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Highly Bioavailable Nasal Calcitonin - Potential for Expanded Use in Analgesia

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INTRODUCTION

Nasal calcitonin is currently indicated for the treatment of postmenopausal osteoporosis in females greater than 5 years post menopause with low bone mass relative to healthy premenopausal females. Injectable calcitonin is indicated for the treatment of Paget's disease and for hypercalcemia, as well as for postmenopausal osteoporosis. Throughout the past 2 decades, numerous reports of the highly effective analgesic properties of calcitonin have appeared.¹⁻²⁰ Because calcitonin increases plasma beta-endorphin levels, acting at the hypothalamic and/or at the pituitary level, it is able to relieve pain independently of its peripheral effects on bone.¹

Calcitonin is regarded as a highly safe drug because single doses of salmon calcitonin nasal spray up to 1600 IU, doses up to 800 IU per day for 3 days, and chronic administration of doses up to 600 IU per day have been studied without serious adverse effects.²¹ However, the bioavailability of current FDA-approved nasal salmon calcitonin products is poor, averaging only 3% compared to the bioavailability achieved via the alternate subcutaneous injection route, with a two-order-of-magnitude variable range of 0.3% to 30.3%.²¹ As a result of the low bioavailability and high variability, most studies related to the use of calcitonin in ameliorating pain have been conducted using injected calcitonin rather than the nasally administered calcitonin, presumably to move up the dose-response curve in anticipation that higher systemic blood levels are more efficacious, and to avoid unacceptable variability in systemic blood levels.

The advent of highly effective and non-irritating alkylsaccharide absorption enhancement agents, designated Intravail® excipients, affords a practical opportunity to reconsider the broader use of calcitonin as a highly effective non-invasive analgesic for a variety of bone pain indications.²²⁻²⁸

HIGHLY BIOAVAILABLE NASAL CALCITONIN

Salmon calcitonin has been shown to be highly effective for reducing osteoporotic and vertebral bone fracture pain, opioid resistant metastatic bone cancer pain, post-operative phantom limb pain (acute), sickle-cell bone crisis, post herpetic (shingles) pain, and neuropathic pain.² While injected calcitonin achieves substantially higher circulating blood levels, a non-invasive format is preferred in terms of patient compliance, convenience, ease of self-administration, and avoidance of

needlestick injuries for patients or caregivers.

An open label, balanced, randomized, three-treatment, three-period, three-sequence, single-dose, cross-over bioavailability study to compare the bioavailability of calcitonin from three different formulations (two nasal sprays and a subcutaneous formulation) in 10 healthy adult human subjects under fed conditions was conducted to determine the enhancement in bioavailability resulting from inclusion of Intravail A3 (n-dodecyl- β -D-maltoside) in a standard salmon calcitonin formulation. The addition of Intravail

A3 to a standard metered nasal spray calcitonin formulation resulted in a five-fold increase in average bioavailability from 6.6% for the control without Intravail A3 to 35.9%. Increased systemic bioavailability is expected to increase the clinical usefulness of calcitonin as a safe, non-invasive, non-opioid, analgesic in a number of important underserved clinical indications.

INTRAVAIL® TRANSMUCOSAL ABSORPTION ENHANCERS

Intravail alkylsaccharide excipients comprise a new class of transmucosal

ABSORPTION ENHANCEMENT

absorption enhancers that allow intranasal delivery, or more broadly, transmucosal delivery, of peptide, protein, and non-protein macromolecular therapeutics having molecular weights up to and in excess of 20 KDa, with bioavailabilities up to and in excess of 50% compared to injection.^{26,29}

The particular alkylsaccharides shown to be effective absorption enhancers are non-toxic, non-irritating, chemically synthesized molecules composed of a sugar, typically a disaccharide, and an alkyl chain, typically 10 to 16 carbon atoms in length, linked by an ester or glycosidic bond metabolized to CO₂ and H₂O through the corresponding sugar and fatty acid.³⁰ They provide controlled transient permeation of the nasal mucosal barrier with no irritation.

Preclinical studies in animal models have shown that selected alkylglycosides increase intranasal absorption of salmon calcitonin in a dose-dependent manner. For example, at a tetradecylmaltoside (TDM) concentration of 0.125%, intranasal absorption of salmon calcitonin in the rat is approximately 52% compared to intravenous administration.²⁵ Similar results for increased bioavailability of nasally administered peptides are observed for Intravail A3 at the same concentration.²⁷ Rapid onset of action in 7.5 to 10 minutes was observed, which is important for pain applications. While studies of transmucosal absorption in the rat or rabbit are useful indicators of comparative bioavailability trends, direct extrapolation from animal model bioavailability to bioavailability in humans is not possible.

The purpose of the present study

| Test Article | Cmax | AUC (0-4 hrs) | Tmax | Relative Bioavailability |
|---|-------|---------------|------|--------------------------|
| | pg/mL | pg.hr/mL | hrs | |
| sCalcitonin Injection (Formulation A) | 52.62 | 78.92 | 0.75 | 100% |
| sCalcitonin Nasal Spray Without Dodecylmaltoside (Formulation B) | 12.89 | 5.22 | 0.5 | 6.6% |
| sCalcitonin Nasal Spray With 0.18% Dodecylmaltoside (Formulation C) | 26.18 | 28.35 | 0.25 | 35.9% |

Effect of A3 Upon Pharmacokinetic Parameters for Intranasal Salmon Calcitonin Administration in 10 Normal Female Subjects

was to determine the effectiveness of Intravail A3 alkylsaccharide in increasing absorption of calcitonin in humans in anticipation of its possible use in non-invasive treatment of a number of underserved or orphan indications. The addition of Intravail A3 excipient to a standard metered nasal spray calcitonin formulation resulted in an average bioavailability of 35.9% compared to 6.6% for the control, a five-fold increase in bioavailability. These results are highly encouraging and suggest the clinical use of nasally administered calcitonin may be extended to include a number of important indications in the pain management area.

MATERIALS

Salmon calcitonin is commercially available as nasal spray and injectable formulations sold under the brand names Calcimar and Miacalcin. The concentration of salmon calcitonin in the intranasal formulation is 2200 IU/mL, providing 200 IU of synthetic salmon calcitonin per 91 microliter spray dose.

The concentration of salmon calcitonin in the injectable dosage form is 200 IU/mL.

METHODOLOGY

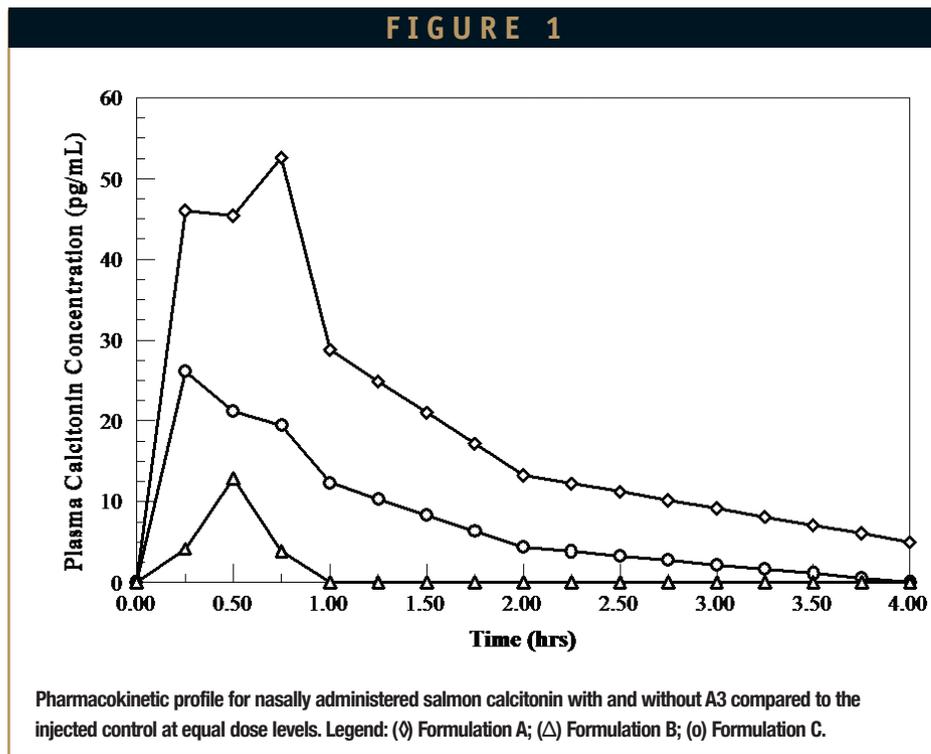
Formulation Preparation

The injectable salmon calcitonin control used in this study (Formulation A) consisted of a 0.33-mL subcutaneous injection of unaltered commercially available injectable salmon calcitonin administered according to the manufacturer's directions, providing a total dose of salmon calcitonin of 66.6 IU, corresponding to 11.1 micrograms based on the international standard value of 6,000 IU/mg.³¹

The nasal salmon calcitonin test articles for this study were obtained by diluting the commercial nasal formulation 1:3 with 30 mM pH 4.5 sodium acetate buffer containing either a) no Intravail, or b) 0.27% (2.7 mg/mL) Intravail A3 excipient in sterile distilled water to yield a first test article containing a final concentration of 733.3 IU/mL salmon calcitonin and no Intravail

ABSORPTION ENHANCEMENT

FIGURE 1



(Formulation B), and a second test article containing 733.3 IU/mL salmon calcitonin and 0.18% Intravail A3 (Formulation C). Each of these two test articles provides 66.6 IU of salmon calcitonin per each 91 microliter spray dose. 1-mL aliquots of each of the two test articles were placed into amber screw-top vials and closed by attaching a 91-microliter metered spray pump manufactured by Pfeiffer GmbH (Radolfzell, Germany) for each subject. Vials were stored and used in an upright position, and the spray pump was primed to displace air bubbles in the pump chamber by depressing the pump two or three times until a uniform fine spray was observed, immediately or shortly prior to administration to the subjects.

Subjects/Study Center

The study was conducted at Apothecaries Limited, 579, Devli, East

Sainik Farms, New Delhi 110 018, India. All participating subjects gave their informed consent. This research was carried out according to the Good Clinical Practice Guidelines as enunciated by the Indian Council of Medical Research (ICMR) and the principles as enunciated in the Declaration of Helsinki, 2000. Informed consent documents, Protocols, and Investigator's Brochures were reviewed and approved by the Institutional Ethics Committee.

A total of 10 adult healthy postmenopausal female subjects between the ages of 40 to 55 years were selected for participation in this study. Subjects were randomized into two sequence groups. The randomization was performed in such a way that each subject received either of the nasal test articles during the first period and the other during the second period. All subjects received the

injectable test article in the third period. All test articles were administered in the study center by the study personnel to ensure compliance with the study protocol. Blood samples were drawn at appropriate intervals to allow determination of plasma salmon calcitonin concentrations.

Analytical Methods

Salmon calcitonin levels in plasma were measured using a commercial Ultrasensitive ELISA salmon calcitonin enzyme immunoassay kit (No. DSL-10-3600) manufactured by Diagnostic Systems Laboratories, Inc., a Division of Beckman Coulter, from DSL India. The theoretical sensitivity, or minimum detection limit, as calculated by interpolation of the mean minus two standard deviations of 13 replicates of the 0 pg/mL salmon calcitonin standard, is 4.2 pg/mL. The standards supplied with the kit for estimation of salmon calcitonin were subjected to a linearity test using the standards provided with the kit, and found to be linear in the range of 7.0 pg/ml to 330 pg/ml of salmon calcitonin. The intra assay precision was $\leq 5\%$, and the inter assay precision was $\leq 9.1\%$. The AUC, T_{max} , C_{max} , and bioavailability was calculated from the plasma level data.

RESULTS & DISCUSSION

The pharmacokinetics profile for the three formulations of calcitonin tested are shown in Figure 1. Six out of 10 subjects showed complete calcitonin clearance at the 4-hour data point, and two subjects showed complete clearance at 2 hours. Therefore, AUC (0 to 4 hrs) was

ABSORPTION ENHANCEMENT

calculated for all 10 subjects. The average values are shown in Table 1.

The AUC (0 to 4) was found to be 5.22 pg.hr/ml for Formulation B and 28.35 pg.hr/ml for Formulation C. The relative bioavailability of Formulation B was 6.6%, and Formulation C was 35.9% with respect to Formulation A after 4 hours of drug administration. The presence of Intravail in Formulation C increases the overall relative bioavailability of calcitonin in plasma. The C_{max} for the injection was approximately 2 to 4 times the values obtained for the nasal spray formulations. The T_{max} ranged from 0.25 to 0.75 hours for the three formulations. The addition of A3 to the salmon calcitonin formulation resulted in a five-fold increase in bioavailability compared to the commercial injectable formulation without A3. The precision observed for the formulation containing Intravail was ± 17%.

In contrast, for the current commercial product, which provides 3% average bioavailability, the precision spans two-orders of magnitude, from 0.3% to 30.6% (Novartis Package Insert). Hence, it can be concluded that Formulation C (calcitonin with A3) showed comparatively better bioavailability compared to Formulation B (calcitonin without Intravail). The presence of Intravail excipient in Formulation C increases the overall relative bioavailability of calcitonin in plasma.

More than two dozen scientific publications throughout the past 2 decades have indicated that salmon calcitonin has significant potential in the

| TABLE 2 | | | | |
|---|-------------------------------|--------------------------|-------------------------|-----------|
| Test Article | Excipient | Absolute Bioavailability | Total Dose Administered | Reference |
| Intravenous Calcitonin Control | None | 100% | 10 IU/kg | 32 |
| Nasal Calcitonin, in pH 4 Isotonic Phosphate Buffer | 1% Chitosan Free Amine | 2.45% | 10 IU/kg | |
| Nasal Calcitonin in pH 4 Isotonic Phosphate Buffer | 5% Dimethyl-beta-cyclodextrin | 1.91% | 10 IU/kg | |
| Nasal Calcitonin in pH 4 Isotonic Phosphate Buffer Control | None | 1.22% | 10 IU/kg | |
| Nasal Calcitonin in pH 3.75 6 mM Sodium Acetate, 0.9% Sodium Chloride | 0.125% Tetradecyl Maltoside | 52% | 8 IU/kg | 25 |

Comparison of Observed Bioavailabilities for Calcitonin in the Rat

treatment of a variety of underserved pain treatment applications, such as opioid-resistant metastatic bone cancer pain, acute phantom limb pain following amputation, vertebral fractures, and Sickle Cell disease-related bone pain. Most of the reported studies utilized injectable calcitonin, presumably because of the very low bioavailability and high variability observed with the current commercially available metered nasal spray products.

Calcitonin has been shown to be a safe and effective drug for current applications, even at concentrations considerably higher than those used to induce analgesia. Because of its peptidic nature, calcitonin is essentially free of any chemical toxicity issues. Calcitonin is also non-addictive and therefore not subject to abuse or diversion to non-clinical applications. In addition, it is free of many undesirable side effects associated with opioid administration, such as respiratory and circulatory

depression, apnea, respiratory arrest, constipation, light-headedness, dizziness, and sedation.

Previously, a number of attempts have been made to overcome these limitations, in particular using two classes of extensively studied absorption-enhancement excipients, namely the chitosans and the cyclodextrins. In a side-by-side comparison, the absorption-enhancement properties of representative members of each of these families for calcitonin compared to intravenous administration were studied, allowing an absolute intranasal bioavailability in the rat to be determined.³² The results for chitosan, dimethyl beta cyclodextrin, and tetradecyl maltoside, a representative alkylsaccharide excipient, are summarized and compared in Table 2. The formulation containing alkylsaccharide excipient is significantly more effective than either of the formulations containing chitosan or dimethyl beta cyclodextrin.

ABSORPTION ENHANCEMENT

CONCLUSION

Salmon calcitonin formulations containing Intravail provide greatly increased systemic bioavailability. The increased bioavailability and decreased variability may provide opportunities for increased non-invasive applications of salmon calcitonin in the treatment of pain for a number of underserved clinical indications, such as opioid-resistant metastatic bone cancer pain, acute phantom limb pain following amputation, vertebral fractures, and Sickle Cell disease-related bone pain.

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BIOGRAPHIES



Dr. Edward T. Maggio currently serves as the CEO of Aegis Therapeutics. He has been a founder and board member of 7 public and private life science companies in San Diego and one in Copenhagen. He serves on various

departmental advisory boards for NYU's Polytechnic Institute, the University of California, Cal State University. Dr. Maggio has co-authored more than 30 book chapters and scientific articles and is an inventor on more than three dozen issued and pending US and foreign patents in the biotechnology area.



Dr. Elias Meezan is Professor Emeritus and former Chairman of Pharmacology and Toxicology at the University of Alabama at Birmingham and is a co-inventor of the patented Intravail® drug delivery

technology. He has authored or co-authored more than 100 scientific publications in areas including the development of widely used methods for the isolation of brain and retinal microvessels and the biochemical pharmacology of alkylmaltosides and their applications in treating diabetes and cystic fibrosis.



Dr. DKS Ghambeer is a Clinical Trials Investigator at Apothecaries Limited in New Delhi, India, and has conducted sponsored clinical research studies on a range of medical devices and drugs for Apothecaries' multinational client base.



Dr. Dennis Pillion is Professor of Pharmacology & Toxicology at the UAB School of Medicine and is co-inventor of the patented Intravail® drug delivery technology. He has been a diabetes researcher and educator for the past 30

years and is studying non-invasive delivery of long-acting and short-acting insulins and other peptide therapeutics using ocular, nasal, or oral formulations containing novel absorption-enhancing agents.