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RESEARCH**

APPLICATION NUMBER:

21-406

PHARMACOLOGY REVIEW

**PHARMACOLOGY AND TOXICOLOGY REVIEW OF NDA
(RESUBMISSION)**

NDA #: 21-406

Product Name : Fortical® Nasal Spray (calcitonin-salmon)

Sponsor: Unigene Laboratories, Inc., Fairfield, New Jersey

Indication: Osteoporosis

Division: HFD-510 (DMEDP)

Submission date: September 15, 2004

Submission type 3S (complete response)

Reviewer: Gemma Kuijpers

Date: February 1, 2005

EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability

Approval (AP)

From the point of view of Pharmacology/Toxicology, the Sponsor has provided adequate and sufficient nonclinical information to support the safety of Fortical Nasal Spray according to recommended clinical use. Upon agreement on the proposed labeling, the product can be approved.

1.2 Recommendation for nonclinical studies

No further studies are required.

1.3 Recommendations on labeling

See 3.7. APPENDIX, LABEL

2. Summary of nonclinical findings

The original NDA (#21-406) for this product (Fortical® Nasal Spray) was submitted on March 5, 2003 as a 505b(2) application, for the indication of treatment of osteoporosis. This NDA was reviewed by the Pharmacology/Toxicology Reviewer (NDA Review, NDA #21-406, December 4, 2003).

Data obtained in non-clinical studies indicated that recombinant salmon calcitonin (rsCT) in Fortical Nasal Spray is bioequivalent with synthetic sCT. Toxicological evaluation did not provide any indication that the drug product will cause unexpected toxicity. An immunogenicity study in rats showed that there was no tachyphylaxis with either rsCT or ssCT (NDA Review, NDA #21-406, December 4, 2003).

Upon review of the original NDA #21-406, Reviewer concluded that the nonclinical toxicology testing was adequate and that the data indicated that the recombinant salmon calcitonin product (Fortical® Nasal Spray) is not likely to produce unexpected toxicities. Therefore, the Pharmacology/Toxicology Reviewer recommended that NDA #21-406 be approved. An action letter was sent to the company on Dec 31, 2003, with an approvable (AE) decision based on the lack of clinical immunogenicity study data.

In a complete response to the AE action letter, the sponsor submitted results from patients enrolled in clinical study UGL-N9904, from serum obtained at time 0 (screening) and at 24 weeks. New preclinical data were not submitted.

2.2 Nonclinical safety issues relevant to clinical use

Based on nonclinical studies, Fortical® Nasal Spray may cause an increased incidence of rhinitis.

3. Pharmacology/Toxicology Review

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

The data obtained in non-clinical studies submitted to NDA 21-406 indicate that recombinant salmon calcitonin (rsCT) in Fortical® Nasal Spray is bioequivalent with synthetic sCT. Toxicological evaluation did not provide any indication that the drug product will cause unexpected toxicity.

Recommendations:

AP

Labeling:

In the Pharmacology/Toxicology review of the original NDA, Reviewer concluded that the findings from carcinogenicity and reproductive toxicity studies with the marketed synthetic product (Miacalcin®) can be included in the Fortical® label. In addition, Reviewer suggested a number of revisions to the label. The Sponsor submitted a new label in the (re)submission of the NDA on September 15, 2004, in which the sections relevant for pharmacology/toxicology were not changed as compared to the label submitted with the original NDA 21-406.

Since the application is a 505(b)2 application, the label needs to be identical to the innovator's product's label (Novartis, Miacalcin®, Nasal Spray). The Sponsor carried out their own genotoxicity studies (modified Ames test, chromosome aberration test in CHO cells) with Fortical®, but the data are similar to those obtained in studies submitted for the Miacalcin® NDA. Thus, the changes previously suggested by the Reviewer (Review December 4, 2003) are not required or recommended, and the label for Fortical® can be the same as the one for Miacalcin®.

Attached are the submitted labeling sections relevant for Pharmacology/Toxicology, including changes proposed by Reviewer in the "PRECAUTIONS" section.

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 X Draft Labeling

 Deliberative Process

COMMENT TO LABEL

The clinical pharmacology section of the label contains mostly information from the Miacalcin label, including animal data on the action of endogenous calcitonin. This section of the label needs reorganization upon review by Clinical Pharmacology and Medical Reviewers.

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/s/

Gemma Kuijpers
2/1/05 11:50:35 AM
PHARMACOLOGIST

Karen Davis-Bruno
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PHARMACOLOGIST
concur with recommendations

PHARMACOLOGY AND TOXICOLOGY REVIEW OF NDA

NDA #: 21-406

Product Name : Fortical® Nasal Spray (calcitonin-salmon)

Sponsor: Unigene Laboratories, Inc., Fairfield, New Jersey

Indication: Osteoporosis

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Reviewer: Gemma Kuijpers

Date: December 4, 2003

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EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability

Approval (AP)

From the perspective of Pharmacology/Toxicology, the Sponsor has provided adequate and sufficient nonclinical information to support the safety of Fortical Nasal Spray according to recommended clinical use. Upon agreement on the proposed labeling, the product can be approved.

1.2 Recommendation for nonclinical studies

No further studies are required.

1.3 Recommendations on labeling

See Label (APPENDIX)

2. Summary of nonclinical findings

2.1 Overview of nonclinical findings

This 505b(2) application is for Fortical (recombinant salmon calcitonin) Intranasal Spray for the indication of treatment of postmenopausal osteoporosis.

Salmon calcitonin, a 32-amino acid peptide hormone, is currently marketed in the US for the treatment of diseases of bone metabolism in a synthetic form, under the name of Miacalcin (Nasal Spray, or Injection) (Novartis Pharmaceuticals). The proposed dose of Fortical Nasal Spray is 200 IU daily (90 uL/day of a 2200IU/mL solution). This is the same as the recommended dose of Miacalcin Nasal Spray.

The chemical structure of recombinant salmon calcitonin (rsCT), the active ingredient in Fortical Nasal Spray, was shown to be identical to that of the chemically synthesized peptide (ssCT). The drug product contains a number of impurities (mainly degradants) that are subject to regulations regarding nonclinical toxicity testing. The nonclinical testing program was discussed with Sponsor in pre-IND and NDA meetings. The main nonclinical requirement was a 28-day intranasal rat toxicity study. This study was not previously submitted to the IND. The information submitted to the NDA was adequate to support the marketing application.

Pharmacology

Fortical was tested in nonclinical pharmacology studies carried out with rsCT produced by the direct expression method ("rsCT-DE") or an earlier process involving a fusion protein ("rsCT-FP"). In *in vivo* studies in rats and dogs, Sponsor demonstrated that recombinant salmon calcitonin (rsCT-FP or rsCT-DE) decreases serum calcium and

increases serum cAMP. The data showed that the recombinant peptide has a pharmacologic action equivalent to that of Miacalcin (synthetic sCT) or a standardized preparation of sCT. The studies support the efficacy of rsCT to inhibit bone resorption and turnover.

In the mouse, rsCT-FP and ssCT at high human dose multiples had no effect on general activity and behavior.

Pharmacokinetics

The biological equivalence of recombinant and synthetic sCT was confirmed in nonclinical pharmacokinetic studies. Both recombinant peptides (rsCT-FP and rsCT-DE) were bioequivalent with ssCT when administered to rats or dogs by the intravenous and subcutaneous route. Studies in the rat showed that rsCT formulated in a solution containing benzyl alcohol, phenylethyl alcohol, Tween-80 and citric acid has similar nasal bioavailability as ssCT formulated with benzalkonium chloride.

Toxicology

Toxicology studies were carried out to ensure that the recombinant product is not associated with unexpected toxicity. An other important objective was the qualification of product-specific impurities and excipients.

In acute toxicity studies in rats and dogs, behavioral inactivity and GI toxicity were observed at very high doses. Although parameters evaluated were limited, results suggested that rsCT produced no unexpected toxicity. The studies were carried out with either rsCT-DE or rsCT-FP. Since the two peptides are chemically and biologically equivalent and their impurity profiles are likely to be similar, studies with either peptide were relevant.

In an acute toxicity study in rats with a high dose of a rsCT formulation with high levels of degradants, there were mild lung findings at gross necropsy. It was unclear whether these findings were drug- or degradant-related. Based on large clinical dose multiples and the absence of this finding in the subchronic rat nasal toxicity study, the finding is unlikely to be of clinical relevance.

In a 28-day intranasal study in the rat, local toxicity of the nasal preparation containing rsCT-DE and excipients (benzyl alcohol, phenylethyl alcohol, Tween-80 and citric acid) was thoroughly evaluated. The data showed that there was a slight increase in the incidence of focal rhinitis in males, possibly related to the Fortical excipients. There were no other adverse effects in the respiratory tract.

Clinically, rhinitis is observed with chronic use of Miacalcin Nasal Spray. Other adverse events of calcitonin include nausea, vomiting, flushing, and are mainly associated with the higher doses administered using the injectable form.

Two *in vitro* mutagenicity studies were carried out with rsCT-FP, a modified Ames test, and a mammalian cell cytogenicity test in Chinese hamster ovary (CHO) cells. The compound tested negative in both assays.

Information on the carcinogenic potential of calcitonin has been obtained in studies with ssCT. Carcinogenicity studies were not performed with the recombinant product, since there were no signals to prompt carcinogenicity testing of the proposed formulation. In a 1-year toxicity study with ssCT an increased incidence of pituitary tumors was observed in Sprague Dawley rats. Sponsor included the information obtained with ssCT (Miacalcin) in the Precautions section of the proposed labeling.

Reproductive toxicity studies were not performed with recombinant product. Sponsor stated that the studies were not needed since the product is for an indication in postmenopausal women. Studies with ssCT showed that calcitonin reduces fetal weight in rabbits, and decreases the lactation index in rats. Sponsor included reproductive study findings with ssCT (Miacalcin) in the proposed label.

An immunogenicity study in rats showed that there was no tachyphylaxis with either rsCT or ssCT.

Reviewer concluded that the nonclinical toxicology testing was adequate. The data indicate that the recombinant salmon calcitonin product (Fortical Nasal Spray) is not likely to produce unexpected toxicities. The findings from carcinogenicity and reproductive toxicity studies with the marketed synthetic product (Miacalcin) can be included in the label.

2.2 Nonclinical safety issues relevant to clinical use

Based on nonclinical studies, Fortical Nasal Spray may cause an increased incidence of rhinitis.

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PHARMACOLOGY/TOXICOLOGY REVIEW

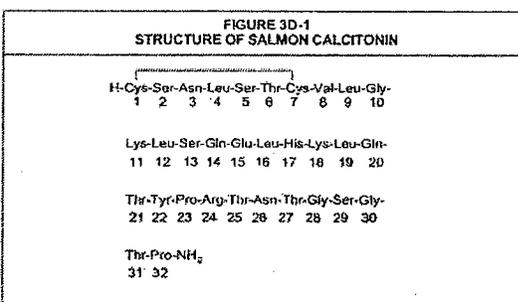
NDA number: 21-406
Submission date/type of submission: March 5, 2003/ 505(b)2
Information to sponsor: Yes (X) (labeling changes)
Sponsor and/or agent: Unigene Laboratories, Inc., New Jersey
Manufacturer for drug substance: Unigene Laboratories, Boonton, NJ

Reviewer name: Gemma Kuijpers
Division name: Division
HFD #: 510
Review number: 1
Review completion date: September

Drug:

Drug substance: Recombinant salmon calcitonin (rsCT)
Trade name: Fortical®
Generic name: Calcitonin
USAN name: salmon calcitonin
INN name: calcitonin (salmon), or calcitonin - salmon
CAS registry number: [47931-85-1]
Molecular formula/molecular weight: C₁₄₅H₂₄₀N₄₄O₄₈S₂ /
acid peptide

Structure:



Relevant INDs/NDAs/DMFs: IND 59,664 (Fortical Nasal Spray)
NDA 20,313 (Miacalcin Nasal Spray)
NDA 17,808 (Miacalcin Injection)
DMF

Drug class: Peptide hormone

Indication: Treatment of postmenopausal osteoporosis

Clinical formulation: Nasal Spray (2200 IU/mL)

Route of administration:

Nasal (0.09 mL per actuation)

Drug product

| Ingredient | Concentration (per vial) |
|-------------------------------|--------------------------|
| rsCT | 0.367 mg/mL (2200 IU/mL) |
| Citric acid, Monohydrate, USP | |
| Polysorbate 80, NF | |
| Benzyl Alcohol, NF | |
| Phenylethyl Alcohol, USP | |
| Sodium Chloride, USP | |
| Sodium Hydroxide, NF | |
| Hydrochloric Acid, NF | |
| Purified Water, USP | |

Proposed use: Product is to be used for the treatment of postmenopausal osteoporosis. Calcitonin is believed to inhibit bone resorption and thus increase bone mineral density (BMD), which may reduce risk of skeletal fractures. Spray is provided in 3.5 mL filled amber glass bottle. Dosage strength is 200 IU of calcitonin-salmon per activation. Recommended dose is one spray (0.09 mL, 200 IU) per day administered intranasally, alternating nostrils daily.

Disclaimer: Part of the tabular and graphical information in this review were copied from the NDA submission.

NDA type: This NDA was submitted as a 505b(2) application and the information was partly obtained from published studies and studies carried out for the marketed product (Miacalcin).

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3.1 INTRODUCTION

Calcitonin

The calciotropic peptide hormone calcitonin is involved in calcium and phosphorus homeostasis. In mammals, the major source of calcitonin is from the parafollicular or C cells in the thyroid gland, but it is also synthesized in a wide variety of other tissues, including the lung and intestinal tract. In birds, fish and amphibians, calcitonin is secreted from the ultimobranchial glands. In comparison to calcitonins from other species, salmon calcitonin is the most similar in structure to human calcitonin and has the greatest biological activity.

Calcitonin lowers blood calcium and phosphorus levels primarily by inhibiting osteoclastic bone resorption. Calcitonin also inhibits renal tubular calcium and phosphorus reabsorption, and mediates deposition of postabsorptive calcium into bone following a meal. The calcitonin receptor is a G-protein coupled receptor coupled by G_s to adenylyl cyclase and the generation of cAMP. Calcitonin causes a rapid and sustained rise in plasma cyclic AMP. Secretion of the hormone by thyroid C-cells is stimulated by increased serum calcium levels through activation of the calcium sensing receptor. Calcitonin secretion is also regulated by other hormones such as glucagon, gastrin and serotonin. Metabolic degradation of calcitonin is thought to occur in kidney and liver. Calcitonin at high doses can paradoxically increase intestinal calcium absorption. Calcitonin can also increase jejunal mineral and water secretion and reduce gastric acid secretion. Although some physiological effects have been characterized, the essential role of calcitonin in terrestrial animals and humans remains unclear. However, a number of therapeutic uses of calcitonin have been developed.

Therapeutic use and formulations

Based on its antiresorptive action in bone, calcitonin is clinically used for the treatment of Paget's disease, hypercalcemia of malignancy and postmenopausal osteoporosis. Since it is a peptide hormone it does not reach the systemic circulation via the oral route. Calcitonin is currently marketed by Novartis Pharmaceuticals as Miacalcin Nasal Spray and Miacalcin Injection. Both products contain synthetic salmon calcitonin as active ingredient. Miacalcin Nasal Spray is indicated for the treatment of postmenopausal osteoporosis in women. Miacalcin Nasal Spray is composed of synthetic salmon calcitonin (ssCT) (2200 IU/mL), benzalkonium chloride (0.2 mg/mL), NaCl (8.5 mg/mL), trace HCl, purified water, nitrogen. Unigene Laboratories, Inc. has developed recombinant DNA processes for producing salmon calcitonin. For the Fortical Nasal Spray, the recombinant salmon calcitonin (rsCT) has been formulated in a solution containing citric acid, Tween 80 (polysorbate 80), phenylethyl alcohol, benzyl alcohol, NaCl, and trace HCl and NaOH.

Fortical Nasal Spray

Calcitonin is a 32-amino acid peptide with an approximate weight of

Unigene produces the recombinant salmon calcitonin peptide under current Good Manufacturing Practices (cGMP).

Nonclinical studies with Fortical

Several of the earlier nonclinical studies (pharmacology, pharmacokinetics, acute toxicity, local tolerance, immunogenicity) were conducted with the recombinant sCT-FP protein, mainly by the SC or IV route. Subsequently, nonclinical testing of rsCT-DE (also called rsCT) was carried out in different formulations with different buffers and excipients (PD and acute toxicity by IV or SC route, PK by nasal route, repeat dose toxicity by nasal route). The main purpose of the pharmacology studies was to demonstrate equivalence with the synthetic peptide (ssCT). The PK studies were carried out to characterize the PK profile of rsCT by different administration routes and compare it with ssCT. The aim of the toxicology studies was to assess the toxicity profile of the recombinant peptide and to compare toxicity of rsCT with ssCT. The 28-day rat

intranasal toxicity study was the principal nonclinical toxicology requirement. It was performed with rsCT formulated as in the commercial product, however, with ~~citric acid~~ rather than ~~citric acid~~.

OVERVIEW OF NONCLINICAL STUDIES (TABEL 3E-1)

| TABLE 3E-1 SUMMARY OF NONCLINICAL STUDIES | | | | |
|--|---------------|-------------------------|---|----------------------|
| Study No. | Type of rsCT | Species and Route | Primary Assessment | Location of Report |
| rsCT P9906Rp | rsCT, rsCT-FP | Rat, IV, SC | Pharmacokinetic, Pharmacology | Volume 10, Page 076 |
| Sinko et al. 1995 | rsCT-FP | Rat, IV, SC | Pharmacology/ Acute Toxicity | Volume 10, Page 070 |
| rsCT Pc9604Rp (Study 1) | rsCT-FP | Rat, SC | Pharmacology | Volume 10, Page 097A |
| rsCT Pc9604Rp (Study 2) | rsCT-FP | Rat, SC | Pharmacology | Volume 10, Page 097A |
| rsCT-Pc9603Rp | rsCT-FP | Rat, SC | Pharmacology/ Pharmacokinetic | Volume 10, Page 146 |
| Study No. 94-3209 | rsCT-FP | Dog, IV | Pharmacology/ Acute Toxicity/ Pharmacokinetic | Volume 10, Page 171 |
| 1470/8-1035 | rsCT-FP | Mouse, IV | Pharmacology | Volume 11, Page 001 |
| rsCT T9905Rp | rsCT | Rat, IV | Acute Toxicity | Volume 11, Page 179 |
| rsCT T9601Dp | rsCT-FP | Dog, IV | Acute Toxicity | Volume 11, Page 271 |
| rsCT T9903Rn | rsCT | Rat, Nasal | Subchronic Toxicity | Volume 12, Page 020 |
| 1470/003-1032 | rsCT-FP | Rat, IV | Special Toxicity/Local Tolerance | Volume 12, Page 123 |
| 1470/009-1032 | rsCT-FP | Rat, IV | Special Toxicity/Local Tolerance | Volume 12, Page 145 |
| 1470/004-1032 | rsCT-FP | Rat, IM | Special Toxicity/Local Tolerance | Volume 12, Page 168 |
| 1470/001-1032 | rsCT-FP | Rabbit, IA ¹ | Special Toxicity/Local Tolerance | Volume 12, Page 191 |
| 1470/002-1032 | rsCT-FP | Rabbit, PV ² | Special Toxicity/Local Tolerance | Volume 12, Page 212 |
| rsCT T9701Rp | rsCT-FP | Rat, IM | Special Toxicity/ Immunogenicity | Volume 12, Page 233 |
| 1470/6-1052 | rsCT-FP | <i>in vitro</i> | Mutagenicity | Volume 13, Page 010 |
| 1470/7-1052 | rsCT-FP | <i>in vitro</i> | Mutagenicity | Volume 13, Page 083 |
| rsCT P9904aRn | rsCT | Rat, Nasal | Pharmacokinetic | Volume 13, Page 211 |
| rsCT P9902aRn | rsCT | Rat, Nasal | Pharmacokinetic | Volume 13, Page 225 |
| rsCT P9601Rp | rsCT-FP | Rat, IV | Pharmacokinetic | Volume 13, Page 242 |
| rsCT P9602Rp | rsCT-FP | Rat, SC | Pharmacokinetic | Volume 14, Page 001 |
| rsCT P9601Dp | rsCT-FP | Dog, IV | Pharmacokinetic | Volume 14, Page 133 |
| rsCT P9602Dp | rsCT-FP | Dog, SC | Pharmacokinetic | Volume 14, Page 153 |

1 = Intra-arterial, 2 = paravenous

UGL-N9904
Phase II/III
Pharmacology
and Tolerability
Study

Fortical B Nasal Spray (CTM0011)
(Same as above)

Volume 30,
Page 004

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3.2 PHARMACOLOGY

3.2.1 Summary

The primary pharmacological action of calcitonin is an inhibition of osteoclast activity and renal Ca reabsorption resulting in decreased serum calcium levels.

In a rat study using subcutaneous dosing, the hypocalcemic effect of rsCT-DE or rsCT-FP was shown to be equivalent to that of the second international standard of calcitonin (2nd IS). Additional studies in rats and dogs showed that rsCT-FP and ssCT have similar calcium-lowering effects when given by intravenous or subcutaneous administration. Both rsCT and ssCT increase serum cAMP. Pharmacologically effective doses in the rat (≥ 0.01 ug/kg, s.c.) were much lower than those used in acute or repeat toxicity studies.

RsCT-FP and ssCT at high human dose multiples had no effect on general activity and behavior in the CD-1 mouse.

Pharmacology studies in animals have shown that calcitonin can suppress bone turnover as indicated by changes in biochemical and histological markers (published data).

3.2.2 Pharmacodynamics

A comparative i.v. PD/PK study of the two recombinant versions of calcitonin, rsCT-FP or rsCT-DE, and the 2nd IS for sCT was carried out in rats (Study rsCT P9906Rp). The data showed that RsCT-DE has the same biologic activity in the standard rat hypocalcemia assay as rsCT-FP and the 2nd International Standard for sCT (Review #1, IND.#59,664, APPENDIX). The highest dose in this study (0.054 ug/kg, SC) is at least 100,000 times lower than the dose tested in the acute toxicity study with 20,000 ug/kg (IV).

The PD response to rsCT-FP as compared to ssCT (synthetic salmon calcitonin) was evaluated upon s.c. and i.v. injection in rats and dogs. The results indicated qualitative and quantitative similarity between rsCT-FP and ssCT in the ability to induce a hypocalcemic response. Data also showed that rsCT-FP and ssCT induce an equivalent increase in plasma cAMP upon s.c. administration in the rat (Study rsCT Pc9603Rp).

| STUDY | TEST MODEL | ROUTE OF ADMIN. | DOSE OR DOSE RANGE | ACTIVITY COMPARISON | RESULTS |
|--|------------|-----------------|---|--------------------------------------|---|
| Study with rsCT made by Direct Expression Process | | | | | |
| rsCT P9906Rp | Rat | SC | 90 - 360 mIU/kg (0.014 - 0.054 μ g/kg) | 2 nd IS, rsCT-FP | demonstrated equivalent hypocalcemic effect for rsCT, rsCT-FP and 2 nd IS |
| Studies with rsCT made by Fusion Protein Process (rsCT-FP) | | | | | |
| Sinko et al, 1995 | Rat | IV | 5.7 - 57 μ g/kg | none | Calcium-lowering effect was dose-dependent |
| Sinko et al, 1995 | Rats | SC | 0.017 - 57 μ g/kg | vehicle control | Calcium-lowering effect was dose-dependent and saturable |
| rsCT Pc9604Rp, Study 1 | Rats | SC | 60 - 240 mIU/kg (0.009 - 0.036 μ g/kg) | ssCT (Miacalcin), 2 nd IS | No significant difference in hypocalcemic response for the three groups |
| rsCT Pc9604Rp, Study 2 | Rats | SC | 75 - 300 mIU/kg (0.011 - 0.045 μ g/kg) | 2 nd IS | No significant difference in hypocalcemic response for the rsCT-FP and 2 nd IS |
| rsCT Pc9603Rp | Rats | SC | 1 μ g (approx. 5.7 μ g/kg) | ssCT (Miacalcin) | Both treatment groups showed a 40% decrease in plasma Ca conc., AUC for two treatments not significantly different |
| Study #94-3209 | Dogs | IV | 16.7 - 68.7 μ g/kg | ssCT (Bachem) | Both treatment groups showed a 25% decrease in plasma Ca conc., response for the two treatments not significantly different |

Sponsor did not conduct preclinical studies using rsCT to evaluate effects on parameters other than plasma calcium and cAMP. However, Sponsor described data from published studies on the effects of sCT on bone quality and turnover. In models of cancellous bone loss such as the OVX rat, sCT increases bone mass, inhibits bone turnover and preserves cancellous bone microarchitecture and strength. In OVX sheep, sCT had no deleterious effects on bone mineralization.

3.2.4 Safety pharmacology

In a study in CD-1 mice (Study No. 1470/8-1035) with 50 ug/kg rsCT-FP or sCT, single dose, IV injection, there were no effects on general activity and behavior in the rsCT-FP group (Irwin screen) as compared to vehicle control or sCT groups. The dose was a multiple of 3333x (mg/kg basis) and 278x (mg/m² basis) times the intended human nasal dose of 200 IU (0.5 ug/kg), assuming 3% bioavailability of a nasal dose relative to an IV dose.

3.2.5 Pharmacodynamic drug interactions

There were no studies on the interaction of rsCT with other drugs.

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3.3 PHARMACOKINETICS/TOXICOKINETICS

3.3.1 Summary

PK studies served to demonstrate bioequivalence of different sCT products and effects of excipients on nasal bioavailability of rsCT.

In a rat study using the intravenous dosing route, it was shown that rsCT-DE and rsCT-FP are bioequivalent (Study rsCT 9906Rp). Studies with rsCT-DE by the intranasal route in rats showed that the preservatives phenylethyl alcohol and benzyl alcohol did not significantly affect absorption (Study rsCT 9904Rn). The addition of citrate to the rsCT formulation, however, increased nasal bioavailability in the rat to levels comparable to those seen with the marketed ssCT formulation containing benzalkonium chloride (Studies rsCT P9902Rn). These studies were reviewed in detail in the IND review (IND 59,664, APPENDIX).

Pharmacokinetic parameters for rsCT-FP and ssCT were determined in IV and SC studies in rats and dogs (Sponsor's Table 30). Data showed dose-linearity of C_{max} and AUC. The PK profile of rsCT-FP as compared to ssCT suggests that the two compounds are bioequivalent.

| STUDY | SPECIES N, SEX | DOSE RANGE | VEHICLE | ROUTE OF ADMIN | PK PARAMETERS |
|-----------------|-------------------|---|---------|----------------------|---|
| rsCT P9902Rn | Rats 61 F | 5 - 20 µg (approx. 20 - 80 µg/kg) | 1 | IN | Comparative study assessing bioavailability obtained with different formulations |
| rsCT P9906Rp | Rats 6 F | 2 µg (approx. 8 µg/kg) | 4 | IV | Comparative study to obtain PK parameters for rsCT and rsCT- FP. No statistical difference in AUC, t _{1/2} , CL, Vd, or K _e for the two peptides. |

1 = Comparative study of different formulations containing different combinations of the following:
benzalkonium chloride, benzy alcohol, phenylethyl alcohol, Tween 80, HCl and citric acid

4 = Physiological saline

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PK parameters for rats and human are summarized in the Reviewer's Tables below.

In humans, mean bioavailability of Miacalcin intranasal spray is estimated to be 3% of that of injectable (s.c.) calcitonin, but is highly variable (range 0.3%-31%) (Miacalcin label). Intramuscular and subcutaneous bioavailability of ssCT in humans is appr. 70% (Beveridge et al, 1976). Thus, absolute BA of intranasal sCT (Miacalcin or Fortical) is <3%. The clinical data submitted to this NDA show that nasal bioavailability of Miacalcin and Fortical in humans is approximately the same. For calculation of dose or exposure multiples in toxicity studies, nasal bioavailability of rsCT or ssCT in humans was assumed to be 3%.

PK parameters in rat

| Study | Species | Route | Vehicle | Test article | Dose (ug/kg) | Cmax (ng/mL) | AUC (ngxmin/mL) | T1/2 (min) | BA (%) |
|---------------|--------------|-------|--------------------------------|--------------|--------------|--------------|-----------------|------------|--------|
| rsCT9906Rp | Rat (Wistar) | IV | saline | rsCT | 8 | - | 479 | | |
| | | | | rsCT-FP | 8 | - | 450 | | |
| rsCT P9601Rp | Rat (Wistar) | IV | saline | rsCT-FP | 4.2 | - | 291 | 47 | |
| | | | | | 24 | - | 1361 | 32 | |
| | | | | | 40 | - | 3298 | 40 | |
| rsCT P9602Rp | Rat (Wistar) | SC | water | rsCT-FP | 6.5 | 0.78 | 97 | 63 | 24 |
| | | | | | 55 | 6.1 | 708 | 86 | 18 |
| | | | | | 125 | 15 | 1186 | 56 | 12 |
| | | | | | 474 | 54 | 5740 | 68 | 14 |
| rsCT Pc9603Rp | Rat (Wistar) | SC | Water, phenol, HAc, NaAc, NaCl | rsCT-FP | 5.7 | 0.38 | 47 | 111 | 28 |
| | | | | ssCT | 5.7 | 0.33 | 46 | 102 | 22 |
| rsCT P0208Rp | Rat | IM | T80, BA, PA, citric acid, NaCl | RsCT-FP | 587 | 141 | 10095 | 71 | 16.8 |

PK parameters in humans

| Study | Species | Route | Vehicle | Test article | Dose | Cmax (pg/mL) | AUC (pgxmin/mL) |
|------------------------|---------|-------|--------------------------------|--------------|---------|--------------|-----------------|
| (Lee et al, 1994) | Human | IN | NaCl, BenzalkCl | Miacalcin | 200 IU | 5 | 294 |
| Thamsborg et al (1990) | Human | IN | NaCl, BenzalkCl | Miacalcin | 200 IU | ? | 1100 |
| UGL-N9901 | Human | IN | NaCl, T80, PA, BA | rsCT-DE | 2000 IU | 39 | 666 |
| | | | NaCl, T80, PA, BA, citric acid | rsCT-DE | 2000 IU | 43 | 733 |
| | | | NaCl, Benz alk Cl | Miacalcin | 2000 IU | 50 | 872 |
| UGL-N9903 | Human | IN | NaCl, T80, PA, BA, citric acid | rsCT-DE | 2400 IU | 69 | 1155 |
| | | | NaCl, T80, PA, BA, citric acid | rsCT-DE | 2400 IU | 67 | 1160 |
| | | | NaCl, Benzalk Cl | Miacalcin | 2400 IU | 54 | 977 |

* marketed formulation

T1/2 = 23 min (N9901), and 25-30 min (N9903)

3.4 TOXICOLOGY

3.4.1 Overall toxicology summary

In an acute rat IV study at a very high dose, rsCT-DE caused shivering and inactivity up to 1h after dosing. In the dog, high IV doses of rsCT-FP and ssCT induced GI toxicity (emesis, salivation) in one study, and retching and lethargy in another study. In these studies, rsCT was administered in saline or water. There were no cardiotoxic effects in the dog. CNS toxicity has been observed with sCT (NDA 17,808), and GI toxicity is an expected event with calcitonin. Since the rsCT-DE and rsCT-FP peptides are chemically and biologically equivalent and their impurity profiles are similar (*Sponsor, personal communication, will be provided*) studies with either peptide are relevant. In conclusion, no unexpected toxicities were observed with the recombinant product.

According to the ICH Guidance for Industry (Q3A, Impurities in New Drug Substances, and Q3B, Impurities in New Drug Products), it is recommended that safety information is provided on product-related impurities and degradation products. The qualification threshold for impurities is — for products dosed at ≤ 2 g/day, and the threshold for degradants is — for doses < 10 mg. Several impurities are present in Fortical Nasal Spray at levels above these thresholds.

An acute rat toxicity study was performed via the intramuscular route with a batch of rsCT-DE that had been stored for 9 months (25°C) and in which the levels of degradation products were near or above specification limits. The vehicle in this study was a solution containing the excipients present in the commercial product. Slight changes were observed in the lung at very high human exposure multiples. It is unclear whether these were due to the degradants or the high doses of calcitonin, or an artifact due to animal sacrifice by CO₂ inhalation (as per Sponsor's explanation). The finding is unlikely to be of clinical significance.

Subchronic toxicity of rsCT-DE was evaluated in a 28-day intranasal toxicity study in the rat using the commercial formulation of Fortical Nasal Spray (including excipients) and the intended route of administration. It was agreed with Sponsor that this would be sufficient to test subchronic toxicity of the Nasal Spray if there was no significant toxicity. A slight increase in rhinitis in male rats was observed as compared to the saline control and Miacalcin, at 30- to 15-fold human exposure. This effect may have been due to the Fortical excipients. Lung changes such as noted in the acute study with degradants were not observed. Rhinitis is an expected adverse reaction to calcitonin Nasal Spray and would be self-limiting with clinical use. In conclusion, this study provided sufficient safety information on the nasal toxicity of the commercial product including its excipients and impurities.

Similarly, in the nonclinical testing program for Miacalcin Spray, performed after approval of injectable form of calcitonin, a 28-day nasal study in dogs with ssCT was considered adequate to provide information on the safety of the nasal formulation.

In this study with ssCT containing benzalkonium chloride (appr. 100 IU/kg/day) (Miacalcin, NDA 20,313), there were lung changes (pneumonitis and exudate) and changes in organ weights (ovary, uterus).

Sponsor provided information on the levels of excipients _____ benzyl alcohol and phenylethyl alcohol, stabilizer Tween-80) in Fortical Nasal Spray and marketed nasal sprays. Levels in Fortical Nasal Spray were similar or lower than in the marketed products. Excipients do not need further toxicity testing.

NOTE: For calculation of dose or exposure multiples in animal studies dosed by the IV route, it was assumed that human nasal bioavailability is 3%. Since 3% is a maximum estimate the multiples are conservative estimates.

3.4.2 Single-dose toxicity

Maximum Tolerated Dose of rsCT-DE in rats (Study Nr. RsCT T9905Rp) (Unigene Labs, NJ)

Methods

Rats (N=3/grp), weight 200-250 g, were administered 0.5 ml of 0.9% saline or 0.9% saline containing rsCT-DE (10 mg/ml). Total dose was 5 mg (ca. 20 mg/kg). Appearance and behaviour were assessed at 30 min, 1h, 4h, and 1, 2, 6, 13 days after administration. Necropsy was not performed.

Results

Clinical signs in rsCT-DE treated rats (3/group)

| Time post dosing | Observations |
|------------------|------------------|
| 30 min | Slight shivering |
| 1h | Inactivity |
| 4h | No effects |
| 1d | No effects |
| 2d | No effects |
| 6d | No effects |
| 13d | No effects |

Conclusion

RsCT-DE at an i.v. dose of 5 mg in rats (20 mg/kg) has slight toxicity up to 1h post dosing in rats. Based on body surface area (mg/m²), and assuming a nasal bioavailability (BA) of 3% in humans, the 20 mg/kg iv rat dose is approximately 215,000x the human 200 IU intranasal dose.

Acute toxicity study of Fortical Nasal Spray containing calcitonin degradation products (Unigene Report F02121) _____ Unigene Study No. T0209Rp, I _____

Rats (5/sex/grp) were doses by intramuscular injection with single doses of either vehicle (— citric acid, polysorbate-80, benzyl alcohol, phenylethyl alcohol, NaCl, — (1.6 ml/kg), or vehicle plus test article (1.6 ml/kg) that had been stored inverted for 9 months at 25°C. (Lot # 0006-1002, 2200 IU/mL). Thus, dose of calcitonin was $1.6 \times 367 = 587$ ug/kg. Degradation products in the sample:

| Degradation product | Content | Specification |
|---------------------|---------|---------------|
|---------------------|---------|---------------|

Gross necropsy at Day 14 were performed (animals sacrificed by CO₂ inhalation). Tissues and organs of thoracic and abdominal cavities were examined.

Results

No mortality, no clinical signs, no body weight gain effects.

Gross necropsy: slightly red lungs in 1/5 m, and mottled red lungs in 1/5 f in the treated group. No other changes in tissues/organs.

Data on intramuscular bioavailability of the same Fortical formulation (Lot # CTM0011) were obtained (Study No. rsCTP0208Rp). At a dose of ca. 587 ug/mL, intramuscular BA was 16.8%, AUC 10,095 ngxmin/mL, Tmax 15 min, Cmax 141 ng/mL, T_{1/2} 71 min. Published data from Lee et al (1994) indicate that a 200 IU intranasal dose of ssCT leads to a Cmax of 5 pg/mL and AUC of 294 pgxmin/mL in humans. Data were used to calculate exposure multiples.

Discussion

There were slight changes in the lung in males and females. It was unclear whether these gross lung changes were coincidental, or due to the active ingredient (rsCT) or the degradants. According to Sponsor, animals sacrificed by CO₂ inhalation can have these types of lung changes. Except for the citric acid adducts, Miacalcin is expected to contain similar degradation products as Fortical (personal communication, J. Gilligan, Unigene Labs).

Based on body surface area (mg/m²), and assuming a relative intranasal bioavailability (BA) of 3% in humans, the 587 ug/kg dose is approximately 6300x the human 200 IU intranasal dose. Based on exposure data the dose is ca. 28,000x (Cmax) or 34,000 times (AUC) the human 200 IU nasal dose. The difference may be due to lower nasal bioavailability than the assumed 3% in humans (0.5-1%).

Conclusions

In a single dose rat IM toxicity study of Fortical Nasal Spray with a maximum of specified degradation contents, slight lung changes were observed in male and female rats, at doses equivalent to >25,000x the human nasal 200IU nasal dose. The cause of the lung changes was unclear. It is unlikely to be of clinical significance.

An acute toxicity study of Fortical in the dog via intravenous administration

Study No. 94-3209)

Methods

Dogs were given single doses of ssCT (2/sex) or rsCT-FP (3/sex) of 0.0167, 0.0333 and 0.0667 mg/kg via IV injection, in sterile purified water. Three doses were given to same animals separated by 90 minute intervals.

Results

No mortality.

Signs: Emesis within 2h after dosing with 0.0667 mg/kg in 1/3m and 1/3f given rsCT and 1/2 f given ssCT. Excessive salivation in 1/3m up until 1.5h after dosing with 0.0667 mg/kg rsCT.

ECG (pretest and 30 min postdose): All records within normal limits with both ssCT and rsCT.

Blood pressure, heart rate (pretest and 30 min): No test article effect

TK (5 min post dose): Similar plasma levels of sCT after dosing with ssCT and rsCT, of (ssCT:) 55-119-187 ng/mL, and (rsCT:) 43-92-186 ng/mL.

Serum calcium: Ca in serum was decreased after the MD (second dose) by ca. 20%, and after the HD (third dose) by ca. 23%. Ca was not affected by the LD.

Conclusion

Acute toxicity of IV ssCT and rsCT was observed in GI tract (emesis, salivation) and was similar for both types of calcitonin.

Based on body surface area (mg/m²), and assuming a relative intranasal bioavailability (BA) of 3% in humans, the iv doses were approximately 550x, 1100x, 2200x the human 200 IU intranasal dose.

Exploratory single dose intravenous administration of rsCT in the dog (Study # rsCT T9601Dp)

Methods

Single doses of rsCT-FP (25 mg) (Lot 16), in sterile saline, were given to 2 male dogs by IV infusion. Dog weight was not given. Assuming a BW of 10 kg, this dose is 2.5 mg/kg. Plasma samples were taken at 0, 4, 8, 12, 15, 21, 30, 45, 60, 90, 120, 180, 240, 360 min and 400-600 min in N=1 for sCT quantitation.

Results

No mortality.

Retching and lethargy throughout sampling (0-600 min) period, resolved within 24h.

No effects upon physical exam.

Plasma levels: Cmax 8-10 ug/mL (T max 15 min). Levels < 1 ug/mL by 60 min.

Approximate dose: $25 \text{ mg}/10 \text{ kg} = 2.5 \text{ mg}/\text{kg} = 2,500 \text{ ug}/\text{kg} = 5,000 \times 33 \times 1/2 =$ equivalent to 82,500 x human 200 IU intranasal dose, based on mg/m² and 3% nasal BA.

Plasma Cmax of 10 ug/mL = appr. 42,000 x human AUC of 240 pg/mL (data from human study with ssCT, Lee et al, 1994) (multiple similar to mg/m² comparison)

Studies with ssCT have not been done with such a high dose.

Conclusion

In dogs, a single IV dose of ca. 2.5 mg/kg rsCT in saline, caused transient retching and lethargy. The 2.5 mg/kg dose is equivalent to 40,000-80,000 times the human intranasal dose on the basis of Cmax or mg/m², respectively.

3.4.3 Repeat-dose toxicity

Study title: 4 Week Intranasal Toxicity Study in Rats

Purpose: Investigate local irritancy of the test formulations in the upper respiratory tract of the rat following repeat daily dosing for 28 days. Fortical Nasal Spray is compared to Miacalcin^R.

Key study findings:

Rats were given Fortical or Miacalcin by intranasal administration for 28 days. There were no effects on clinical signs, body weight or food consumption. Lung weight and necropsy findings were not affected by treatment. Incidence and severity of focal rhinitis in the nasal cavity was increased in the Fortical group. This may have been related to the specific Fortical formulation, i.e. excipients or impurities.

Study no.: rsCT T9903Rn
Volume #, and page #: Vol. 1.12 (pp. 20-97)
Conducting laboratory: _____

Report No. :**Date of study initiation:** September 29, 1999**GLP compliance:** Yes (OECD Principles of GLP)**QA report:** Yes**Drug, lot #, and % purity:** Batch No. 0004-9002 (Fortical Nasal Spray 200IU/dose of 90uL); Impurity content: —
Batch Nos. 372B2016 and 382B2542 (Miacalcin Nasal Spray)**Methods**

Sprague Dawley rats CD^R VAF/PLUS-SPF) (N=6/sex/group), 6-7 weeks old, body weight 175 gr (m) and 165 gr (f), were dosed with Fortical or Miacalcin, **14.8 ug in 40 ul (20 uL/nostril)**, daily, by intranasal administration. Fortical and Miacalcin doses were similar (200 IU per 90 uL). Dose administration procedure was 10 ul to each nostril, repeated after ca. 30 seconds, using a calibrated pipette ("droplet installation" method). The administration of 40 ul/day resulted in a dose of **87 to 40 ug/kg/day (males)**, or **87 to 62 ug/kg/day (females)** over the time course of the study (decrease due to increasing body weight). Groups dosed with test article were undergoing (A) necropsy at 4 weeks, or (B) necropsy at 6 weeks following 2 weeks recovery. Animals in the control group received saline. Since the body weight increased from 170 gr (m,f) at Day -1 to 370 gr (m) and 240 gr (f) over the 4-week study period, the dose in terms of mg/kg decreased over time.

Treatment groups

| Group | | Treatment | Dose | Recovery period (2 weeks) |
|-------|---|------------------|---------------|---------------------------|
| 1 | | Control (saline) | 20 uL/nostril | No |
| 2 | A | Miacalcin | 20 uL/nostril | No |
| | B | Miacalcin | 20 uL/nostril | Yes |
| 3 | A | Fortical | 20 uL/nostril | No |
| | B | Fortical | 20 uL/nostril | Yes |

Calcitonin dose (Fortical or Miacalcin)

| | Rat dose | Multiple of human dose* | |
|--------|------------------------------|-------------------------|-------------|
| | | Mg/kg basis | Mg/m2 basis |
| | 40 uL = 89IU = 14.8 ug | | |
| Day 1 | 87 ug/kg | 174x | 28x |
| Day 28 | 40 ug/kg (m) 62 ug/kg (f) | 80x 124x | 13x 20x |

*Human dose is 200 IU/day, i.e. 33 ug/65 kg=0.5 ug/kg

Fortical formulations

| | Fortical formulation used in intranasal rat study rsCT | Fortical Nasal Spray (intended commercial product) |
|------|--|--|
| | Amount per 90 uL | Concentration |
| rsCT | 200 IU in 90 uL | 200 IU per 90 uL dose |

| | | | |
|---------------------|--|--|--|
| Citric acid | | | |
| Tween 80 | | | |
| Benzyl alcohol | | | |
| Phenylethyl alcohol | | | |
| NaCl | | | |
| HCl** | | | |
| NaOH** | | | |
| Purified water | | | |

* buffered to pH 3.6
 **To adjust to pH 3.5-3.9

Fortical and Miacalcin formulations

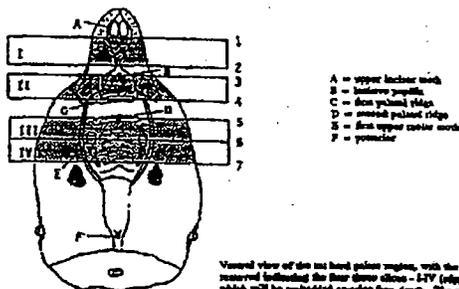
| | Fortical formulation used in intranasal rat study rsCT (FORTICAL C) | Fortical Nasal Spray (intended commercial product) (FORTICAL B) | Miacalcin Nasal Spray (marketed product) |
|---------------------|---|---|--|
| rsCT | | | |
| Citric acid | | | |
| Tween 80 | | | |
| Benzyl alcohol | | | |
| Phenylethyl alcohol | | | |
| Benzalkonium | | | |
| NaCl | | | |
| HCl** | | | |
| NaOH** | | | |
| Purified water | | | |

* buffered to pH 3.6
 **To adjust to pH 3.5-3.9

Necropsy consisted of complete external and internal examination. Organ weight was recorded for lungs. Tissues from respiratory tract were preserved (bronchial and cervical lymph nodes, larynx, lungs, nasal cavity, pharynx, trachea). Histopathology examination was carried out of lungs, nasal cavity and pharynx. After flushing, 4 transverse sections of the nasal cavity were produced and evaluated (levels I, II, III, IV). Statistical analyses were performed for data on body weight, food consumption, organ weight (absolute and relative) and histological incidences.

Tissues for Preservation/Evaluation

| Tissues | Weighed | Examined | Comments |
|----------------------|---------|----------|---|
| Bronchia: Lymph Node | | | |
| Cervical Lymph Node | | | |
| Implant Sites | | | Implant retained for identification purposes. |
| Larynx | | | |
| Lungs | x | x | Perfused after weighing. |
| Nasal Cavity | | x | After dissection from the carcass, the nasal cavity was gently flushed whilst the head submerged in order to ensure removal of air pockets from within the nasal cavity. Four transverse sections (Levels I, II, III and IV) of the nasal cavity were produced and evaluated (see diagram below). Sections included nasal associated lymphoid tissue. |
| Pharynx | | x | |
| Trachea (anterior) | | | |
| Trachea (posterior) | | | |



Ventral view of the rat head (nasal region), with the lower jaw removed indicating the four transverse (I-IV) (coloured areas) which will be completed another five days. The numbers on the right-hand side indicate the levels of the slices that necessary to produce the four slices.

Best Possible Copy

Results

Mortality:

None

Clinical signs:

No treatment-related effects

Body weights:

No treatment effects. Body weight varied from 170 gr on Day 1 to 370 gr (males) and 240 gr (females) on Day 28.

Food consumption:

No treatment effects

Gross pathology:

No treatment effects

Organ weights:

No significant effects on absolute or relative lung weight

Histopathology:

MALES (6/grp)

| Group | | control | Miacalcin | Fortical |
|--------------------------|---------|---------|-----------------|-----------------|
| Group No. | | 1 | 2 | 3 |
| | | | A (no recovery) | A (no recovery) |
| Nasal cavity | | | | |
| Rhinitis, level I, focal | Minimal | 1 | 0 | 1 |
| | Mild | 0 | 0 | 1 |
| | Total | 1 | 0 | 2 |

There were no findings in the lung.

It is unclear whether the slightly higher incidence of focal rhinitis in the nasal cavity at the level anterior to the incisive papilla (2/6 in Fortical group vs. 1/6 in saline control group) was related to the excipients. The pharmacologically active compound is the same in Fortical and Miacalcin spray (sCT).

Toxicokinetics:

Not performed

Dose multiples

The administration of 40 ul/day resulted in a dose of 87 to 40 ug/kg/day (males), or 87 to 62 ug/kg/day (females) over the time course of the study. This is equivalent (on mg/m² basis) to 28→13 or 28→20 times the human 200 IU dose, if we assume similar

bioavailability in rats and humans (rat nasal bioavailability is ca. 3%, human nasal BA is <3%). For assessment of local toxicity, the relevant comparative dose parameter would be exposure per surface unit of nasal epithelium. Thus, the best dose comparison would most likely be on the basis of mg/m² body surface area.

Conclusion

In a 28-day repeat dose intranasal toxicity study with Fortical Nasal Spray (proposed marketing formulation) and Miacalcin Spray in rats, an increased incidence of rhinitis (anatomic level I) was observed in male rats treated with Fortical. This may have been related to the Fortical-specific excipients. The dose (14.8 ug/day) varied over the course of the study due to growth of the animals. Dose multiples (mg/m² basis) were approximately 30-15 times. No other toxicity was observed.

Excipients

The concentrations of the preservatives phenylethyl alcohol, benzyl alcohol, and benzalkonium chloride of various marketed prescription and OTC Nasal Spray products were compared to the concentrations of the preservatives in Fortical Nasal Spray (Review IND 59,664, APPENDIX).

Nasal Spray formulations

| Product | Flonase (Rx) | Beconase AQ (Rx) | Afrin nasal decongestant (OTC) | Ocean nasal mist Saline (OTC) | Saline Nasal Spray (Rite Aid, OTC) | Fortical | Miacalcin |
|-----------------------|-------------------------|-------------------------|--------------------------------|-------------------------------|------------------------------------|----------|-----------|
| Daily Dose (ul) | 100-200 ug (200-400 ul) | 100-200 ug (200-400 ul) | | | | | |
| Benzalkonium chloride | 0.2 mg/ml | Not listed | Not listed | Not listed | - | | 0.2 mg/ml |
| Benzyl alcohol | - | - | 2.4 mg/ml | 3.0 mg/ml | 5.2 mg/ml | | - |
| Phenylethyl alcohol | 2.5 mg/ml | 2.5 mg/ml | - | - | - | | - |
| Tween 80 | Present | present | ? | ? | ? | | - |

Benzyl and phenylethyl alcohol are present in marketed products at similar or higher concentrations as in Fortical. Prescription drug dose volumes are 200-400 ul, compared to 90 ul Fortical.

According to the CDER Inactive Ingredient Database, the ~~Polysorbate-80~~ Polysorbate-80 is present in Nasal Spray formulations at 0.004% and 10% (metered spray). It is generally recognized as non-toxic and is widely used in foods and oral drugs. Based on this information, the excipients in Fortical Nasal Spray do not need further toxicity testing.

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3.4.4. Genetic toxicology

Two *in vitro* mutagenicity studies have been carried out with rsCT-FP, a modified Ames test, and a mammalian cell cytogenicity test in CHO cells. The compound tested negative in both assays. The test solutions contained undeterminate amounts of impurities.

Reverse mutation Assay: Salmonella typhimurium/Escherichia coli (Ames test)

—Study No. 1470/6-1052)

Method

A modified Ames plate-incorporation test (Study No. 1470/6-1052) was carried out in *S. typhimurium* (strains TA 1535, TA 1537, TA98, TA 100), or *E. coli* (WP2 pKM101, WP2 uvrA pKM101) and consisted of two independent experiments. Test article was recombinant salmon calcitonin, rsCT-FP (Batch No. 1100-D-5022). Bacterial suspensions (2 mL) were preincubated for 1h at 37°C with test article (EXP. 1: 8, 40, 200, 1000, 5000 ug/ml, EXP. 2: 312.5, 625, 1250, 2500, 5000 ug/mL) or control vehicle, in absence and presence of metabolic activation (phosphate buffer or S-9 mix). The modified method was used to prevent amino-acid feeding effect when bacteria are incubated with test article for prolonged periods which causes thickening of background lawn. Positive controls (-S9) were 4-nitroquinoline 1-oxide (TA100), 2-nitrofluorene (T98), N-methyl-N'-nitro-N-nitrosoguanidine (TA 1535), ICR-191 (TA1537), and positive control (+S9) was 2-aminoanthracene. Negative control was sterile purified water. Bacteria were pelleted and resuspended and aliquots of 0.1 mL were added to 2 mL agar, and incubated (mutagenicity plates for 3 days, viability plates overnight) at 37°C. Plate counts of revertant colonies were analysed using Dunnett's test (comparison of each dose with control). Linear regression analysis was performed to test for dose response.

Results

All positive controls caused a statistically significant increase in the number of revertants in both experiments.

Solvent (negative) control counts were within range of historical control values for spontaneous revertant frequencies.

Study was valid, based on negative and positive control data and loss of no more than 5% of plates through contamination or other causes.

In some strains, there was cytotoxicity mainly at the highest dose of 5000 ug/mL, with or without metabolic activation, evidenced as reduction in viable cell numbers and in some cases as thinning of background lawn. This toxicity did not affect the mutagenicity evaluation.

There were no increases in revertant numbers in any test strain, in the absence or presence of metabolic activation (S9), that were significant according to Dunnett's test (1% level), and there were no positive dose responses according to regression analysis.

Conclusions

Under the conditions of the study, rsCT did not induce mutations in *S. typhimurium* or *E. coli*, in the absence or presence of metabolic activation.

Method

An in vitro cytogenetics assay was carried out in Chinese Hamster Ovary (CHO) cells using duplicate cultures in 2 independent experiments. In Experiment 1, cells were exposed to rsCT-FP (Batch Nr. 1100-D-5021) for 20h (2h + S9 and 18h -S9, or 20h - S9), and chromosome aberrations were analysed at doses of 580, 893, 1373 ug/mL (-S9) or 2113, 3250, 5000 ug/mL (+S9). In Experiment 2, doses were 1507, 1674, 1860 ug/mL (-S9) or 2813, 3750, 5000 ug/mL (+S9). An additional sampling time (44h) was utilized with doses of 1507 ug/mL (-S9) or 5000 ug/mL (+S9). Also, a 2h-pulse treatment (-S9, 2h + 18h) was included using a dose of 5000 ug/mL. Doses were selected based on toxicity as evidenced by mitotic index. Positive controls were cyclophosphamide (+S9), and 4-nitroquinoline 1-oxide (-S9).

Proportion of cells with aberrations (structural including gaps, structural excluding gaps, numerical) were determined. Aberrations excluding gaps were compared with negative controls by Fisher's exact test.

Results

Positive controls caused a statistically significant increase in the proportion of cells with structural aberrations excluding gaps in both experiments.

Solvent (negative) control numbers (except 1) were within range of historical negative control values.

Study was valid based on negative and positive control data, variability in frequency of aberrations in replicate negative control cultures, and number of analyzable cells. Dose selection was adequate.

In Experiment 2 (2h +S9, 19h recovery) there were statistically significant increases in proportion of aberrant cells excluding gaps at 2813 and 5000 ug/mL, but not at 3750 ug/mL. Thus, this was not a dose-dependent effect. In 1 out of 4 cultures from the two positive dose groups, the aberration frequency was above historical control range (6/100 in 1 culture treated with 2813 ug/mL). There was no positive response at any dose in Experiment 1. Since this result was not reproducible and not dose-dependent it was considered not biologically significant.

Exp 2, 2h+S9, 18h recovery

| Treatment (ug/mL) | Cells scored | Cells with aberrations excluding gaps | Cells with aberrations excluding gaps |
|-------------------|--------------|---------------------------------------|---------------------------------------|
| | | (n/100 + n/100) | (n/200 cells) |
| 0 | 200 | 0+2 | 2 |
| 2813 | 200 | 3+6 | 9* |
| 3750 | 200 | 2+0 | 2 |
| 5000 | 200 | 4+4 | 8* |
| | | (n/25 + n/25) | (n/50 cells) |
| CPA, 2.5 ug/mL | 50 | 11+8 | 19** |

*p<0.05; **p<0.001; historical negative control range (N/100 cells): 0-4

Exp 1, 2h+S9, 18h recovery

| Treatment (ug/mL) | Cells scored | Cells with aberrations excluding gaps (n/100 + n/100) | Cells with aberrations excluding gaps (n/200 cells) |
|-------------------|--------------|--|--|
| 0 | 200 | 1+2 | 3 |
| 2113 | 200 | 0+0 | 0 |
| 3250 | 200 | 1+2 | 3 |
| 5000 | 200 | 3+3 | 6 |
| | | (n/25 + n/25) | (n/50 cells) |
| CPA, 2.5 ug/mL | 50 | 7+13 | 20** |

*p<0.05; **p<0.001; historical negative control range (N/100 cells): 0-4

There was an increase in the frequency of cells with numerical aberrations at the 20-h sampling time following pulse treatments, in both absence and presence of S-9 (Exp 1, +S9, 2113 ug/mL and 5000 ug/mL, Exp 2 +S9, 5000 ug/mL, Exp 2, -S9, 5000 ug/mL). Frequencies per 100 cells were: 7.4 and 8.7, 10.1, 8.7, which was outside the historical control range of 0-6/100 cells. The effect represented increases in endoreplication and polyploidy. The biological significance of this finding is not clear.

The increase in aberrations in presence of S9 in Experiment 2 was not considered significant.

Conclusion

Under the conditions of the study, rsCT did not induce structural chromosome aberrations in cultured Chinese hamster ovary (CHO) cells, in the absence or presence of metabolic activation.

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3.4.5. Carcinogenicity

Carcinogenicity studies with rsCT were not conducted. However, information of the carcinogenic potential of synthetic sCT has been obtained in studies with Calcimar (synthetic sCT) injection (NDA #17,769). At the end of a 1-year toxicity study in Sprague Dawley and Fisher 344 rats with doses of 1.25, 5 and 80 IU/kg/day by subcutaneous injection, a higher incidence of pituitary tumors (adenoma, adenohypophysis) was noted in Sprague -Dawley rats treated with the high dose of 80IU/kg/day. Incidence of hyperplasia was not increased. As indicated in the label, it was concluded that sCT probably reduced the latency period for development of pituitary tumors.

Immunohistochemical analysis of the pituitary tissue from sCT treated rats suggested that most tumors were "null cell" tumors (tumors that do not secrete PRL). In the review of the Miacalcin Nasal Spray NDA #20,313 (A. Jordan, April 20, 1994), the Pharmacology/ Toxicology Reviewer concluded that the information from the original 1-year study and follow up studies submitted to NDA #20,313 show that sCT treatment results in development of pituitary tumors, but that the significance of the results from the follow up studies is not clear.

As stated in the Miacalcin label, the dose of 80 IU/kg is equivalent to 16-19 times the recommended human parenteral dose and about 130-160 times the human intranasal dose based on body surface area, mg/m². The dose of 5 IU/kg is equivalent to about 8-10 times the human intranasal dose based on body surface area, mg/m². These calculations are based on a parenteral dose of 50 IU, or an intranasal dose of 200 IU with 3% bioavailability, and a 60-75 kg human body weight.

80IU/kg rat \approx 13IU/kg human

50 IU/60kg \approx 0.84IU/kg

200IU/60kg/33.3 \approx 0.1 IU/kg

The original pituitary tumor finding with ssCT has been included in the Miacalcin label. Sponsor proposed to include the finding in the label for Fortical Nasal Spray. Reviewer agrees with this proposal. However, Reviewer proposes some changes to the label.

3.4.6 Reproductive and developmental toxicology

Reproduction studies were not conducted with rsCT, since the product is intended for use in postmenopausal women. Information on the reprotoxicity of synthetic sCT has been obtained in studies with Calcimar Injection (NDA 17,808).

In a Segment 2 study, rabbits were dosed with 20 and 80 MRC units (IU)/kg/day (GD6-GD28), by subcutaneous injection. Placental and fetal weights were reduced at the high dose. Litter sizes were reduced in treated groups in dose-related fashion. There were no fetal anomalies.

Rabbit doses of 20-80 MRC units/kg/d are equivalent to 8-33 times the human SC dose of 50 IU, and 70-278 times the human nasal dose of 200 IU (assuming 3% bioavailability), based on body surface area, mg/m².

In a Segment 2 study in rats (doses 0, 5, 20, 80 MRC units/kg/day, GD6-15) there were no adverse findings.

In a Segment 3 study rats were dosed subcutaneously with 5, 20, 80 MRC units/kg/d (GD 15-PPD21). The lactation index (also called weaning index, i.e., number of pups alive on PPD 21/number of pups alive on PPD 4) was reduced in the treated dams. In the same study, maternal toxicity was observed (mortality and BW effects in HD, and kidney histopathology in LD, MD, HD). There was also an increase in pups born dead in MD and HD groups. The pup mortality findings may have been due to maternal toxicity.

Sponsor proposes to include the reprotoxicity findings mentioned in the Miacalcin label in the label for Fortical Nasal Spray. The fetal weight reduction observed in the rabbit Segment 2 study with multiples of the human dose was mentioned in the Miacalcin label. A finding of reduced lactation was included in the Miacalcin label ("*Calcitonin has been shown to inhibit lactation in animals*") in the Nursing Mothers section. However, the rat Segment 3 study results show no effect on lactation but on lactation index. Since there was maternal toxicity in this study at all dose, Reviewer proposes not to include the reduction in lactation index in the label and to delete the animal finding of lactation inhibition. The rabbit finding of reduced fetal weight can be included.

Reviewer proposes changes to the label as appended.

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3.4.7 Local tolerance

Local tolerance studies have been performed in rats and rabbits with rsCT-FP. In rabbits, intra-arterial or paravenous injection of 50 IU or 10 IU Fortical did not cause local or systemic toxicity other than minimal erythematous reaction. In rats, IV injection in the tail vein (50 IU) did not cause local irritation. In rats, intramuscular injection of Fortical or Miacalcin (5 IU) in the thigh caused mild irritation evidenced by myofiber degeneration.

These studies are not directly relevant to the current NDA.

3.4.8 Special toxicology studies

An immunogenicity study was performed in rats with rsCT-FP and ssCT (Miacalcin). Rats were given daily intramuscular injections of rsCT-FP or ssCT, doses 0.1 or 1 ug/kg, or vehicle, for 7 to 14 days. These doses are equivalent to 0.2x and 2x the clinical nasal dose of 200 IU, based on mg/m² comparison, and assuming 3% intranasal (human) and 16% intramuscular (rat) bioavailability. No circulating anti-sCT antibodies were detected in the blood 7-10 days after final injection. Subcutaneous injections of rsCT-FP (10 ng/100g), or Miacalcin (50IU/100 g) given 10 days after final injection, elicited a hypocalcemic response in treated groups similar to the vehicle control group. The data indicate the absence of neutralizing antibodies. There is no tachyphylaxis (definition: rapid development of tolerance or immunity to the effects of a drug) in either rsCT or ssCT treated rats.

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3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

The data obtained in non-clinical studies indicate that recombinant salmon calcitonin (rsCT) in Fortical Nasal Spray is bioequivalent with synthetic sCT. Toxicological evaluation did not provide any indication that the drug product will cause unexpected toxicity.

Recommendations:

AP

Suggested labeling:

Attached

Reviewer Signature _____

Supervisor Signature _____ Concurrence Yes ___ No ___

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3.7. APPENDIX/ATTACHMENTS

- LABEL
- IND review

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4 Page(s) Withheld

 Trade Secret / Confidential

X Draft Labeling

 Deliberative Process

Withheld Track Number: Pharm/Tox-

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

KEY WORDS:

Reviewer Name: Gemma A. Kuijpers, Ph.D.
Division Name: Division of Metabolic and Endocrine Drug Products
HFD # 510 (DMEDP)
Review Completion Date: February 9, 2000
Review number: #1

IND NUMBER:

Serial number: 59,664
Date/type of submission: 000
January 4, 2000
Information to sponsor: Yes (x) No ()
Sponsor (or agent): Unigene Laboratories, New Jersey
Manufacturer for drug substance:

DRUG:

Generic Name: Fortical[®] Nasal Spray (recombinant salmon
calcitonin)
Chemical Name: calcitonin
CAS Registry Number: n/a
Molecular Formula: n/a
Molecular Weight: 32-amino acid peptide
Drug Class: calcitropic hormone
Structure:

CLINICAL INFORMATION

Indication: Treatment of postmenopausal osteoporosis
Clinical formulation: Nasal Spray, two formulations (Fortical A and Fortical B)
Excipients: rsCT, NaCl, Tween 80, HCl, NaOH, Citric acid (A:

Strength: B:), Phenylethyl alcohol, Benzyl alcohol
2200 IU rsCT/ml
Route of administration: Intranasal
Proposed clinical protocol or use: Phase I and Phase II protocols
Previous clinical experience: Phase I and Phase II studies

Relevant INDs/NDAs/DMFs: IND 50,747 ((Fortical[®], Unigene Laboratories, Inc.),
calcitonin for injection

BACKGROUND

The calciotropic peptide hormone calcitonin is involved in calcium homeostasis. Specifically, calcitonin decreases the translocation of calcium from the renal tubule and bone fluid compartment into the blood and thus prevents abnormal increases in both serum calcium and serum phosphate. It can be considered a counterregulator of PTH. In bone, it acts as an anti-resorptive agent, and based on that action it is clinically used for the treatment of Paget's disease, hypercalcemia of malignancy and postmenopausal osteoporosis. Calcitonin causes a rapid and sustained rise in plasma cyclic AMP. Apart from its effect on bone and kidney calcitonin has GI effects and analgesic properties.

Human PK studies with salmon calcitonin have shown that calcitonin is rapidly absorbed and eliminated upon parenteral administration. $T_{1/2}$ (absorption) is ca. 10-15 min and $T_{1/2}$ (elimination) is ca. 50-90 min. Animal studies have shown that it is metabolized to inactive fragments in the kidney and in blood and peripheral tissues.

Since it is a peptide calcitonin can not be administered orally. Currently marketed products are injectable synthetic salmon calcitonin products, and an intranasal preparation (Miacalcin Nasal Spray, Novartis Pharmaceuticals). The clinical dose is 50IU-100IU (<20 ug/day) for the injectable products, and 200 IU per day for the nasal one. Bioavailability for intramuscular and subcutaneous administration is 65%-75%, and for intranasal administration less than 5%. Calcitonin in the therapeutic setting is generally considered to be safe.

SYNTHETIC VS. RECOMBINANT PEPTIDE

The currently marketed calcitonin products are synthetic. Unigene Laboratories, the Sponsor of this IND, has developed a recombinant salmon calcitonin product, Fortical™. Fortical Injection has been tested clinically in Phase I and II osteoporosis trials, and according to the Sponsor its pharmacological and pharmacokinetic equivalence to Miacalcic (Salcatorin BP, calcitonin for injection, Novartis Pharmaceuticals) has been established in clinical studies in the U.S. and the UK. In 1999, Forcaltonin, Unigene's injectable recombinant sCT product, was approved for marketing in the EU. Unigene is now developing the rsCT drug substance as a nasal spray. According to the Sponsor recombinant sCT (Fortical^R) and synthetic sCT have comparable pharmacological and safety profiles. For the nasal spray development, a new recombinant DNA production method of direct expression (DE) is used instead of the fusion protein (FP) method used previously for the injectable form of the peptide. Moreover, the excipients in the nasal spray formulation are different than the ones in the injectable formulation. Analytically, rsCT-DE is similar to rsCT-FP and ssCT.

PREVIOUS HUMAN EXPERIENCE

Studies completed with intranasally administered Fortical

Two clinical studies with the nasal preparation have been completed in the UK (Study UGL-N9901), a repeat-dose PK/tolerability intranasal study in healthy volunteers

and a repeat-dose safety/tolerability/PK intranasal study in healthy volunteers (N9901). In both studies, five consecutive doses of 400IU Miacalcin or Fortical were given intranasally (total dose 2000 IU). The dose given was higher than the intended I.N. dose because the bioavailability of nasally administered peptide is very low and highly variable, and the therapeutic dose (200IU) results in blood levels below the LLQ of the available RIA assay.

Safety data on injectable calcitonin (Fortical and Miacalcic)

In a comparative study with injectable rsCT (100 IU, s.c., Fortical or Miacalcic) (Unigene Study) it was found that the most common adverse event was nausea and vomiting. The next frequently observed AE was diarrhea. The events were mostly mild or moderate, although some (occurring in <10% of the study population) were serious (headache, nausea, vomiting). Fortical and Miacalcic were equivalent with respect to their clinical safety and tolerance.

Safety data on intranasal calcitonin (Forcaltonin and Miacalcin)

In study UGL-N9901 (N=14 female volunteers) with intranasally administered Forcaltonin A or B or Miacalcin (total dose 2000 IU), it was found that the most common AE was nausea. Other events were rhinitis, dizziness, vomiting, headache, vasodilation. The latter AE's appeared to be more frequent with Forcaltonin as compared to Miacalcin.

PROPOSED CLINICAL STUDIES

1. BIOEQUIVALENCE STUDY (UGL-N9903)

Study is a cross-over, open label, multi-dose bioequivalence study in healthy female volunteers. Study design will be similar to the two completed studies, UGL-N9901 and UGL-N9901. Formulations tested will be Fortical A, Fortical B and Miacalcin. Dose level is 6 x 400 IU, doses 20 minutes apart, total dose 2400 IU, over a 100-minute period. Patients will receive doses of Fortical A, B, or Miacalcin with a 1-week washout between the 3 treatment phases. Determinations planned are plasma calcitonin, and safety and tolerability parameters.

2. PHARMACOLOGY/TOLERABILITY STUDY (UGL-N9904)

Study is a single-blind, multiple-dose, parallel study comparing safety and tolerability of Fortical and Miacalcin in normal female volunteers (age 18-45). Fortical used will be A or B, containing citric acid. Planned study duration is 1 month, with an extension to 6 months. Planned daily dose is 200 IU/day, for 1 month. Both Fortical and Miacalcin contain 200IU/90ul spray. Determinations planned are safety and tolerability parameters. Bone markers and PTH will also be monitored. At the end of 6 months BMD will be determined by DXA.

FORTICAL FORMULATION

Fortical and Miacalcin Nasal Spray formulations

Formulations of Fortical A,B,C, and Miacalcin

| Ingredient | FORTICAL | FORTICAL | MIACALCIN |
|-----------------------|-----------------|----------|-----------|
| | Per 90 ul spray | Per ml | Per ml |
| RsCT | | | |
| Citric acid | | | |
| Tween 80 | | | |
| Benzalkonium chloride | | | |
| Benzyl alcohol | | | |
| Phenylethyl alcohol | | | |
| Sodium Chloride | | | |
| Sodium hydroxide | | | |
| HCl | | | |
| Purified water | | | |

*Fortical C is used in the 28-day rat nasal local tolerance study (see page 5)

PHARMACOLOGY/TOXICOLOGY

In the Pharmacology/Toxicology section of the current IND (IND #59,664, Item 8) the Sponsor presents 11 preclinical studies (APPENDICES A-K). Four of the studies (APPENDICES A-D) were conducted for the current IND, and seven studies (Appendices G-K) were previously also submitted to IND #50,747 (rsCT, for injection). The studies were aimed to demonstrate the *in vitro* biological activity of the recombinant form of salmon calcitonin (rsCT), and its biological equivalence to synthetic salmon calcitonin (ssCT).

This review includes a summary review of the preclinical studies with rsCT for injection (rsCT-FP), carried out previously in dogs, rats and rabbit, and *in vitro* (mutagenicity studies), that were submitted to IND #50,747. These studies include the ones described in APPENDICES G-K of the current IND, i.e., two mutagenicity studies and five local tolerance studies with the injectable form of rsCT. The review also covers a detailed evaluation of the studies conducted with the newly developed drug substance (rsCT-DE) that will be used for the nasal preparation of Fortical. The latter studies include two PK studies (APPENDIX A, B), one PK/PD study (APPENDIX D), and one toxicology study (APPENDIX C), all carried out in rats. Furthermore, one proposed local tolerance study in rats (APPENDIX L), and the nasal spray formulation is evaluated (APPENDIX M). The safety of the proposed clinical studies is assessed on the basis of the submitted information.

Pharmacodynamics

The PD response to rsCT-FP as compared to ssCT has previously been evaluated in studies of plasma calcium and cAMP changes upon s.c. injection in rats and dogs. The results indicated qualitative and quantitative similarity between rsCT-FP and ssCT (IND #50,474). A comparative i.v. PD/PK study of the two recombinant versions of calcitonin, rsCT-FP and rsCT-DE, and the 2nd IS for sCT was carried out in rats and described in the current IND

Pharmacokinetics

The pharmacokinetic profile of rsCT-FP as compared to ssCT suggests the bioequivalence of the two compounds (IND #50,474).

A comparative PK study of rsCT-FP and rsCT-DE, i.v., was carried out in rats.

The PK parameters for the two calcitonins were very similar.

Two comparative PK studies were carried out with rsCT using the intranasal administration route

In these studies the effect of different excipients on the PK parameters of rsCT was determined using three different formulations of rsCT. One of the 3 formulations was also tested against rsCT in NaCl only (

Toxicity

General toxicity

Toxicity studies in dogs have been carried out with rsCT-FP, one single dose infusion study, and one acute toxicity study in dogs given three escalating doses at 90-min intervals. In the second study the effects were compared to those of ssCT. There were no detailed reports of these studies in this IND. The results are summarized below.

An i.v. toxicity study with rsCT-DE was carried out in rats and the results were reported in this IND

Mutagenicity

Two *in vitro* mutagenicity studies have been carried out with rsCT-FP. A modified Ames test (APPENDIX E), and a mammalian cell cytogenicity test in CHO cells (APPENDIX F). Results are summarized below.

Local tolerance

- A local intra-arterial tolerance study was done with rsCT-FP in rabbits: intra-arterial injection in the ear (APPENDIX G)

- Local venous and paravenous tissue tolerance to Fortical rsCT was studied in rabbits (APPENDIX H).
- Local tolerance following IM administration of Fortical rsCT and Miacalcic ssCT was studied in rats. (APPENDIX I)
- Local tolerance following IV administration in the tail vein of Fortical rsCT was studied in rats. (APPENDIX J, and repeat study APPENDIX K)
(The results of these five studies are summarized below)
- A 28-day local intranasal tolerance study in rats comparing Fortical Nasal Spray with Miacalcin Nasal Spray is proposed (Study protocol, APPENDIX L). In the proposed clinical studies, two different formulations of Fortical, Fortical A and B, will be used (A 10 mM citric acid, B 20 mM citric acid). In this local tolerance study in rats a third formulation, Fortical C will be used. Fortical C is a formulation with 25 mM citric acid.

Preservatives/Stabilizers

The concentrations of the preservatives phenylethyl alcohol, benzyl alcohol, and benzalkonium chloride of various marketed prescription and OTC Nasal Spray products are compared to the concentrations of the preservatives in Fortical Nasal Spray (APPENDIX M).

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SUMMARY REVIEW OF PREVIOUS STUDIES WITH rsCT FOR INJECTION

Pharmacodynamics

The acute pharmacologic response following parenteral salmon calcitonin administration is a decrease in circulating calcium levels. This "hypocalcemic response" was assessed in several studies in rats and dogs in order to compare the biologic activity of recombinant sCT (rsCT) and synthetic sCT (ssCT). In both species, rsCT produced by the fusion protein process was shown to elicit a hypocalcemic response similar in magnitude, duration and onset to that of ssCT.

Pharmacokinetics and Metabolism

Salmon CT is primarily metabolized to inactive fragments in the kidney and to some extent in blood and peripheral tissues. The disposition of rsCT was evaluated in rats and dogs. Plasma CT concentrations were determined by RIA.

In rats, i.v. administration showed peak levels at the first time point with rapidly declining levels thereafter. AUC was linear with respect to dose. $T_{1/2,el}$ was 32-48 minutes. In rats, s.c. administration also showed linear dose dependence of AUC and C_{max} . Upon s.c. administration T_{max} was ca. 35 minutes, $T_{1/2,el}$ ranged from 56 to 86 minutes, and absolute bioavailability was 12-24%. In an s.c. study in rats, bioequivalence was established between rsCT and ssCT (Miacalcin injection) with respect to AUC, C_{max} , T_{max} , $T_{1/2,el}$ and BA.

In dogs, i.v. administration showed peak levels at the first time point with rapidly declining levels thereafter. AUC was linear with respect to dose. $T_{1/2,el}$ was ca. 17 minutes. In the dog, s.c. administration also showed linear dose dependence of AUC and C_{max} . T_{max} was 24 minutes, $T_{1/2,el}$ ranged from 20-80 minutes and absolute BA ranged from 18-80%. The results of a single, escalating dose i.v. study in dogs supported bioequivalence between rsCT and ssCT based on similarity of plasma levels at 5 minutes post dosing.

Toxicology

In dogs given single i.v. doses of 0.0167, 0.033 and 0.067 mg/kg rsCT or ssCT the high dose of 0.067 mg/kg rsCT or ssCT caused excessive salivation and emesis. The doses had no cardiotoxic, blood pressure or heart rate effects. The doses are 70, 40, and 280 times the human dose on the basis of kg body weight (Human dose of 100 IU s.c. = equivalent to approximately 72 IU i.v. = 14.4 ug = 0.24 ug/kg = 0.00024 ug/kg).

A 25 mg i.v. dose of rsCT in dogs (125,000 IU) (ie >10,000 times the therapeutic human s.c. dose of 100 IU on the basis of kg body weight) caused severe retching for 4 h post dosing and lethargy. Subsequently, dogs maintained good health for at least one year. This 25 mg dose caused peak plasma levels of > 8 ug/ml, i.e., more than 20,000 times the maximum clinical blood levels (0.4 ng/ml) of sCT in humans.

Mutagenicity

In a modified Ames test with rsCT-FP, cultures were preincubated for 1h at 37°C with each test article concentration (8-5000 ug/ml) or control vehicle, both in absence and presence of metabolic activation. At the highest dose there was evidence of cytotoxicity particularly at the highest dose both with and without activation. There was no evidence of mutagenicity.

In a Chinese Hamster Ovary (CHO) cell cytogenetics test, with and without metabolic activation (S-9), cells were exposed to rsCT-FP at doses of up to 5000 ug/ml. Positive controls were cyclophosphamide in the presence of S-9, and 4-nitroquinoline 1-oxide in the absence of S-9. There were no increases in cells with structural chromosomal aberrations. However, there was an increase in the frequency of cells with numerical aberrations at the 20-h sampling time following pulse treatments, in both absence and presence of S-9. The effect represented increases in endoreplication and polyploidy.

Local tolerance

Local tolerance studies have been performed in rats and rabbits. In rabbits, intra-arterial or paravenous injection of 50IU or 10IU Fortical Injection did not cause any obvious drug-related

irritation or other tissue damage. In rats, intramuscular injection of Fortical or Miacalcin (5 IU) in the thigh did not cause drug-related irritation.
I.V. injection in the tail vein of rats (50 IU) also did not cause irritation or systemic toxicity.

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REVIEW OF STUDIES WITH rsCT FOR NASAL ADMINISTRATION SUBMITTED TO THE CURRENT IND

PHARMACODYNAMICS

Comparative Pharmacokinetics and Pharmacodynamics of rsCT Derived as either a Fusion Protein or by a Direct Expression Method

(Study No. rsCT P9906Rp)

Methods

Sprague Dawley rats, weight 80-130g, N=18/ dose group/ test article, were given 9, 18, or 36 mIU/100g, s.c., of either rsCT-FP, rsCT-DE, or the Second International Standard for sCT (2nd IS) in 1% gelatin and 1% Na-acetate. The dose levels are equivalent to 90, 180, 360 mIU/kg, or 0.018, 0.036, 0.072 ug/kg. Determined were serum Ca levels at 60 minutes post dosing. The assay used was the rat hypocalcemia assay, Ph.Eur.1997:0471.

The human s.c. dose of 50 IU is equivalent to 0.170 ug/kg, which is ca. 2.5 times the highest dose used in this rat study on the basis of kg body weight. The reason for the relatively low animal dose is unclear.

Results

Mean serum Ca levels upon sCT administration

| Dose (mIU/kg) | N/group | Serum Ca level (mg/100ml) | | |
|---------------|---------|------------------------------|---------|---------|
| | | 2 nd Intl. Stdrd. | RsCT-FP | RsCT-DE |
| 9 | 18 | 9.2 | 9.3 | 9.4 |
| 18 | 18 | 8.6 | 8.6 | 8.6 |
| 36 | 18 | 7.7 | 7.8 | 7.8 |

Potency estimates of different rsCT preparations (Rat Hypocalcemia Assay)

| Test group | 2 nd Intl. Stdrd. | RsCT-FP (Rstd #4*) | RsCT-DE |
|-------------------------------|------------------------------|--------------------|---------|
| Weighted Mean Potency (IU/mg) | _____ | _____ | _____ |

RsCT-FP is also called reference standard #4

Conclusions

RsCT-DE has the same biologic activity in the standard rat hypocalcemia assay as rsCT-FP and the 2nd International Standard for sCT.

PHARMACOKINETICS

The Pharmacokinetics of Nasal Formulations of rsCT in the rat

(Study No. rsCT P9904Rn) (Unigene Labs, NJ)

Methods

Four doses of rsCT, 5 ug each, in 25 ul vehicle, 30 minutes apart, were given to 12 rats (Formulation I) or 8 rats (Formulation II), intranasally, with a pipette inserted 8mm into the rat's nostril. The dose of 5 ug is appr. 0.02 mg/kg, or 20 ug/kg, for a 250 mg rat. The total dose is 20 ug (80 ug/kg). Blood was sampled up to 150 minutes post dosing, and C_{max}, T_{max} and F were determined. RsCT was assayed by RIA (Unigene PRO.012).

Components of Formulation I and II (per 25 ul)

| Formulation | I | II |
|--------------------------|-------|-------|
| sCT (ug) | _____ | _____ |
| Benzyl alcohol (ug) | _____ | _____ |
| Phenylethyl Alcohol (ug) | _____ | _____ |
| Tween 80 (ug) | _____ | _____ |
| HCl (to adjust pH) | _____ | _____ |
| NaCl (ug) | _____ | _____ |

Components of Formulation I and II (ug/ml)

| Formulation | I | II |
|-----------------------------|---|----|
| sCT (ug/ml) | | |
| Benzyl alcohol (ug/ml) | | |
| Phenylethyl Alcohol (ug/ml) | | |
| Tween 80 (ug/ml) | | |
| HCl (to adjust pH) | | |
| NaCl (ug/ml) | | |

PK parameters

| Formulation | I | II |
|--------------------------|------|------|
| N (animals) | 12 | 8 |
| C _{max} (ng/ml) | 1.42 | 1.27 |
| T _{max} (min) | 108 | 116 |
| F (%) | 1.3 | 1.1 |

Conclusion

The preservatives phenylethyl alcohol and benzyl alcohol had a slight but not statistically significant effect on the absorption of intranasally administered sCT

The Pharmacokinetics of Nasal Formulations of rsCT in the rat
(Study No. rsCT P9902Rn) (Unigene Labs, NJ)

Methods

Either a single or four doses of 5 ug rsCT in 25 ul vehicle, were given to 6-20 rats, intranasally, with a pipette inserted 8mm into the rat's nostril. Multiple doses were given 30 minutes apart. The rsCT was formulated in one of three formulations, A1, A2 or A3.

Blood was sampled up to 150 minutes post dosing, and C_{max}, T_{max} and F were determined. RsCT was assayed by RIA (Unigene PRO.012).

Components of Formulations A1, A2, A3 (per 25 ul)

| Formulation | A1 | A2 | A3 |
|-----------------------------|----|----|----|
| sCT (ug) | | | |
| Benzalkonium Cl (mg) | | | |
| Benzyl alcohol (ug/ml) | | | |
| Phenylethyl Alcohol (ug/ml) | | | |
| Tween 80 (ug/ml) | | | |
| Citrate (mM) | | | |
| HCl (mM) | | | |
| NaCl (mg) | | | |

Results

PK parameters

| Formulation | A1 | A2 | A3 | A1 | A2 | A3 |
|--------------------------|------|------|------|------|------|------|
| SCT dose (ug) | 5 | 5 | 5 | 20 | 20 | 20 |
| N (animals) | 6 | 6 | 6 | 11 | 12 | 20 |
| C _{max} (ng/ml) | 0.65 | 0.25 | 0.67 | 3.13 | 1.42 | 3.92 |
| T _{max} (min) | 22.5 | 7 | 25 | 115 | 108 | 116 |
| F (%) | 1.57 | 0.26 | 1.85 | 3.13 | 1.31 | 3.77 |

Conclusion

Bioavailability was highest for formulations A3 (benzyl and phenylethyl alcohol and citrate) and A1 (benzalkonium and HCl). Bioavailability of Formulation of A2 was relatively low.

Comparative Pharmacokinetics and Pharmacodynamics of rsCT Derived as either a Fusion Protein or by a Direct Expression Method

(Study No. rsCT P9906Rp) (Unigene Labs, NJ)

Methods

Female Wistar rats (225-250g), N=9/test group, were fasted overnight and administered a single dose of 2 ug rsCT-FP (Lot Nr. 1100-9001) or rsCT-DE (Lot Nr. 1300-9005), in 200 ul 0.85% NaCl, i.v. in the jugular vein. This dose is equivalent to ca. 8 ug/kg.

Results

Pharmacokinetic parameters for rsCT-FP and rsCT-DE

| | RsCT-FP | RsCT-DE | P value |
|------------------------|---------|---------|---------|
| AUC (ng x min/ml) | 450 | 479 | 0.34 |
| T _{1/2} (min) | 17.4 | 20.8 | 0.25 |
| CL (ml/min) | 4.53 | 4.23 | 0.30 |
| V _d (ml) | 114 | 129 | 0.48 |
| k _e (min) | 25.1 | 30.1 | 0.25 |

Conclusions

In the rat, there were no differences in the pharmacokinetics of rsCT-FP and rsCT-DE.

TOXICOLOGY

Maximum Tolerated Dose of rsCT-DE in rats

(Study Nr. RsCT T9905Rp) (Unigene Labs, NJ)

Methods

Three rats were infused i.v. with 0.5 ml of 0.9% saline, with or without rsCT-DE (10 mg/ml). Total dose was 5 mg (ca. 20 mg/kg). The clinical dose of injectable (s.c.) calcitonin is 100 IU every other day. Animal behaviour was assessed up til 13 days after administration.

Results

Clinical signs in rsCT-DE treated rats (3/group)

| Time post dosing | Observations |
|------------------|------------------|
| 30 min | Slight shivering |
| 1h | Inactivity |
| 4h | No effects |
| 1d | No effects |
| 2d | No effects |
| 6d | No effects |
| 13d | No effects |

Conclusion

RsCT-DE at an i.v. dose of 5 mg in rats (20 mg/kg), or approximately 60.000x the currently used injectable human dose of 100 IU (0.33 ug/kg) on the basis of body weight, has slight toxicity up to 1h post dosing in rats. The dose comparison is approximate since the human dose is injected s.c. or i.m. and the animal dose in this study was given i.v. However, s.c. bioavailability is ca. 70%, so the real dose multiple is only ca. 30% smaller (40.000x).

4 Week Intranasal Toxicity Study in Rats

Study No. rsCT T9903Rn

Study facility:

Purpose

Investigate local irritancy of the test formulations in the URT of the rat following repeat daily dosing for 28 days. Fortical Nasal will be compared to Miacalcin^R

Methods

Sprague Dawley rats (CD^R VAF/PLUS-SPF) (N=6/sex/group), 6-7 weeks old, will be dosed with Fortical or Miacalcin, 17.8 ug in 40 ul, daily, by intranasal administration. Dose

administration procedure will be 10 ul to each nostril, repeated after ca. 30 seconds. Groups dosed with test article will be undergoing (A) necropsy at 4 weeks, or (B) necropsy at 6 weeks following 2 weeks recovery. Animals in the control group will receive saline.

- Necropsy: Macroscopic examination will be carried out of all animals (4- and 6-week necropsy) with close examination of nasal cavity.
- Organ weight: Lungs will be weighed.
- Histopathology: Histopathology will be carried out for all animals in the control and 4-week necropsy groups of the following nasopharyngeal tissues: Lungs, nasal cavity, pharynx. Nasal cavity will be sectioned at four transverse levels. If any abnormality is seen in the 4-week necropsy groups the 6-week necropsy groups will also be examined. Other tissues preserved will be: bronchial and cervical lymph node, larynx, trachea (anterior and posterior).

Time plan

Dosing of animals is expected to be started on January 13, 2000. Terminal studies are planned for February 10 (A groups) and February 24 (B groups). Draft report will be issued to the Sponsor by the end of March, 2000.

Comment on planned dose in 4-week intranasal toxicity study in rats

17.8 ug in 40 ul is equivalent to 40 ug in 90 ul. This (40 ug in 90 ul) is the amount in a standard clinical dose of 90 ul nasal spray (200 IU). CORRECTION: Thus, the drug concentration in the animal dosing formulation is the same as in the human formulation. However, in terms of mg/kg or mg/m², the animal dose is larger than the human dose. A dose of 17.8 ug in the rat (300 g) is approximately 60ug/kg. The human dose in 40ug/60kg= 0.7 ug/kg. Thus the rat dose of 17.8 ug (total) or 60 ug/kg is about 90 times the human dose of 0.7 ug/kg on the basis of mg/kg. On the basis of mg/m², the rat dose of 17.8 ug is approximately 15 times the human dose of 40 ug.

For assessment of local toxicity, the relevant comparative dose parameter would be exposure per surface unit of nasal epithelium. The best dose comparison would most likely be on the basis of mg/m² body surface area.

NOTE added for NDA (#21,406) review: The above paragraph in the IND review was corrected since there was an error in the dose calculation.

PRESERVATIVES

Preservative Content of Marketed Nasal Products

Unigene Study Report F99010 (APPENDIX M)

Purpose of study

Compare preservative concentrations of Fortical Nasal Spray to those in other nasal spray formulations (Rx and OTC)

Nasal Spray formulations

| Product | Flonase (Rx) | Beconase AQ (Rx) | Afrin nasal decongest ant (OTC) | Ocean nasal mist Saline (OTC) | Saline Nasal Spray (Rite Aid, OTC) | Fortical | Miacalcin |
|-----------------------|--------------|------------------|---------------------------------|-------------------------------|------------------------------------|----------|-----------|
| Benzalkonium chloride | 0.2 mg/ml | Not listed | Not listed | Not listed | - | | 0.2 mg/ml |
| Benzyl alcohol | - | - | 2.4 mg/ml | 3.0 mg/ml | 5.2 mg/ml | | - |
| Phenylethyl alcohol | 2.5 mg/ml | 2.5 mg/ml | - | - | - | | - |
| Tween 80 | Present | present | ? | ? | ? | | - |

These results show that the concentration (mg/ml) of benzyl alcohol and phenylethyl alcohol in Fortical nasal spray is similar to those in some other nasal spray products.

Flonase and Beconase AQ are anti-inflammatory steroids indicated for allergic rhinitis. They contain a similar concentration of phenylethyl alcohol as Fortical. The daily administered dose of

Flonase and Beconase (100mg suspension) is similar to the dose of Fortical (90 ul). Thus the total dose of administered phenyl ethyl alcohol is also similar for these products.

Saline Nasal Spray (Rite Aid) is used to introduce moisture in the nose and contains a similar concentration of benzyl alcohol as Fortical does. Dosage and administration of this OTC product is as needed. Thus the daily dose of benzyl alcohol could well be exceeding the one of Fortical at least for some period of time.

Tween 80 (polysorbate 80) is present in Flonase and Beconase. However, the concentration is not listed in PDR.

SUMMARY AND EVALUATION

PHARMACOLOGY/TOXICOLOGY

The results of previous pharmacology and toxicology studies conducted with the injectable form of Fortical have indicated that the recombinant form of sCT as formulated by the Sponsor (rsCT-FP) is pharmacologically effective and bioequivalent to the synthetic form of the peptide.

The rsCT contained in the nasal formulation (Fortical Nasal Spray) manufactured by a direct expression process (rsCT-DE) also appears to be bioequivalent to rsCT-FP used in the injectable form of Fortical, both with respect to biologic activity and pharmacokinetic profile.

A comparative bioavailability study of rsCT showed that formulation in benzyl alcohol, phenyl alcohol and Tween 80 with citrate buffer was similar to a formulation in benzylalkonium chloride (which is used in the Miacalcin Nasal Spray formulation). Omission of citrate greatly reduced bioavailability.

Systemic toxicity of the rsCT-DE in the rat was extremely low. Doses of ca. 40,000x the intended human dose caused minimal toxic effects.

A 4-week repeat dose intranasal study is currently carried out on the local tolerance of daily nasal doses of Fortical Nasal Spray in rats. The study is expected to be finished at the end of February, 2000. The one dose used in this study is the same as the intended clinical dose of 200 IU daily, on the basis of kg body weight.

The preservative content of the Fortical Nasal Spray formulation to be used in the clinical studies is different from Miacalcin Nasal Spray. However, other prescription and OTC drug products on the market contain similar concentrations of the preservatives as Fortical.

EVALUATION OF INTENDED CLINICAL PROTOCOLS

The intended clinical doses of Fortical Nasal Spray in the two proposed clinical studies are:

1. Bioequivalence study (UGL-N9903)

Dose level **6 x 400 IU**. Doses will be given 20 minutes apart (total dose 2400 IU).

2. Pharmacology/tolerability study (UGL-N9904)

Daily dose **200 IU/day**, for 1 month.

Human therapeutic doses of calcitonin (nasal administration)

| IU | Ug (approximately) | IU/kg | ug/kg (approximately) |
|-----|--------------------|-------|-----------------------|
| 50 | 10 | 0.83 | 0.17 |
| 100 | 20 | 1.67 | 0.33 |
| 200 | 40 | 3.3 | 0.67 |
| 400 | 80 | 6.7 | 1.34 |

1. In the first proposed clinical study a total of 2400IU will be administered nasally. Animal toxicity data on the local effect of this dose are not available. However, The Sponsor has conducted two clinical studies in the UK (UGL-N9901 and N9901) with doses of 2000 IU of either Fortical or Miacalcin. The safety data available from these studies suggest the expected systemic AE of nausea, dizziness, vomiting, headache and vasodilation, and local rhinitis. It thus appears reasonably safe to initiate this study.

2. In the second study daily doses of 200 IU will be administered. The results of the ongoing 28-day rat local tolerance study are relevant to evaluate the toxicity of this dose regimen and will be available by the end of March. Therefore, and also because ENT examinations will be done of the trial subjects, this Reviewer feels the study can be initiated but will ask the Sponsor to submit a draft report of the rat study as soon as it is available.

Additional Comment

The Clinical Investigator Brochure makes no mention of the results of the mutagenicity studies.

TO BE COMMUNICATED TO SPONSOR

1. Please provide the study results of the ongoing 28-day intranasal toxicity study in rats (Study No. _____) as soon as possible.
2. It is recommended to mention the results of the mutagenicity studies in the Investigator's Brochure.

Gemma Kuijpers, Ph.D.

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/s/

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PHARMACOLOGIST

ready to go

Karen Davis-Bruno
12/5/03 01:06:56 PM
PHARMACOLOGIST
Concur with recommendations

NDA Filing Meeting Checklist

NDA #: 21-406
Sponsor: Unigene Laboratories
Drug: Fortical (recombinant salmon calcitonin, rsCT), Nasal Spray
Indication: Treatment of postmenopausal osteoporosis
Product: Nasal Spray (200 IU per 90 uL)
Volumes: 83 (Pharm Tox Volumes 1, 2, 10-15)
NDA type: 505b(2) application (based on Miacalcin® Nasal Spray, synthetic salmon calcitonin)
IND#: 59,664
DMF#: 14,426

NONCLINICAL PHARMACOLOGY/TOXICOLOGY

| ITEM | YES | NO | COMMENT |
|--|-----|----|---------|
| 1) Does this section of the NDA appear to be organized (according to 21 CFR 314 and current guidelines for format and content) in a manner that would allow a substantive review to be completed? | X | | |
| 2) Is this section of the NDA indexed and paginated in a manner to enable a timely and substantive review? | X | | |
| 3) Is this section of the NDA sufficiently legible so that a substantive review can be done? Has the data been presented in an appropriate manner (consider tables, graphs, complete study reports, inclusion of individual animal data, appropriate data analysis, etc.)? | X | | |

| | | |
|--|---|---|
| <p>4) Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA? (Please itemize the critical studies included and indicate any significant studies that were omitted from the NDA - e.g., safety pharm, genotox, reprotox, chronic tox, carcinogenicity)</p> | X | <p>Preclinical studies include pharmacology/PD studies (i.v., s.c., rat and dog), safety pharmacology study (i.v., mouse), PK studies (i.v., s.c., rat and dog), acute toxicity studies (i.v., s.c., rat and dog), 28-day repeat dose toxicity study (i.n., rat), special toxicity/ local tolerance/ immunogenicity studies (i.v., i.m., i.a., p.v., rat, rabbit), in vitro genotoxicity studies. Reprotoxicity studies were not performed with rsCT (data from rabbit studies with synthetic calcitonin are in Miacalcin label and included in Fortical label). Carcinogenicity studies were not performed with rsCT (see below). Bone quality studies were not performed with rsCT. Toxicology testing requirements were met.</p> |
| <p>5) Have electronic files of the carcinogenicity studies been submitted for statistical review?</p> | X | <p>Carcinogenicity studies are not required for this application. Calcitonin is marketed for the indication of osteoporosis (Miacalcin®, intranasal spray and s.c. injection). Data on tumorigenicity from chronic toxicity studies with s.c. calcitonin in the rat are described in the Miacalcin label and included in the Fortical label.</p> |

| | | |
|---|---|--|
| <p>6) Were the studies adequately designed (ie., appropriate number of animals, adequate monitoring consistent with the proposed clinical use, state-of-the art protocols, etc.)?</p> | X | |
|---|---|--|

| | | | |
|---|---|--|---|
| 7) If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the sponsor clearly defined the differences and submitted reviewable supportive data (ie., adequate repeat studies using the marketed product and/or adequate justification for why such repetition would not be necessary)? | X | | |
| 8) Does the route of administration used in animal studies appear to be the same as the intended human exposure route? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route? | X | | One 28-day study by the intranasal route was carried out in rats to test for route-specific toxicity of the clinical formulation. |
| 9) Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.577? Is information available to express human dose multiples in either mg/m ² or comparative serum/plasma AUC levels? | X | | |
| 10) From a pharmacology/toxicology perspective, is this NDA fileable? If not, please state in item # 10 below why it is not. | X | | |

11) Reasons for refusal to file: N/A

Comments:

The drug product, Fortical Nasal Spray, is a solution containing recombinant salmon calcitonin (rsCT). The recommended dose is one spray (90 uL, 200 IU) per day administered intranasally, in alternating nostrils daily. The reference drug is Miacalcin Nasal Spray (200IU per 90 uL), which contains synthetic salmon calcitonin. The recombinant calcitonin in Fortical Nasal Spray is authentic with the calcitonin drug substance in Miacalcin Nasal Spray. It is pharmacologically effective and appears to be bioequivalent with the synthetic form of the peptide.

Studies carried out in the preclinical program with various rsCT formulations were summarized in Table 3E-1 (Vol. 1.2, p.110).

At the preNDA meeting the need for impurity testing for Pharmacology/Toxicology was mentioned. The composition of the clinical Fortical and Miacalcin preparations are listed below. Impurities include ~~entities~~ and other compounds in the drug substance, while excipients include buffers and preservatives in the drug product, as specified in the CMC section of the NDA.

Composition of Fortical and Miacalcin:

| | Fortical Nasal Spray | Miacalcin Nasal Spray |
|---------------------------|----------------------|-----------------------|
| RsCT or sCT | | 2200 IU/mL |
| Citric acid | | - |
| Polysorbate 80 (Tween 80) | | - |
| Benzalkonium chloride | | Trace |
| Benzyl alcohol | | - |
| Phenylethyl alcohol | | - |
| NaCl | | - |
| NaOH | | - |
| HCl | | Trace |
| Water | | |

The Fortical solutions tested in the preclinical pharmacology and toxicology studies were different from the solution to be marketed in terms of impurities ~~(impurities)~~ and in terms of excipients (buffers and preservatives). Formulations were described in the study reports. Preclinical test solutions contained rsCT made by two different recombinant methodologies (fusion protein, rsCT-FP, or direct expression protein, rsCT). The to-be-marketed rsCT is manufactured by the direct expression method. Excipients in the preclinical studies were either different or present at other concentrations than in the clinical formulation. However, the pivotal 28-day repeat dose intranasal toxicity study in the rat was carried out with the clinical to-be-marketed formulation. The impurity/excipient issue will be reviewed in detail in the NDA review.

Gemma Kuijpers
 Reviewing Pharmacologist

Karen Davis-Bruno
Supervisory Pharmacologist

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/s/

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Karen Davis-Bruno
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