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Effect of chitosan on the intranasal absorption of salmon calcitonin in sheep

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Abstract

The effects of a chitosan-based delivery system on the pharmacokinetics of intranasally administered salmon calcitonin (sCT) were investigated in a sheep model. In particular, the feasibility of producing a formulation with a comparable or improved bioavailability and/or less variability than the currently marketed nasal product (Miacalcin nasal spray, Novartis Pharmaceuticals) was assessed. A comparator (control) formulation comprising sCT solution was also tested. Sheep ($n = 6$) were dosed intranasally according to a randomized crossover design. The intranasal sCT dose was 1100 IU (equivalent to approximately 17 IU kg^{-1}). After completion of the nasal dosing legs, five of the sheep received 300 IU sCT (equivalent to approximately 5 IU kg^{-1}) by subcutaneous injection to estimate relative bioavailability. After intranasal or subcutaneous dosing, serial blood samples were taken and plasma separated by centrifugation before measuring sCT concentrations by ELISA. Pharmacokinetic (non-compartmental) and statistical (analysis of variance or non-parametric alternative) analyses were performed. No systemic or local adverse effects were observed following intranasal or subcutaneous administration of sCT. The mean relative bioavailability of sCT from the chitosan solution was improved twofold compared with Miacalcin nasal spray and threefold compared with sCT control solution. Inter-animal variability in sCT absorption appeared to be lower with use of the chitosan-based solution compared with the control solution or commercial product. Based on the reported sheep data, a chitosan delivery system could offer the potential to significantly improve the intranasal absorption of sCT and reduce the variability in absorption. In the clinical setting, this may allow relatively lower doses of the drug to be given intranasally and/or lead to improvements in the efficacy or quality of intranasal therapy.

Introduction

Salmon calcitonin (sCT) nasal spray (Miacalcin nasal spray, Novartis Pharmaceuticals) is indicated for the prevention of bone loss and to reduce bone turnover during the treatment of postmenopausal osteoporosis (Overgaard et al 1989; Overgaard & Riis 1994; Physician's Desk Reference 2003). The drug may also be of benefit for the treatment of other conditions affecting bone such as corticosteroid-induced or idiopathic osteoporosis, Paget's disease and osteogenesis imperfecta, and as a preventative treatment in early postmenopausal women (Kurose et al 1987; Rizzato et al 1989; O'Doherty et al 1990; Overgaard 1994; Reginster et al 1994; Trovas et al 2002).

The currently available nasal preparation contains synthetic sCT, a 32 amino acid polypeptide which comprises the same linear sequence of amino acids found in the natural hormone and has 16 amino acids in common with natural or synthetic human calcitonin (hCT) (Azria 1989; Gennari et al 1990). In the clinical setting, it is possible to administer sCT intranasally due to its greater (approximately 30-fold) biological activity compared with hCT despite the relatively poor absorption of exogenous calcitonins via this route (Kurose et al 1987; Pontiroli et al 1989; Lee et al 1994).

Much of the clinical research into the pharmacokinetics of intranasally administered hCT or sCT has been undertaken in healthy volunteers (Pontiroli et al 1985, 1989; Buclin et al 1987; Kurose et al 1987; Overgaard et al 1991; Lee et al 1994). The absorption of sCT from the commercial nasal preparation tends to be variable and low; bioavailability, relative to intramuscular (i.m.) injection, is acknowledged to be in the range 0.3–30.6%

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(average 3%) for intranasal sCT (Physician's Desk Reference 2003). In an independent nasal dosing study in healthy volunteers, sCT was only detectable in the sera from five of ten subjects and the average bioavailability, relative to intramuscular dosing, was reported to be 1.6% (Lee et al 1994). The coefficient of variation (CV) of values of area under the serum sCT concentration–time curve (AUC) was 150% compared with 47% after intramuscular injection indicating considerable variability in the overall absorption of sCT via the nasal route.

A number of approaches have been investigated in volunteers or animal models as a means to improve the extent and reproducibility of intranasal calcitonin absorption. These include the use of permeation enhancers such as bile salts or their derivatives (e.g. sodium glycocholate, sodium taurocholate or sodium taurodihydrofusidate), fatty acid derivates (e.g. acylcarnitines), non-ionic surfactants (namely alkylglycosides e.g. tetradecylmaltoside), and cyclic oligosaccharides (namely cyclodextrins e.g. dimethyl-β- and hydroxypropyl-β-cyclodextrin) (Pontiroli et al 1989; Lee et al 1994; Kagatani et al 1996; Ahsan et al 2001; Yetkin et al 2001; Sinswat & Tengamnuay 2003). Generally, however, at the concentrations required to promote nasal drug absorption, the aforementioned agents have been associated with damage to cell membranes and in some cases toxicity to cilia which would probably preclude their use in commercial products (Merkus et al 1993; Marttin et al 1995; Marttin 1997; Hinchcliffe & Illum 1999). An exception to this is the use of cyclodextrins as solubility enhancers, rather than as absorption promoters, thus facilitating formulation of relatively poorly soluble drugs into a nasal product. Modified cyclodextrins reportedly have better safety profiles than traditional types (a commercial nasal estradiol formulation, Aerodiol nasal spray (Servier) contains randomly methylated-β-cyclodextrin). Other approaches that have been investigated for improving nasal calcitonin absorption include the use of powder formulations based on water-insoluble calcium carbonate or gelatin microspheres, proteolytic enzyme inhibitors (e.g. aprotinin) and altering pharmaceutical properties such as tonicity or pH in aqueous formulations (Yamamoto et al 1987; Morimoto et al 1995, 2001; Dua et al 1997; Ishikawa et al 2001). Chitosan is also under investigation as a nasal delivery system for sCT.

Chitosan is a bioadhesive/mucoadhesive linear cationic polysaccharide comprising copolymers of glucosamine and N-acetylglucosamine (Lehr et al 1992). The polymer is obtained by deacetylation of chitin, a natural material found in abundance in the shells of decapod crustaceans such as crab and shrimp. The application of chitosan as a means of improving the nasal absorption of polypeptide drugs was first published by Illum (1992) and later by Illum et al (1994). The use and safety of chitosan and chitosan derivatives as pharmaceutical excipients and transmucosal delivery systems has been comprehensively reviewed and a monograph for chitosan exists in the European pharmacopoeia (Dornish 1997; Illum 1998; Junginger & Verhoef 1998; Bernkop-Schnürch 2000; Illum et al 2001; Singla & Chawla 2001; European Pharmacopoeia 2002). The principal mechanism of action of chitosan as an absorption promoter is thought to be related to its bioadhesive properties thereby prolonging the residence of formulations in the nasal cavity as demonstrated in man and sheep (Soane et al 1999, 2001). In-vitro studies have indicated that chitosan may transiently open epithelial tight junctions thereby promoting paracellular transport of drugs (Artursson et al 1994; Schipper et al 1997; Junginger & Verhoef 1998; Dodane et al 1999; Ranaldi et al 2002; Smith et al 2004). Previously, chitosan (glutamate salt, pH 4) was shown to provide a two- or fourfold increase in nasal sCT absorption (determined indirectly from the hypocalcaemic action of the formulations) compared with 'chitosan-free' controls in sheep and rat models, respectively (Hinchcliffe 1996). More recently, Sinswat & Tengamnuay (2003) reported a 2.5-fold increase in sCT absorption in the rat with use of chitosan (free amine, pH 4). The principal aim of this study was to determine the effects of an intranasal aqueous delivery system based on chitosan (as the glutamate salt) on the pharmacokinetics of sCT in the sheep model compared with a chitosan-free nasal sCT solution and a current marketed product (Miacalcin nasal spray). As an adjunct, sCT was given to sheep by subcutaneous injection of Miacalcic injection (Novartis) for the purpose of calculating relative bioavailability of the nasally administered drug.

Materials and Methods

Chemicals and reagents

EMLA cream (AstraZeneca UK Ltd) and Ketaset (100 mg mL^{-1} ketamine hydrochloride, Fort Dodge Animal Health Ltd, UK) were purchased from a local hospital stores and veterinary practice, respectively, via the School of Biosciences, Nottingham University, Nottingham, UK. Ethylenediaminetetraacetic acid (EDTA) tubes (5 mL, containing 1.6 mg mL^{-1} EDTA per tube) and 3.5 mL polypropylene tubes were obtained from Sarstedt Ltd (Leicester, UK). Aprotinin (6750 kallikrein inhibitor units (KIU) per mg dry weight) was obtained from Calbiochem-Novabiochem UK Ltd (Nottingham, UK) and sodium chloride 0.9% w/v solution (saline; Steriflex intravenous infusion BP) from Fresenius Health Care Group (Basingstoke, UK). Miacalcin nasal spray (Novartis, USA) was supplied by West Pharmaceutical Services (Lionville, PA). Salmon calcitonin (sCT) solution and sCT/chitosan solution were manufactured by West Pharmaceutical Services (Lionville, PA) using salmon calcitonin, Ph. Eur. (molecular weight 3430 Da, biological activity 6874 IU mg^{-1}) obtained from PolyPeptide Laboratories (Torrance, CA) and chitosan (chitosan glutamate, Protasan UP G213) obtained from Pronova Biopolymer AS (Biomedical Division, Oslo, Norway). Miacalcic injection (Novartis, UK) was obtained via a local pharmacy. Salmon Calcitonin assay (ELISA) kits were purchased from Tepnel Biosystems (Deeside, Flintshire, UK). All other reagents used in the preparation of the formulations were of GMP grade and those used in the analytical procedures were of analytical grade or equivalent.

Animals

Six castrated male (Leicester Mule & Texel crossbred) sheep were obtained from Nottingham University Farm, UK, for use in this study. The sheep weighed (mean ± standard deviation (s.d.)) 64 ± 6 kg over the course of the study. The animals were group housed in a heat and ventilation air conditioned (HVAC) facility maintained at approximately 20°C and 50% relative humidity with a 12-h light–dark cycle. The sheep were acclimatized for seven days before commencing the study. The animals were fed 'Grower' ewe nuts and hay with water freely available. The animal study had received local (School of Biosciences, Nottingham University) ethical approval and was performed under a valid UK Government (Home Office) Project Licence.

Intranasal sCT dosing study

The study was performed as a randomized crossover in the six sheep with a wash-out period of at least two days between successive dosing legs. Details of the formulations tested are given in Table 1. To facilitate dosing and as a counter measure against the animal sneezing during nasal administration, the sheep were sedated by injection of 2.25 mg kg^{-1} ketamine hydrochloride via a jugular vein. Typically, nasal doses were given approximately 2 min after ketamine administration and the total period of sedation lasted approximately 3 min. The nasal doses were divided equally between both nostrils and administered via a commercial spray nozzle (CB-18 from Valois Division Pharmacie, Le Vaudreuil, France), customized for use in sheep and inserted 6–7 cm into the nasal cavity. A fixed nominal dose of 1100 IU sCT was administered intranasally to each sheep equating to a dose of 17 IU kg^{-1} based on the average (i.e. 64 kg) animal. All animals were observed throughout each study day period for signs of general systemic adverse effects by looking for deviations from normal appearance or behaviour. Since nasal dose administration could cause local irritation, any incidences of nasal discharge and snorting/sneezing in the sheep were recorded.

Table 1 Nominal composition of sCT formulations administered to sheep

Formulation description	Composition
sCT/chitosan nasal solution (F1)[†]	2200 IU mL^{-1} sCT and 5 mg mL^{-1} chitosan glutamate preserved with 0.15 mg mL^{-1} benzethonium chloride[‡]
sCT control nasal solution (F2)[†]	2200 IU mL^{-1} sCT preserved with 0.15 mg mL^{-1} benzethonium chloride[‡]
Miacalcin nasal spray (F3)[†]	2200 IU mL^{-1} sCT preserved with benzalkonium chloride
Miacalcic injection (F4)	200 IU mL^{-1} sCT solution

[†]Drug potency confirmed by HPLC. [‡]Formulations were isotonic (adjusted with sodium chloride) and at pH 4 (adjusted with hydrochloric acid).

Blood samples (4 mL) were collected by venepuncture of a cephalic vein under local anaesthesia (topical application of EMLA cream) at 0 (before sCT administration), 5, 10, 15, 30, 45, 60, 90, 120, 180 and 240 min post-administration. Each blood sample was dispensed into an EDTA tube containing 61 KIU aprotinin (from a 610 KIU mL^{-1} solution prepared in saline) and plasma separated by centrifugation at 4°C and approximately 3200 rev min^{-1} (1800 *g*) for 10 min. The plasma samples were stored in polypropylene tubes at −80°C until bioanalysis.

Subcutaneous injection of sCT

After completion of the nasal dosing study, five of the six sheep were administered sCT by subcutaneous injection into the flank under ketamine sedation as before (for consistency with the nasal dosing study) to estimate relative bioavailability of the nasally administered drug. Blood samples were collected from a cephalic vein at 0, 15, 30, 45, 60, 90, 120, 240, 360, 480 and 1500 min and samples processed as above.

Bioanalysis of sCT

sCT concentrations in sheep plasma were determined by an ELISA method using a standard curve prepared in blank sheep plasma (Harlan Sera-Lab Ltd, UK). This was a non-competitive sandwich-type enzyme immunoassay technique which utilized a biotin–avidin enhancement system. The assay was linear over the sCT concentration range of 5–340 pg mL^{-1} with a correlation coefficient of 0.995 ± 0.004 (mean ± s.d.). The lower limit of quantitation (LLOQ) was set at 10 pg mL^{-1} and the intra- and inter-day variation and accuracy were shown to be within acceptable limits (< 20%).

Pharmacokinetic and statistical analysis

Pharmacokinetic (non-compartmental) analysis of the plasma sCT data was undertaken using WinNonlin Version 1.1 (Scientific Consulting Inc., NC). The principal pharmacokinetic measures used to evaluate the efficacy of sCT absorption from the various formulations were: maximum plasma concentration (C_{max}), time of maximum concentration ($T_{max,}$), and area under the plasma concentration–time curve (AUC) calculated up to the last measurable concentration using a trapezoidal method (AUC_t). (Generally there were too few data points above the LLOQ to reliably and consistently calculate the rate constant associated with the terminal elimination phase. Thus, values of AUC could not be extrapolated to infinity as preferred during calculation of F_{rel}.) For each animal, the data were normalized to a nasal dose of 17 IU kg^{-1} sCT and subcutaneous dose of 5 IU kg^{-1}.

The bioavailability of sCT from the intranasal (in) formulations, relative to subcutaneous(sc) injection (F_{rel}) was calculated for each individual animal that received both nasal and subcutaneous sCT (n = 5) as follows:

$$F_{rel} = (AUC_{t(in)} \times Dose_{(sc)})/(AUC_{t(sc)} \times Dose_{(in)}) \times 100 \quad (1)$$

For each dose group, descriptive statistics (i.e. mean, s.d., standard error of the mean (s.e.m.) and CV) were summarized for the above pharmacokinetic measures. Inferential statistical analyses on the nasal pharmacokinetic data were undertaken using GraphPad Instat Version 3.01 (GraphPad Inc., San Diego, CA). A one-way analysis of variance with Tukey–Kramer Multiple Comparisons Test was performed on log-transformed values of C_{max} and AUC_t. Values of T_{max} were analysed using a Kruskal–Wallis test (non-parametric analysis of variance). Throughout, the significance level was set at 5% (i.e. $\alpha = 0.05$).

Results and Discussion

There were no apparent adverse systemic events in the sheep following intranasal sCT administration and all formulations appeared to be well tolerated locally with no incidences of sneezing/snorting or mucus discharge recorded after dosing. The subcutaneous injection appeared to be equally well tolerated by the sheep.

The plasma sCT concentration–time curves after the administration of the three nasal sCT solutions to sheep are presented in Figure 1. The corresponding curve following subcutaneous injection is shown in Figure 2. The principal pharmacokinetic data are summarized in Table 2. The average T_{max} values obtained were comparable for each nasal sCT formulation ranging from 11 to 13 min, although it was extended to approximately 45 min after subcutaneous injection of sCT. There were trends towards higher C_{max}, AUC_t and F_{rel} values after dosing sCT/chitosan compared with Miacalcin nasal spray or sCT control solution. C_{max} and AUC_t values were significantly (2- or 3-fold) higher after the administration of sCT/chitosan solution compared with Miacalcin nasal spray or sCT solution, respectively. Mean C_{max} values peaked at around 99 pg mL^{-1} (range 50–107 pg mL^{-1}) with sCT/chitosan compared with 33 pg mL^{-1} (range 13–49 pg mL^{-1}; $P < 0.01$) and 42 pg mL^{-1} (range 15–79 pg mL^{-1}; $P < 0.01$) after dosing sCT control solution and Miacalcin nasal spray, respectively. After dosing sCT/chitosan solution, the average AUC_t value (3220 pg min mL^{-1}, range 1606–4972 pg min mL^{-1}) was increased 3.5-fold compared with sCT solution (943 pg min mL^{-1}, range 198–2519 pg min mL^{-1}; $P < 0.05$)) and 2-fold relative to Miacalcin nasal spray (1636 pg min mL^{-1}, range 87–3792 pg min mL^{-1}, $P > 0.05$). Average values of C_{max} or AUC_t obtained in the nasal control dose group were not significantly different ($P > 0.05$) to those obtained after dosing Miacalcin nasal spray. Values of C_{max} and AUC_t obtained after subcutaneous injection of 5 IU kg^{-1} sCT were around 462 pg mL^{-1} (range 367–577 pg mL^{-1}) and 107 495 pg min mL^{-1} (range 75 287–129 389 pg min mL^{-1}), respectively. To put these results into perspective, after subcutaneous injection of Miacalcic, C_{max} values were close to 40-fold higher than those obtained after intranasal dosing of the commercial nasal formulation and AUC values in the region of 200-fold greater when all data were normalized to a 17 IU kg^{-1} dose (data not shown).

The bioavailability (F_{rel}) of intranasally administered sCT was low when compared with subcutaneous injection and could be ranked in the order: sCT/chitosan solution (1.0%) > Miacalcin nasal spray (0.6%) > sCT solution (0.3%). Thus, bioavailability was improved approximately 2-fold in the sheep after the intranasal administration of the chitosan-based formulation compared with Miacalcin nasal spray and approximately 3-fold relative to the 'chitosan-free' sCT control solution.

Values of coefficient of variation (CV) of C_{max} and AUC_t apparently indicated greater variability between animals after nasal sCT administration than was observed following subcutaneous injection of the drug. CV values after subcutaneous injection were 18% for C_{max} and 20% for AUC_t. In comparison, after nasal administration of Miacalcin nasal spray, CV values for C_{max} and AUC_t were 57% and 97%, respectively, suggesting relatively poor reproducibility of sCT delivery. Similar values were obtained after intranasal administration of sCT control solution (42% and 104%, respectively). There was apparently less variability in sCT delivery from the chitosan-based formulation; CV values of 27% and 43% were

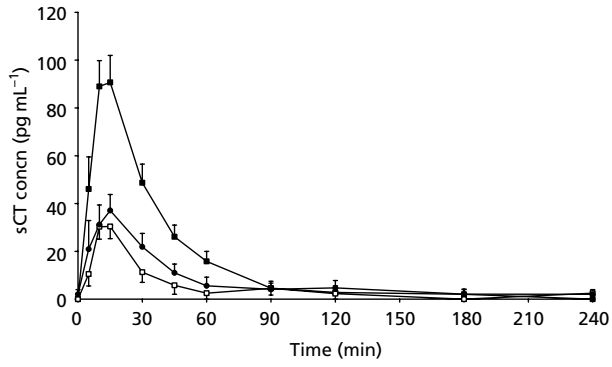

Figure 1 Plasma sCT concentration–time curves after intranasal administration of various sCT formulations to sheep: nasal sCT/chitosan solution (F1, ■); nasal sCT control solution (F2, □); and Miacalcin nasal spray (F3, ●). Each data point represents the mean + or − s.e.m. of six animals.

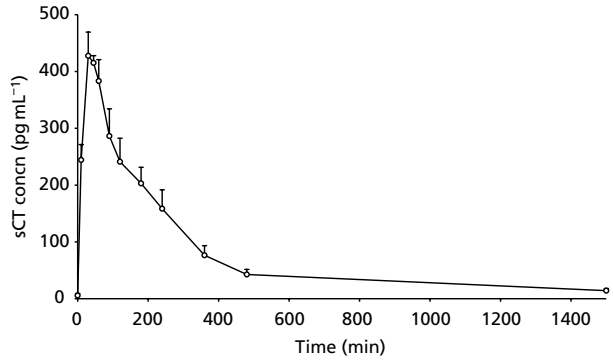

Figure 2 Plasma sCT concentration–time curve after subcutaneous administration of Miacalcic injection to sheep. Each data point represents the mean + s.e.m. of five animals.

Table 2 Summary of pharmacokinetic measures and statistical findings after administration of various sCT formulations to sheep

Formulation	Normalized sCT dose (IU kg^{-1})	C_{max} (pg mL^{-1})	T_{max} (min)	AUC_t (pg min mL^{-1})	F_{rel} (%)
sCT/chitosan nasal solution (F1)[a]	17	99 ± 27 (27%)	13 ± 3 (23%)	3220 ± 1400 (43%)	1.0 ± 0.2 (20%)
sCT control nasal solution (F2)[a]	17	33 ± 14 (42%)	11 ± 2 (18%)	943 ± 984 (104%)	0.3 ± 0.3 (101%)
Miacalcin nasal spray (F3)[a]	17	42 ± 23 (57%)	13 ± 4 (31%)	1636 ± 1580 (97%)	0.6 ± 0.6 (106%)
Miacalcic injection (F4)[b]	5	462 ± 84 (18%)	45 ± 11 (24%)	107 495 ± 22 036 (20%)	100
Statistical evaluation of F1–F3		$P = 0.002$[c]	$P = 0.138$[d]	$P = 0.043$[e]	NT[f]

Data presented as the mean ± s.d. with CV given in parentheses ([a]n = 6; [b]n = 5). [c]One-way analysis of variance on log-transformed values ($\alpha = 0.05$): Tukey–Kramer simultaneous tests (post-analysis of variance): F1 vs F2 $P < 0.01$; F1 vs F3 $P < 0.01$; F2 vs F3 $P > 0.05$. [d]Kruskal–Wallis test (non-parametric analysis of variance) ($\alpha = 0.05$). [e]One-way analysis of variance on log-transformed values ($\alpha = 0.05$): Tukey simultaneous tests (post-analysis of variance): F1 vs F2 $P < 0.05$; F1 vs F3 $P > 0.05$; F2 vs F3 $P > 0.05$. [f]NT = Not tested. C_{max}, maximum observed plasma concentration; T_{max}, time of maximum observed concentration; AUC_t, area under the plasma sCT concentration–time curve to the last measurable concentration; $F_{rel,}$ bioavailability relative to subcutaneous injection (F4); IU, international units; s.d., standard deviation; CV, coefficient of variation; n, sample size.

recorded for C_{max} and AUC_t, respectively. For F_{rel}, a CV of 20% was recorded for the sCT/chitosan solution compared with values of over 100% for sCT control and Miacalcin. It should be noted that a decreased CV may not purely be due to formulation effects, but can arise in part from a lower assay variability at higher sCT concentration and AUC. CV values are commonly used as a means of comparing variation between treatment groups and, thus, are included herein. However, since the theoretical aspects of its calculation are complex, comparisons can be subjective (Petrie & Watson 1999). In particular, low CV values can arise as a result of high mean values, although for the nasal dose groups since mean and s.d. values of C_{max}/AUC_t were of a similar order of magnitude, then CV values should give a reasonable estimation of variation between treatments. In contrast, values obtained following subcutaneous injection were up to 200-fold higher than those after nasal administration and consequently caution should be taken when comparing the two routes of delivery. Less variability in intranasal drug absorption with use of sCT/chitosan could arise through greater consistency in nasal residence time with use of the chitosan-based formulation (see below for further details).

In man, intranasal administration of 200 IU sCT in the form of Calcitonin-Sandoz (comparable with the Miacalcin used herein) produced C_{max} and AUC_t values of 5 pg mL^{-1} and 294 pg min mL^{-1}, respectively (n = 10 although sCT was only detectable in the plasma of five of the subjects) (Lee et al 1994). These data were consistent with preliminary data obtained by our research group following intranasal administration of a commercial formulation to volunteers at comparable dosage (unpublished data). Normalizing to the dosage given to sheep (17 IU kg^{-1}), this would equate to a C_{max} and AUC of approximately 30 pg mL^{-1} and 1750 pg min mL^{-1}, respectively, comparable with values obtained herein in the sheep (42 pg mL^{-1} and 1636 pg min mL^{-1}). For AUC, a CV of 148% was reported for the commercial nasal spray compared with 47% after intramuscular injection. Such variability observed in man is comparable with that in the sheep (97%) after nasal dosing. Lee et al (1994) evaluated nasal formulations based on the permeation enhancer sodium tauro-24,25-dihydrofusidate (STDHF) in man and reported up to 4-fold improvement in bioavailability of sCT compared with the commercial nasal formulation. With use of STDHF, it was reported that inter-subject variability could be reduced; a CV of 68% was obtained for the AUC values after administering a nasal dose of 205 IU which the authors concluded was acceptable. The C_{max} and AUC values reported by Lee et al (1994) were significantly lower than those reported by Kurose et al (1987) following administration of 200 IU sCT as a nasal spray to healthy volunteers (n = 3); a C_{max} of approximately 70 pg mL^{-1} was obtained with AUC up to 2 h of approximately 5000 pg min mL^{-1}. This equates to a 14-fold difference in terms of C_{max} and more than 17-fold difference in terms of AUC between the two studies. Soane (1999) observed relatively high values in man (C_{max} 73 pg mL^{-1}, AUC 4930 pg min mL^{-1}, normalized to a 200 IU dose, n = 7) after dosing a nasal sCT solution. These data highlight the potential high variability associated with simple aqueous solution formulations of sCT, indeed the manufacturer claims a bioavailability in the range of 0.3% to 30.6% for Miacalcin nasal spray representing a potential 100-fold difference between minimum and maximum bioavailability over this range (Physician's Desk Reference 2003).

Other research groups have administered sCT intranasally to animal models. Dua et al (1997) administered 2000 IU sCT as an isotonic solution to 3 kg rabbits (n = 5) and reported a C_{max} of 10 ng mL^{-1} and AUC of 714 ng min mL^{-1}. Normalized to a 17 IU kg^{-1} dose, this equates to approximately 250 pg mL^{-1} and 18 200 pg min mL^{-1}, respectively. In the rat, intranasal sCT solution (without enhancers)

produced a C_{max} of 64 pg mL^{-1} and an AUC up to 180 min of 6195 pg min mL^{-1} (normalized to a 17 IU kg^{-1} dose) (Sinswat & Tengamnuay 2003). Thus, in view of differences between species in terms of C_{max} and AUC values, care should be taken when utilizing animal data to predict drug absorption in man. However, each species would appear to have merits during proof of principle investigations provided that appropriate comparators are tested.

The magnitude of improvement in nasal sCT absorption demonstrated herein by co-administration with chitosan was of a similar order of magnitude to that previously reported in animals. Based on measurement of hypocalcaemic responses, a twofold increase in nasal sCT absorption in the sheep and fourfold increase in the rat were reported with use of a chitosan glutamate-based solution compared with 'chitosan-free' sCT control solution (Hinchcliffe 1996). Sinswat & Tengamnuay (2003) demonstrated more than a twofold increase in sCT absorption in the rat with use of 10 mg mL^{-1} chitosan (free amine).

The improved absorption of sCT afforded through the use of chitosan could in part to be due to its bioadhesive/mucoadhesive or viscosity enhancing properties, thereby prolonging the residence time of the formulation in the sheep nasal cavity. Soane et al (1999, 2001) demonstrated decreased clearance of chitosan solution from the nasal cavities of man and sheep using the non-invasive technique of gamma-scintigraphy. In man, the half-life of clearance was 21 min for the control solution and 41 min for the chitosan-based solution compared with 15 and 43 min, respectively, in the sheep. It is reasonable to assume that the sCT solution formulations would behave similarly to 'drug-free' formulations in terms of their clearance characteristics. The role of viscosity on nasal sCT absorption was investigated by Dua et al (1997) using methylcellulose, which is a weak bioadhesive/mucoadhesive compared with chitosan. No improvement in sCT absorption was observed in the rabbit with the use of viscous formulations. This could indicate that bioadhesive/mucoadhesive properties rather than the viscosity itself were important characteristics for improved nasal drug delivery. It may indicate that other mechanisms of action may be important. Based on in-vitro studies, chitosan has been reported to transiently open epithelial tight junctions thereby facilitating the paracellular transport of drugs (Artursson et al 1994; Borchard et al 1996; Schipper et al 1997; Junginger & Verhoef 1998; Ranaldi et al 2002; Smith et al 2004). The mechanism of action in-vitro appeared to be related to interaction of (polycationic) chitosan with negatively charged epithelial cells resulting in structural changes in tight junction-associated proteins. However, how relevant this phenomenon is to the ability of chitosan to improve drug absorption in-vivo is unclear.

Conclusions

There were no apparent systemic or local adverse effects observed following intranasal administration of sCT to sheep. After administration of a sCT/chitosan solution, the average bioavailability of sCT was improved twofold compared with a commercially available nasal spray and threefold relative to sCT solution. Drug absorption from the 'chitosan-free' sCT solution did not differ significantly from that attained with Miacalcin nasal spray. Also, there was apparently less variability in intranasal sCT absorption in the sheep after administration of the chitosan-based solution. Based on the sheep data reported herein, a chitosan delivery system could offer the potential to improve the bioavailability of intranasally administered sCT and reduce the variability in absorption. In the clinical setting, this may allow relatively lower doses of the drug to be given intranasally and lead to improvements in the efficacy or quality of intranasal therapy.

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