PRODUCT MONOGRAPH

[Pr] REGRANEX*

becaplermin gel 0.01%

Wound Healing Growth Factor

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Date of Preparation:
December 30, 1998

Date of Revision

Submission Control No.: 128967

Date of Authorization: July 15, 2009

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CLINICAL PHARMACOLOGY

REGRANEX becaplermin is a recombinant human platelet-derived growth factor (rhPDGF). It is a homodimer produced by insertion of the gene for the B chain of platelet-derived growth factor into the yeast, *Saccharomyces cerevisiae.* Becaplermin has a molecular weight of approximately 25 kD and is composed of two identical polypeptide chains (each composed of 109 amino acids) that are bound together by disulfide bonds. It has biological activity similar to that of naturally occurring platelet-derived growth factor, which includes promoting the chemotactic recruitment and proliferation of cells involved in wound repair. In animal wound models, the predominant effect of becaplermin is to enhance the formation of granulation tissue.

REGRANEX becaplermin is a preserved gel, containing 100 μg of becaplermin per gram of gel. REGRANEX becaplermin contains the following inactive ingredients: sodium carboxy-methylcellulose, methylparaben, propylparaben, *m*-cresol and L-lysine hydrochloride.

REGRANEX becaplermin has been shown to be effective in increasing the incidence of complete wound healing and in decreasing the time to complete wound healing of full-thickness, lower extremity diabetic ulcers.

Pharmacokinetics

Ten patients with Stage III or IV [as defined in the International Association of Enterostomal Therapy (IAET) guide to chronic wound staging] lower extremity diabetic ulcers received topical applications of becaplermin at a dose of 0.32-2.95 μg/kg (7 μg/cm^2) daily for 14 days. Six patients had non-quantifiable PDGF levels at baseline and throughout the study, two patients had PDGF levels at baseline which did not increase substantially, and two patients had PDGF levels that increased sporadically above their baseline

values during the 14-day study period.

INDICATIONS AND CLINICAL USE

REGRANEX becaplermin is indicated to promote healing of full-thickness, lower extremity diabetic ulcers. REGRANEX becaplermin increases the incidence of complete wound healing and decreases the time to complete wound healing. 100% wound closure was more frequently observed in patients with ulcer size (length x width) < 7 cm^2 (see **Clinical Experience**).

REGRANEX therapy should be used in conjunction with good wound care practices. A good wound care program consists of: initial debridement (to remove all callus and necrotic tissue), pressure relief, moist dressings changed at a frequency to sufficiently maintain a moist wound-healing environment, systemic treatment of wound-related infection if present, and additional debridement as necessary.

Clinical Experience

The effects of REGRANEX therapy on the incidence of and time to complete healing in lower extremity diabetic ulcers were assessed in four randomized controlled studies. A total of 922 patients with diabetes, without severe peripheral arterial insufficiency, who had full-thickness, lower extremity diabetic ulcers were enrolled. Comparators were placebo gel and/or good wound care alone. In each trial, all patients received a standard regimen of good wound care. REGRANEX becaplermin was applied once daily. Patients were treated until complete healing, or for a maximum period of 20 weeks. Data from the primary and integrated analyses are presented below.

Three hundred and eighty-two (382) patients with Stage III or IV (IAET guide to chronic wound staging) lower extremity diabetic ulcers, over 90% of whom had Stage III ulcers, were enrolled in the pivotal double-blind study. The results from the study demonstrated that REGRANEX treatment significantly increased the incidence of complete healing by 43% when compared to placebo gel (p=0.007, 50% in the REGRANEX group vs. 35% in the placebo group). REGRANEX treatment also significantly decreased the time to achieve complete healing by 32% (p=0.013; 86 days for REGRANEX group vs. 127 days for placebo gel, estimated 35th percentile). The best results were seen with ulcers sized < 5 cm^2 measured by planimetry, which is equivalent to approximately 7 cm^2 when measured length x width.

An integrated analysis of the four controlled trials was conducted in patients with baseline ulcer areas (length x width) of up to approximately 7 cm^2. Seven hundred and seventy-four (774) patients (84% of the study population) in the four trials had baseline ulcer areas within this range. In these patients, REGRANEX treatment significantly increased the incidence of complete healing compared to placebo gel (p=0.009, logistic regression) and significantly reduced the time to complete healing by 30% (p=0.008; 92 days for REGRANEX group vs. 131 days for placebo gel, estimated 35^{th} percentile).

CONTRAINDICATIONS

REGRANEX becaplermin is contraindicated in patients with:

- known hypersensitivity to any component of this product (e.g., parabens);
- known neoplasm(s) at the site(s) of application.

WARNINGS AND PRECAUTIONS

Serious Warnings and Precautions

Malignancies distant from the site of application have occurred in becaplermin users in both a clinical study and in postmarketing use.

An increased rate of mortality secondary to malignancy was observed in patients treated with 3 or more tubes of REGRANEX Gel in a post-marketing retrospective cohort study. REGRANEX Gel should only be used when the benefits can be expected to outweigh the risks. (See **CONTRAINDICATIONS** and **WARNINGS and PRECAUTIONS**).

REGRANEX Gel contains becaplermin, a recombinant human platelet-derived growth factor, which promotes cellular proliferation and angiogenesis (See **CLINICAL PHARMACOLOGY**). Malignancies distant from the site of application have occurred in becaplermin users in both a clinical study and in postmarketing use, and an increased rate of death from systemic malignancies was seen in patients who have received 3 or more tubes of REGRANEX Gel. The benefits and risks of becaplermin treatment should be carefully evaluated before prescribing.

Application Site Reactions

If application site reactions occur, the possibility of sensitization or irritation caused by parabens or *m*-

cresol should be considered.

Immunogenicity

Becaplermin may have a very small potential to elicit an antibody response when applied topically once daily for up to 20 weeks; however, none of the patients that received REGRANEX therapy developed neutralizing antibodies against becaplermin.

Drug Interactions

It is not known whether becaplermin interacts with other topical medications applied to the ulcer site. Consequently, it is recommended that REGRANEX not be applied to the ulcer site in conjunction with other topical medications.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Becaplermin was not genotoxic in a battery of *in vitro* assay testing including endpoints of bacterial and mammalian cell point mutation, chromosomal aberration, and DNA damage/repair. Becaplermin was also not mutagenic in an *in vivo* assay for the induction of micronuclei in mouse bone marrow cells.

Carcinogenesis and reproductive toxicity studies have not been conducted with becaplermin.

Pregnancy

Animal reproductive toxicity studies have not been conducted with becaplermin. There are no adequate and well-controlled studies in pregnant women. Therefore, REGRANEX therapy should not be used in pregnant women.

Nursing Mothers

It is not known whether becaplermin is excreted in human milk. Therefore, REGRANEX therapy should be used with caution in nursing women.

Pediatric Use

Safety and effectiveness in children and adolescents under the age of 18 years have not been established.

Potential Effect on Bones and Connective Tissues

The effects of becaplermin on exposed joints, tendons, ligaments and bone have not been established in

humans. Data in rats suggests that, when injected near bones, becaplermin may cause a temporal and potentially reversible accelerated bone modelling (See **TOXICOLOGY, Special Toxicity**).

ADVERSE REACTIONS

Clinical Trial Data

The safety of REGRANEX Gel was evaluated in 1883 adult patients who participated in 17 clinical trials (diabetic ulcer trials with 1292 patients and non-diabetic ulcer trials with 591 patients) of REGRANEX and placebo and/or standard therapy (saline dressing). These 1883 patients had at least one topical administration of REGRANEX and provided safety data.

Adverse drug reactions reported by ≥1% of patients treated with REGRANEX in these trials are shown in Table 1.

Table 1: Adverse Drug Reactions Reported by ≥1% REGRANEX-treated 17 Clinical Trials of REGRANEX

System/Organ Class Adverse Reaction	**REGRANEX (n=1883) %**	**Placebo (n=1069) %**	**Standard therapy (n=190) %**	**p-value†**
Infections and Infestations				
Infected skin ulcer	12.3	11.9	18.9	0.725
Cellulitis	10.3	7.5	18.9	0.011
Osteomyelitis	7.2	5.4	14.2	0.058

† Comparison between REGRANEX and placebo.

Postmarketing Data

Adverse drug reactions first identified during postmarketing experience with REGRANEX are included in Table 2. The frequencies are provided according to the following convention:

Very common	≥1/10
Common	≥1/100 and <1/10
Uncommon	≥ 1/ 1,000 and <1/100
Rare	≥1/10,000 and <1/1,000
Very rare	<1/10,000, including isolated reports.

In Table 2, ADRs are presented by frequency category based on spontaneous reporting rates.

Table 2. Adverse Drug Reactions Identified During Postmarketing Experience with REGRANEX by Frequency Category Estimated from Spontaneous Reporting Rates

Nervous System Disorders	
Rare	Burning sensation[1]
Skin and Subcutaneous Tissue Disorders	
Very rare	Rash
	Erythema[2]

[1] The bundled term burning sensation consists of the preferred terms burning sensation, skin burning sensation, and application site irritation, all of which referred specifically to burning at the application site.
[2] Refers to erythema at the application site.

In a follow-up study, 491 (75%) of 651 subjects from two randomized, controlled trials of becaplermin gel 0.01% were followed for a median of approximately 20 months to identify malignancies diagnosed after the end of the trials. Eight of 291 subjects (3%) from the becaplermin group and two of 200 subjects (1%) from the vehicle/standard of care group were diagnosed with cancers during the follow-up period, a relative risk of 2.7, (95% confidence interval 0.6-12.8). The types of cancers varied and all were remote from the treatment site.

In a retrospective study of a medical claims database, patients who received REGRANEX between January 1, 1998 and June 30, 2003 were followed through December 31, 2003. Cancer rates and overall cancer mortality were compared between 1,622 patients who used REGRANEX Gel and 2,809 matched comparators. Estimates of the incidence rates reported below may be under-reported due to limited follow-up for each individual.

- The incidence rate for all cancers was 10.2 per 1,000 person years for patients treated with REGRANEX Gel and 9.1 per 1,000 person years for the comparators. Adjusted for several possible confounders, the rate ratio was 1.2, (95% confidence interval 0.7-1.9). Types of cancers varied and

were remote from the site of treatment.

- The incidence rate for mortality from all cancers was 1.6 per 1,000 person years for those who received REGRANEX Gel and 0.9 per 1,000 person years for the comparators. The adjusted rate ratio was 1.8 (95% confidence interval 0.7-4.9). Additional follow-up (not part of the original study plan) to include deaths through 2006 changed these mortality estimates to a rate of 0.9 per 1,000 person years for those who received REGRANEX gel, and 1.0 per 1,000 person years for the comparators, an adjusted rate ratio of 1.0 (95% confidence interval 0.5-2.3).
- The incidence rate for mortality from all cancers among patients who received 3 or more tubes of REGRANEX gel was 3.9 per 1,000 person years and 0.9 per 1,000 person years in the comparators. The adjusted rate ratio for cancer mortality among those who received 3 or more tubes relative to those who received none was 5.2, (95% confidence interval 1.6-17.6). Additional follow-up (not part of the original study plan) to include deaths through 2006 changed these mortality estimates to a rate of 2.0 per 1,000 person years for those who received three or more tubes, and 1.0 per 1,000 person years for the comparators, an adjusted rate ratio of 2.4 (95% confidence interval 0.8-7.4).

SYMPTOMS AND TREATMENT OF OVERDOSAGE

There are limited data on the effects of becaplermin overdose.

DOSAGE AND ADMINISTRATION

REGRANEX becaplermin should be applied topically as a continuous thin layer to the entire ulcerated area(s) once daily using a clean application aid (e.g., tongue depressor or cotton swab). The site(s) of application should then be covered with a saline dressing that maintains a moist wound-healing environment and left in place for approximately 12 hours. The dressing should then be removed and the ulcer rinsed with saline or water to remove residual gel and covered again with a second moist dressing (without REGRANEX Gel) for the remainder of the day.

REGRANEX treatment should continue once daily until complete healing is achieved.

REGRANEX should be discontinued if complete healing has not occurred after 20 weeks of therapy or if a 30% reduction in ulcer size is not observed after 10 weeks of treatment (see **WARNINGS AND PRECAUTIONS**).

PHARMACEUTICAL INFORMATION

i) Drug Substance

Common Name:	Becaplermin (rhPDGF-BB)
Structure:	Two identical polypeptide chains, each composed of 109 amino acids, that are bound together by disulfide bonds.
Molecular Weight:	Approximately 25 kD
Description:	A clear, colourless to straw-coloured preserved gel.

ii) Composition

Each gram of REGRANEX becaplermin contains 100 µg of becaplermin, rhPDGF-BB. Other ingredients include: sodium carboxymethylcellulose; sodium chloride; sodium acetate trihydrate; glacial acetic acid; methylparaben; propylparaben; *m*-cresol, 99%; L-lysine hydrochloride; Water for Injection.

iii) Stability and Storage Recommendations

Store refrigerated, 2° - 8°C. DO NOT FREEZE.

AVAILABILITY OF DOSAGE FORMS

REGRANEX becaplermin, supplied as a clear, colourless to straw-coloured preserved gel containing 100 μg of becaplermin per gram of gel, is available in the following tube sizes:

2 gram, 7.5 gram, and 15 gram.

INFORMATION FOR THE PATIENT

Please read this information carefully because it contains important details about your medicine. If there is anything that you do not understand or if you need further information or advice, you should ask your doctor, pharmacist, nurse, podiatrist or other health care professional.

As you may need to refer to it again, do not throw out this information.

WHAT IS REGRANEX becaplermin?

REGRANEX becaplermin is a protein made in the laboratory. It acts like a substance naturally produced in your body called platelet-derived growth factor (PDGF). PDGF helps wounds to heal.

REGRANEX becaplermin is a clear, colourless to straw-coloured preserved gel and is supplied in 2, 7.5, or 15 gram tubes.

WHAT IS REGRANEX THERAPY FOR?

REGRANEX therapy promotes the healing of skin ulcers which are due to the complications of diabetes. With REGRANEX treatment, it is more likely that your ulcers will heal completely and more quickly. **REGRANEX becaplermin is for external use only.**

IMPORTANT POINTS TO NOTE BEFORE STARTING REGRANEX THERAPY

When NOT to use REGRANEX becaplermin

Do NOT use this product if you know you are allergic to parabens or to any of the other ingredients in the product. If in doubt, consult your doctor or pharmacist.

Do NOT use REGRANEX treatment if you have a skin tumour at the site of application.

Precautions

You should consult your doctor before starting REGRANEX treatment if:

- you have had or now have any form of cancer;
- you are pregnant, or think you are;
- you are breast-feeding.

There is no clinical information to support the use of REGRANEX becaplermin in children and adolescents under the age of 18 years.

Do not apply REGRANEX becaplermin together with other topical medications to the ulcer site.

HOW TO USE REGRANEX becaplermin

Prior to REGRANEX treatment, your ulcer should be cleaned by your doctor or health care professional. It is very important to make sure that while you are on REGRANEX therapy you also take very good care of your ulcer to help it heal as quickly and fully as possible. You should keep pressure off your ulcer by following your doctor's or health care professional's instructions. This may involve wearing special shoes or shoe inserts. Relieving pressure on your wound will help it to heal. If you experience signs of infection of the ulcer (e.g., redness, swelling, pain, odour, fever), you should consult your doctor immediately for specific treatment.

Application instructions for REGRANEX therapy. Follow these instructions once each day:

1. Wash your hands thoroughly before applying REGRANEX becaplermin.
2. Remove the dressing on your wound; rinse the wound gently to remove any residual gel from previous applications.
3. Using an applicator such as a cotton swab or a tongue depressor, apply a thin, even layer to the entire surface of the wound. The tip of the tube should not come in contact with the wound or any other surface; the tube should be recapped tightly after each use. Applying more REGRANEX becaplermin will not further enhance healing.
4. Once REGRANEX becaplermin has been applied, cover the wound with a dressing that will keep the area moist. Moist dressings will help your wound to heal. The dressing should be left in place for approximately 12 hours after which it should then be removed and the ulcer rinsed with saline

or water to remove residual gel and covered again with a second moist dressing (without REGRANEX Gel) for the remainder of the day.

5. Return the REGRANEX tube to the refrigerator after each use; the REGRANEX tube should be stored in the refrigerator but not frozen.

Continue to apply REGRANEX becaplermin once daily until your wound has completely healed or until your doctor or health care professional tells you to stop.

UNDESIRED EFFECTS

During REGRANEX treatment, some patients may experience redness or rash at the application site. If you start to feel unwell or notice any undesirable effects, including effects not mentioned in this insert, or if you are not sure about the effect of this product, contact your doctor or caregiver promptly.

HOW TO STORE REGRANEX becaplermin

REGRANEX becaplermin should be stored in the refrigerator (2° to 8°C). DO NOT FREEZE.
After use, close the cap tightly and always refrigerate the tube.

Do not use REGRANEX becaplermin after it has expired (as indicated by the date on the crimp of the tube), even if it has been stored properly.

A REMINDER

This medicine has been prescribed for you. Use it only as directed. Do not give REGRANEX becaplermin to anyone else, even if his/her symptoms are similar to yours.

Keep this and other medications out of the reach of children.

Product Monograph available to health care professionals on request.

PHARMACOLOGY

The predominant effect of becaplermin is to increase the amount of granulation tissue (the newly formed connective tissue that fills the wound defect). The two parameters directly contributing to wound closure, epithelialization and contraction, were evaluated in several wound-healing models. The two most reliable models for evaluating the efficacy of becaplermin (on granulation tissue formation) were the guinea pig partial-thickness skin excision model and the rat sponge implantation model.

Partial-Thickness Skin Excision Models

Partial-thickness skin excision models, involving the use of a dermatome to remove the epidermis and the upper dermis, were used in guinea pigs and pigs to evaluate the efficacy of becaplermin in wound healing. Evaluation of the efficacy of becaplermin in the guinea pig model was restricted to enhanced granulation tissue formation. In the pig models, granulation tissue thickness was the primary efficacy endpoint, although in one model only epithelialization was evaluated.

In the partial-thickness skin excision models, becaplermin showed efficacy in the 3 to 10 μg/mL range, often with indications of an effect at 1 μg/mL. Overall, increasing concentrations of becaplermin tended to be associated with increasing granulation tissue thickness in this model. There were no indications of any inhibitory effects of high concentrations of becaplermin, although in occasional studies the highest concentration tested did not give the most pronounced response.

A set of studies directly comparing guinea pig and pig full- and partial-thickness skin excision models using becaplermin at 100 μg/mL in saline or 100 μg/g in sodium carboxymethylcellulose (NaCMC) gel revealed that each model and formulation was able to show the effects of becaplermin on granulation tissue.

Sponge Implantation Models

The sponge implantation models, where becaplermin solution was injected into the sponges on alternate days, were particularly reliable for demonstrating the effects of becaplermin on enhancing granulation tissue formation. This model was limited to saline-based or 5% dextrose-based formulations and to granulation tissue evaluations. The granulation tissue response was characterized by an increase in connective tissue ingrowth into the sponges and/or an increase in connective tissue

cellularity, especially in the sponge capsule. The response to becaplermin tended to be more cellular than collagenous. Regardless of species, becaplermin concentrations of 3 to 10 μg/mL and higher were effective in these sponge models.

Becaplermin-induced granulation tissue in excision wounds generally appeared identical to that of granulation tissue in control wounds except in amount. However, in the sponge implantation models (mouse, rat, and guinea pig), becaplermin tended to induce a granulation tissue response in the sponge capsule that was particularly cellular, with comparatively little extracellular matrix. Indeed, in the mouse sponge implantation model, efficacy was based strictly upon this cellular capsular response.

Pharmacokinetics

Absorption of topically administered becaplermin in male Fischer rats and subcutaneous administration in monkeys after single and multiple doses was extremely limited or negligible. Therefore, studies to evaluate the distribution, metabolism, and excretion of becaplermin in laboratory animals were not performed.

TOXICOLOGY

The potential toxicity of becaplermin has been evaluated in acute, multidose, sensitization, local irritation, and mutagenicity studies in a variety of animal species.

Acute Toxicity

The potential acute toxicity of becaplermin was evaluated after intravenous and subcutaneous administration in mice and rats, and after subcutaneous administration in monkeys. The results from these studies are summarized in Table 3.

Table 3: Acute Toxicity Studies - Protocol Summaries and Results

Strain/ Species	No./ Group	Route	Vehicle	Dose Levels[a]	Lethality	Summary of Toxic Signs
SW/Mouse	5M	i.v.	Saline	0	0/5	No significant acute toxicity.
				3	0/5	
	5F			0	0/5	
				3	0/5	
SW/Mouse	5M	i.v.	Saline	0	0/5	Transient, rapidly reversible, peripheral
				10	0/5	vasodilation and CNS depression, and low
				30	1/5	mortality.
				100	0/5	No effects on body weights or gross observations in abdominal, pelvic, and
	5F			0	0/5	thoracic cavities. No significant
				10	0/5	histomorphologic changes in mice that
				30	0/5	died during the study.
				100	1/5	
SW/Mouse	5M	s.c.	Saline	0	0/5	No acute toxicity.
				3	0/5	
	5F			0	0/5	
				3	0/5	
F 344/Rat	5M	i.v.	Saline	0	0/5	No acute toxicity.
				3	0/5	
	5F			0	0/5	
				3	0/5	
F 344/Rat	5M	s.c.	Saline	0	0/5	No acute toxicity.
				3	0/5	
	5F			0	0/5	
				3	0/5	
Cynomolgus/ Monkey	1M	s.c.	Saline	0.3	0/1	No acute toxicity.
	1M			1.0	0/1	
	3Mb			3.0	0/3	
	1F			0.3	0/1	
	1F			1.0	0/1	
	3F[b]			3.0	0/3	

[a] mg/kg for mice and rats. In monkeys, applied dose for males and females in each group were approximately 3.7, 11.0, and 35.5 mg/m^2 and 3.4, 11.5, and 34.0 mg/m^2, respectively. Doses expressed as mg/m^2 are mean dosages for male and female monkeys, respectively, according to calculations based on body surface area measurement in m^2 and body weight in grams.

[b] Escalating doses; 0.3 and 1.0 mg/kg group monkeys received a second becaplermin administration of 3 mg/kg on Day 8. Thus, 3 males and 3 females received the highest dose of 3 mg/kg.

s.c. = subcutaneous; CNS = central nervous system; strains: SW = Swiss Webster; F = Fischer.

Pretest/predose evaluations conducted on all animals.

Long-term Toxicity

The toxicity of becaplermin has been investigated in intravenous, topical, and subcutaneous multidose studies in mice, rabbits, and monkeys, respectively. The duration of these studies was up to 13 weeks.

In mice, daily intravenous administration of becaplermin at doses of 0.3, 1, and 3 mg/kg for two weeks resulted in the death of one female treated with 3 mg/kg, as well as rapidly reversible vasodilation and CNS depression in animals given 1 and 3 mg/kg, and a slight but significant increase in mean body weight gain at 3 mg/kg.

In male and female rabbits, no evidence of systemic toxicity or dermal changes were observed when becaplermin (10, 30, or 100 μg/mL) was applied topically, 6 hours per day for 21 days. Dermal irritation observed in both vehicle- and becaplermin-treated rabbits was attributed to excessive epidermal/dermal hydration with an aqueous vehicle, the occlusive dressing technique, and the specific hair pattern of the individual rabbits.

In a dose range finding toxicity study in monkeys, subcutaneous administration of becaplermin daily for three weeks at dosages of 30, 100, and 300 μg/mL resulted in drug-related swelling at the injection site. There was also a possible association of temporary mild anemia and splenomegaly at the higher dosage level. Plasma PDGF concentrations increased linearly with dosage.

In a definitive cynomolgus monkey study, no systemic toxicity attributable to becaplermin treatment was observed following 13 weeks of subcutaneous dosing of up to 150 μg/kg. Appearance of subcutaneous fibroplasia and inflammation at the injection site, and expected appearance of antibodies to the recombinant human protein were observed. Although antibodies to becaplermin developed in one control and all becaplermin-treated monkeys during the course of the study, only 1 of 20 monkey serum samples (evaluated by the mitogenic assay) produced antibodies capable of neutralizing the mitogenic activity of 625 ng/mL becaplermin. There were no drug-related clinical findings or effects on body weight. Dose-related increases in plasma PDGF concentrations were observed in both studies performed in monkeys.

Reproduction and Carcinogenicity

No reproductive toxicity studies were conducted. Carcinogenicity studies were also not conducted for this compound. The potential formation of antibodies and the subsequent inactivation or immune

complexation may negate or complicate the interpretation of the results of a rodent carcinogenicity study.

Special Toxicity

Special toxicity studies have been conducted to evaluate the bone toxicity, neutralizing antibody production, sensitization, and the dermal or ocular irritation potential of becaplermin.

In a bone toxicity study in rats, injection of becaplermin near the right femur and right metatarsus on alternate days for 13 days at concentrations of 10, 30, and 100 μg/mL produced histomorphologic changes suggestive of accelerated bone remodelling judged to be a temporal and potentially reversible alteration. These findings were not unexpected, based on the pharmacologic activity of becaplermin in stimulating connective tissue growth.

Daily subcutaneous administration of becaplermin to rats for one month, at doses ranging from 1.5 to 150 μg/kg, resulted in the production of antibody response in some rats in a dose-related manner. None of the 20 rats that demonstrated antibody response produced neutralizing activity using becaplermin at a concentration of 625 ng/mL in the mitogenic assay. However, at a lower concentration of becaplermin (62.5 ng/mL), 4/20 rats demonstrated 30-60% neutralizing activity of becaplermin. In addition, minimal changes occurred in the subcutis of some rats in the 150 μg/kg group which ranged from minimal to mild mixed inflammatory cell infiltrate with varying degrees of fibroblast activation, to minimal fibroplasia. Since the effects of becaplermin were so slight in the tissues examined, evaluation of lower dosages or the recovery sites was not warranted.

In guinea pigs, becaplermin was tested in both the Modified Maximization Design and the Landsteiner/Draize Model. The Modified Maximization Test involved initial intradermal induction in association with Freund's complete adjuvant (FCA), followed by a topical induction, and then by topical challenge and intradermal rechallenge. The standard Landsteiner/Draize Test involved 10 repeated intradermal injections of becaplermin without FCA, given three times/week, followed by a single intradermal challenge 14 days after the last injection. In both test systems, becaplermin was found to be a sensitizing agent. These findings of delayed contact sensitization are not unexpected for a recombinant human protein.

In three dermal irritation studies, becaplermin at concentrations of 10 to 110 μg/g did not cause significant dermal irritation or systemic toxicity in rabbits when applied topically to intact and abraded skin in

saline or NaCMC gel (2.4, 2.5, 2.75, or 4.0%) preserved with methylparaben, propylparaben, and *m*-cresol (0.5% lysine hydrochloride added to 2.4% NaCMC gel in one study). Becaplermin at 110 μg/g in preserved 2.75% NaCMC also did not produce ocular irritation in rabbits up to seven days post-instillation.

Mutagenicity Studies

Becaplermin was not genotoxic in a variety of *in vitro* assays including endpoints of bacterial and mammalian cell point mutation, chromosomal aberration, and DNA damage/repair. Becaplermin was also not mutagenic in the *in vivo* micronucleus assay.

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