed and approved by the Institutional Animal Care and Use Committee, University of Rhode Island, Kingston, RI.

2.2.5. Experimental procedure

Five of the rabbits were fasted for 36 h prior to each experiment with free access to water. The rabbits were anesthetized with an i.m. dose of 0.25 mg/kg of ketamine hydrochloride and 2.5 mg/kg of acepopromazine maleate. Rabbits were kept lying on the thermal rugs during the experiment. A catheter was placed in the rabbit's median ear artery and a 0.8–1 ml blood sample was collected after −5, 10, 20, 30, 40, 50, 60, 75, 90, 120, 180, 240 and 300 min of the administration of the formulation. The samples were allowed to clot for at least 30 min at room temperature and then centrifuged at 3000 rpm for 10 min and stored at −20°C until assayed.

The control group was used to measure any interference from administration of anesthesia or handling on the inherent calcitonin level. The rabbits were subjected to the same conditions, however calcitonin was not administered. The values obtained were subtracted from the levels obtained after administration of calcitonin formulations.

2.2.6. Study design

Calcitonin was administered as a spray to the rabbits as per a randomized cross-over design. A wash out period of at least one week was allowed between treatments.

2.2.7. Analytical methods

2.2.7.1. Serum calcitonin. All serum samples were assayed with a commercial RIA kit developed at Diagnostics Systems Labortories (Webster, TX). Guinea pig anti-sCT antibodies were used as primary antibodies and have demonstrated less than 2% w/w cross reactivity. The sigmoidal standard curve of B/Bo vs. [sCT], typical of competitive binding assays was linearized using the log transformation. The best fit line was determined using a non-weighted least square regression analysis. The radioimmunoassay quantitation range was 100–5000 pg/ml. The square of the coefficient of

determination (r^2) in this range for the B/Bo vs. log[sCT] plots was greater than 0.993 for all assays performed in duplicate.

2.2.7.2. Serum calcium levels. Serum calcium levels were determined with a DMA Calcium Plus Procedure® utilizing arsenazo III to bind the calcium at an acid pH and form a bluish purple complex. Serum calcium was quantified spectrophotometrically at 650 nm and the range of the linearity was determined to be between 5 and 25 mg/dl. The square of the correlation (r^2) in this range for the absorbance vs. concentration (mg/dl) was 0.999 for all the assays performed in duplicate during the course of study.

2.2.8. Data analysis

The concentration of calcitonin was measured in the serum over a period of 5 h after administration of salmon calcitonin formulations. Salmon calcitonin activity was determined as a function of lowering of calcium. Nasal bioavailability of calcitonin was calculated relative to the serum calcitonin levels (over a period of 5 h after injecting calcitonin intravenously). C_{max} values are the peak serum calcitonin concentrations observed at time T_{max} after adminstration of calcitonin.

$$\%\text{AbsoluteBioavailability} = \frac{(\text{AUC}_{in} - \text{AUC}_{control})}{(\text{AUC}_{iv} - \text{AUC}_{control})} \times \frac{\text{Dose}_{in}}{\text{Dose}_{iv}} \times 100$$

The hypocalcemic effect was measured in terms of maximal decrease ($\%\text{max}_d$). The maximal decrease was the highest percentage of reduction in calcium levels as compared to the basal values. The total decrease in serum calcium level ($D\%$) was calculated using a modified method, by Hirai et al., 1981 ,

$$D\% = \frac{(\text{AUC}_{control} - \text{AUC}_{in})}{\text{AUC}_{control}} \times 100$$

where AUC_{in}, $\text{AUC}_{control}$ and AUC_{iv} refer to the area under the curve calculated by the linear trapezoidal rule. AUC_{in} refers to area under the curve calculated after the intranasal administration of calcitonin formulations.

The data was analyzed by ANOVA using Statistical Analytical Software (SAS). The formulation effects were compared with the controls using Dunnett's test. Multiple comparison among the treatment effects were determined using Scheffe's multiple comparison test. Differences among the treatment were assumed to be significant for values of $P < 0.05$.

2.2.9. Determination of optimum formulations

One of the objectives of this investigation was to study the effects of tonicity and viscosity on the intranasal bioavailability. Tonicity was investigated at three levels of 100, 300 and 600 mOsm and viscosity was studied at two levels of 1 and 76 cps. In order to optimize the effects of the independent variables of viscosity and tonicity on the dependent factor, area under the curve, a full factorial statistical design, was used. A factorial design was used to study the primary effects of viscosity and tonicity and the secondary effects or interactions of viscosity and tonicity on the intranasal absorption of sCT.

In the simplest of cases, techniques used for optimization involve the determination of a maximum or minimum; but when the relationship for the response is given as a function of two or more than two independent variables, the system becomes more complicated. In the present case, response surface methodology (RSM) was applied to estimate the maximum or minimum response so as to determine the optimum formulations for the nasal delivery of salmon calcitonin. The SAS/RSREG software was used to fit the parameters of the complete quadratic reponse surface obtained from the three levels of tonicity incorporated in the formulations. The fitted surface was analyzed to determine the level of the independent variable providing the optimum response. The predicted optimal value can be found from the estimated surface, which usually shows a minimum, maximum or saddle point, if there is no clear maximum or minimum. Since the independent variable should be at three or more levels, tonicity was designated an independent variable with area under the curve as a response surface and viscosity was used as a covariate that was investigated at two levels (SAS/STAT User's guide).

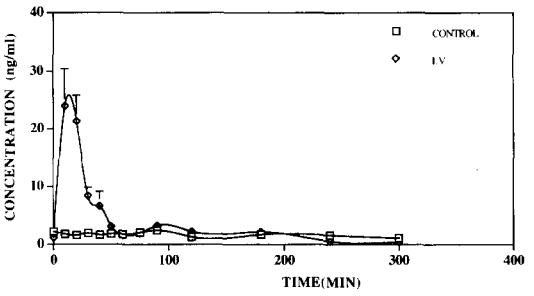

Fig. 1. Concentration time profile for i.v. administration of salmon calcitonin ($n = 5$)

3. Results and discussion

3.1. Intravenous administration

The average serum sCT concentration vs. time profile for 5 I.U. of i.v. administered dose is shown in Fig. 1. The average C_{max} was 25 ± 2.9 ng/ml with a T_{max} of 14 ± 2.4 min. The area under the curve (AUC) was 1102.1 ± 19.8 ng min/ml as compared to control group in which the average C_{max}concentration was 2 ng/ml and the AUC was 401 ± 75.3 ng min/ml. The salmon calcitonin levels obtained in the serum were significantly different from the control level at $P < 0.05$

3.2. Intranasal administration

3.2.1. Effect of tonicity with varying viscosity

3.2.1.1. Influence of isotonicity. To determine the effect of tonicity on the absorption of salmon calcitonin, a base formulation containing 2000 I.U. salmon calcitonin per 200 μl in a 0.03 M pH 4 acetate buffer with a viscosity of 1 cps and adjusted with NaCl to be either isotonic, hypotonic or hypertonic were administered to the rabbits. The AUC, C_{max}, T_{max} and bioavailability of all the formulations differing in tonicity are summarized in Table 1. The AUC of the isotonic formulations at a viscosity of 1 cps was 714 ± 15.7 ng min/ml with a bioavailability of 0.16. The C_{max}obtained for this formulation was 10 ± 4.2 ng/ml at a T_{max} of 40 min.

The higher viscosity (76 cps) isotonic (300 mOsm) formulations prepared with 1% methycellulose at pH 4 containing 2000 I.U. salmon calcitonin in 200 μl when sprayed into the nasal cavity of the rabbits elicited a $C_{max}$of 12 ± 9.7 ng/ml with a T_{max} of 58 ± 8.3 min as shown in Fig. 2. The AUC for the higher viscosity isotonic formulations was 604.6 ± 97.74 ng min/ml with a bioavailability of 0.14.

The bioavailability obtained after intranasal administration of salmon calcitonin for the low and high viscosity isotonic formulations was extremely

Table 1
Pharmacokinetic parameters in New Zealand rabbits after intranasal administration of salmon calcitonin ($n = 5$); Dose 2000 I.U.

Variable	T_{max} (min)	C_{max} (ng/ml)	AUC (ng min/ml)	Bioavailability (%)
Control			400 ± 75.3	
IV (5 I.U.)	14	25 ± 2.9	1102 ± 19.8[a]	100
Intranasal low viscosity				
Isotonic	40	10 ± 4.2	714.2 ± 15.7[c]	0.16[c]
Hypertonic	90	42 ± 19.6	3508 ± 1280[b]	0.80
Hypotonic	90	26 ± 7.6	3171 ± 258[b]	0.71
Intranasal high viscosity				
Isotonic	58	12 ± 9.7	604 ± 97.74[c]	0.14[c]
Hypertonic	90	32 ± 19.6	2183 ± 258[b]	0.62
Hypotonic	120	23 ± 2.4	3579 ± 1030[b]	0.81

[a] Significantly different from control at $P < 0.05$.
[b] Significantly different at low and high viscosity at $P < 0.5$.
[c] No Significant difference at $P < 0.05$.

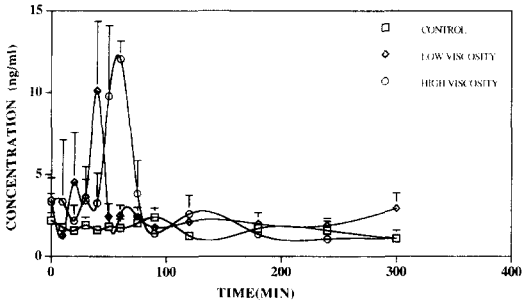

Fig. 2. The influence of viscosity on the intranasal absorption of salmon calcitonin for isotonic solutions

low and is typical of that found for higher molecular weight polypeptides (Lee et al., 1994).

3.2.1.2. Influence of hypertonicity. The low viscosity hypertonic (600 mOsm) formulations shown in Fig. 3 showed a $C_{max}$of 42 ± 19.6 ng/ml with a T_{max} of 90 ± 12 min as compared to a $C_{max}$of 10 ± 4.2 ng/ml and a T_{max} of 40 min for the low viscosity isotonic (300 mOsm) formulations. Thus, deviation from isotonicity for low viscosity formulations elicited a higher concentration of sCT in the serum and extended the time to reach T_{max}. The AUC calculated for the low viscosity hypertonic salmon calcitonin formulations was 3508 ± 1280 ng min/ml with a bioavailability of 0.80. The bioavailability obtained was about five times higher than that obtained for the low viscosity isotonic formulations.

The higher viscosity (76 cps) hypertonic (600 mOsm) formulations prepared with 1% methycellulose when sprayed into the nasal cavity of rabbits elicited a C_{max} of 32 ± 19.6 ng/ml at T_{max} of

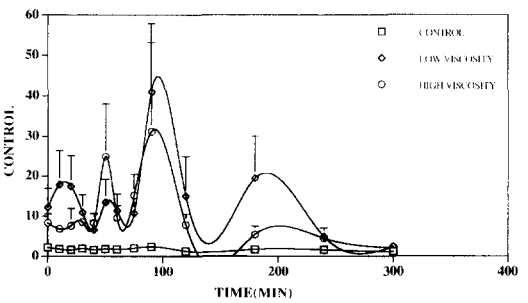

Fig. 3. The influence of viscosity on the intranasal absorption of salmon calcitonin for hypertonic solutions

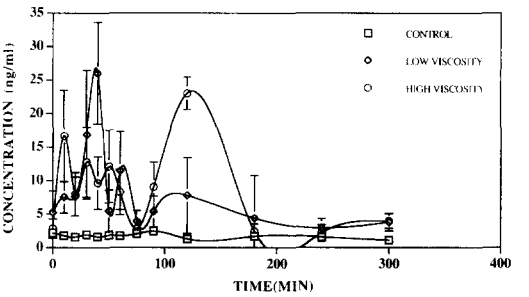

Fig. 4. The influence of viscosity on the intranasal absorption of salmon calcitonin for hypotonic solutions

90 ± 12 min as shown in Fig. 3. The area under the curve obtained was 2183 ± 258 ng min/ml with a bioavailability of 0.62. Thus, deviation from isotonicity of the higher viscosity formulations also demonstrated an increased level of salmon calcitonin in the serum. The bioavailability obtained by the high viscosity hypertonic salmon calcitonin formulations was significantly different from high viscosity isotonic formulations as determined at $P < 0.05$.

3.2.1.3. Influence of hypotonicity. The low viscosity (1 cps) hypotonic (100 mOsm) formulations sprayed into the nasal cavity of the rabbits produced a C_{max} of 26 ± 7.6 ng/ml at a T_{max} of 38 ± 9.16 min as shown in Fig. 4. The AUC obtained was 3171.33 ± 258 ng min/ml with a bioavailability of 0.71. The AUC obtained for the low viscosity hypotonic formulations was approximately the same magnitude as the low viscosity hypertonic formulations and was significantly different from low viscosity isotonic formulations at $P < 005$.

The higher viscosity hypotonic (100 mOsm) formulations prepared with 1% methylcellulose at pH of 4 produced a C_{max} of 23 ± 2.4 ng/ml at a T_{max} of 120 ± 18 min. The AUC calculated was 3579 ± 1030 ng/ml with a bioavailability of 0.81. Even at high viscosity, a deviation from isotonicity produced a significant increase in the bioavailability. Absorption of hypertonic and hypotonic formulations was significantly different from the isotonic formulations at both low and high viscosity; and the bioavailability of the drug was enhanced in both the hypotonic and hypertonic condition by 4–5 fold.

There have been very few experiments performed to study the effect of tonicity, but the information generated from these studies indicates that deviation from isotonicity influences absorption. One explanation postulated in the literature is that hypertonic solutions which cause shrinkage of the epithelical cells and hypotonic solutions which cause swelling of the cells may result in alteration of pore size within the cell junctions and the permeability of the cell walls thus leading to enhanced absorption (Pujara et al., 1995).

3.2.2. Influence of viscosity

It can be seen from the literature that although low viscosity (1–11 cps) formulations can be administered using metered nasal spray pumps, these devices are limited in their ability to produce a fine uniform spray for high viscosity formulations. In this study, a metered dose spray pump, based on a ball and spring mechanism was used to spray low viscosity (1 cps) formulations; and the nasal micron spray pump designed with an air blast spray nozzle was used to spray the higher viscosity (76 cps) formulations. The droplet size distribution generated by both devices was measured with a Malvern sizer and the M50 which is the droplet size of 50% of the droplets sprayed was used for comparison in this paper because the M50 is a more reliable measure of the mean droplet size of spray with less variability.

Previous studies have shown that increasing viscosity increases the droplet size of the spray. The average droplet size M50 for formulations with a viscosity of 1 cps when sprayed from the metered dose spray pump was 45 ± 1.11 μm. This same pump produced a stream rather than a dispersed spray for the high viscosity formulations. However when sprayed from the micron spray pump a dispersed spray was produced with a the droplet size M50 of 112.48 ± 27.6 μm for formulations with a viscosity of 76 cps. The M50 generated by the two nasal delivery devices for the formulations differing in viscosity by 76 cps was approximately different by 2.5 fold (45 μm vs. 112.48 μm). Interestingly, the droplet size distribution produced by the metered nasal spray pump at 1 cps viscosity was gaussian and unimodal while the nasal micron pump produced a slight deviation from a Gaussian distribution and was bimodal. The bimodal distribution was due to a portion of the spray striking the mouthpiece while leaving the spray nozzle (Dua et al., 1994).

The T_{max} produced by low viscosity isotonic and hypotonic formulations was 40 min and 38 min respectively as compared to the higher viscosity isotonic and hypotonic formulations with T_{max} of 58 and 120 min. These results correlate with a previous study that suggested larger droplet size leads to a more localized deposition and causes slower clearance from the nose (Pennington et al., 1988). However for the hypertonic formulations the T_{max} for both the low viscosity and high viscosity formulations was 90 min, which suggest that viscosity may not be the dominant factor for these formulations.

Harris et al., 1989 when using methycellulose as a viscosity enhancing agent demonstrated no improvement in the bioavailability of drug and actually reported a decrease in absorption that was attributed to the delayed diffusion of drug from the formulation due to the higher viscosity. In this study variation in the viscosity did not show any significant difference in the absorption of sCT.

3.3. Optimization of formulations

Six formulations resulting from using three levels of tonicity and two levels of viscosity were administered to rabbits. Area under the curve was calculated and subjected to statistical analysis using ANOVA and Scheffe's multiple comparison test. The results generated from the experimental data suggested that hypertonic and hypotonic formulations were significantly different from isotonic formulations at both low and high viscosity. Regression was carried out to define the relationship between area under the curve and the independent factors of tonicity and viscosity and the optimum region was determined by the response surface analysis.

A saddle point curve (Fig. 5) was obtained which suggests that there is no optimal maximum or minimum tonicity, but rather that deviation from the mid point (isotonicity) would favor an increase in the area under the curve and ultimately bioavailability. Viscosity, used as a covari-

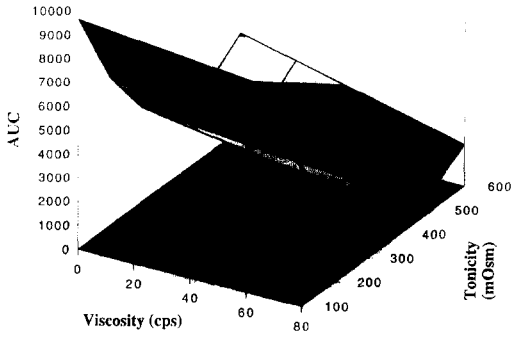

Fig. 5. Three dimensional surface plot showing a saddle point for viscosity and tonicity with respect to area under the curve

ate and plotted along with tonicity and area under the curve demonstrated that there was no influence of viscosity on the area under the curve and subsequently on bioavailability. Fig. 5 indicates an optimum at an AUC of 8000–10 000 ng min/ml for hypotonic formulations with a viscosity ranging from 1 to 80 cps. There is a decrease in the AUC from 8000 to 2000 ng min/ml as the tonicity increases from hypotonic to isotonic (300 mOsm). Again the surface shows an increase as the tonicity increases from isotonic (300 mOsm) to hypertonic (600 mOsm) with a consistent increase in AUC from 2000 to 6000 ng min/ml.

3.4. Determination of calcium

The pharmacodynamic effect of sCT of lowering of calcium is shown in Table 2. The total reduction in calcium level throughout the time period studied is termed as $D\%$. The hypocalcemic effect may also be measured in terms of maximal percent decrease ($\%max_d$) which is the highest percentage of reduction in calcium levels as compared to the basal values. The time at which this reduction takes place is T_{max}. The lowering of calcium for all intranasal formulations ranged from 7.9 to 22% of the basal value as compared to 32% after i.v. administration. The $\%max_d$ for intranasal delivery for all nasal formulations ranged from 12 to 34.5% as compared to 28% for i.v. administration. The similarity in the extent of the hypocalcemic effect observed after administration of different intranasal formulations that varied widely in sCT plasma levels may be attributed to the acute homeostatic mechanism between calcitonin and the parathyroid hormone. The parathyroid hormone counteracts the effects of calcitonin once a critical level of hypocalcemia is attained. Thus the extent of the hypocalcemic effect is controlled by hormonal balance. This feedback control of blood calcium makes it difficult to evaluate the efficacy of sCT delivery systems by observation of the hypocalcemic effect (Mohamadi et al., 1975).

Table 2
Pharmacodynamic parameters in New Zealand rabbits after intranasal administration of salmon calcitonin ($n = 5$); Dose 2000 IU

Variable	T_{max} (min)	Max_d (%)	AUC (mg min/dl)	D (%)
Control			4045.40 ± 255.17	
IV (5 I.U.)	30	28	2732 ± 271.39[a]	32
Intranasal low viscosity				
Isotonic	240	12.96	3724.12 ± 120.99	7.9
Hypertonic	75	28.83	3267.74 ± 31.31[a,b]	19
Hypotonic	180	28.12	3308.0 ± 42.45[a]	18
Intranasal high viscosity				
Isotonic	240	28.81	3242.83 ± 21.66[a]	19
Hypertonic	90	23.52	3696 ± 53.24[a,b]	9.0
Hypotonic	120	34.58	3154.47 ± 102.49[a]	22

[a] Significantly different from control at $P<0.05$.
[b] Significantly different at low and high viscosity at $P<0.5$.

Although the measure of pharmacodynamic effects is preferred especially in the event of low drug bioavailability; the results obtained from the biochemical markers of salmon calcitonin such as lowering in calcium, bone turnover and decrease in the urinary hydroxyproline are difficult to interpret especially during short term studies. This is because they are under hormonal regulation and may or may not differ for healthy subjects and diseased subjects.

In conclusion it seems feasible to optimize the absorption of sCT across the nasal mucosa by controlling formulation variables. In spite of low bioavailability, there is clinical data which supports the efficacy, tolerability and acceptability of intranasal formulations in the treatment of conditions such as Paget's disease and osteoporosis.

Further investigations are required for the evaluation effect of these formulations on the integrity of the nasal mucosa especially during chronic use, as such formulations may have the potential of damaging either the mucociliary system or the underlying epithelium.

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