Pharmacologic and Pharmacokinetic Profile of Repifermin (KGF-2) in Monkeys and Comparative Pharmacokinetics in Humans

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ABSTRACT Repifermin (truncated, recombinant human keratinocyte growth factor-2, KGF-2) was evaluated in cynomolgus monkeys and healthy humans during a phase 1 trial. Monkeys received vehicle or repifermin at 20, 75, or 200 µg/kg IV or 750 µg/kg subcutaneous (SC) daily for 29 days. Clinical observations were made during the entire dosing period. Gross and microscopic changes were assessed at necropsy. Pharmacokinetic parameters and immunogenicity were evaluated in these monkeys and in humans, following a single or 7 daily IV bolus injections of 1, 5, 25, or 50 µg/kg repifermin. In monkeys, repifermin was well tolerated, and histologic evaluation demonstrated dose-dependent, reversible thickening of the mucosa throughout the alimentary tract, except for the stomach. In the alimentary tract tissues, nonepithelial tissues were not affected, indicating a specificity of repifermin for epithelial cells. Pharmacokinetics in both monkeys and humans were dose proportional, showed lack of drug accumulation with repeated daily dosing, and were characterized by high volumes of distribution and clearance rates, indicating substantial tissue binding and metabolism. Repifermin was not markedly immunogenic following multiple daily IV injections in either species. Serum repifermin concentrations in humans were comparable to those attained in monkeys that produced significant pharmacological effects on epithelial cells in the alimentary tract. These findings provide additional support for the ongoing clinical development of repifermin for diseases involving epithelial injury.

KEY WORDS: fibroblast growth factor, keratinocyte growth factor, pharmacology, pharmacokinetics clinical trial.

INTRODUCTION

Repifermin is a truncated, recombinant form of human keratinocyte growth factor-2 (KGF-2) obtained by high-throughput sequencing of prostate and fetal lung Cdna

libraries.¹ KGF-2 is a member of the fibroblast growth factor (FGF) family of proteins and is also described in the literature as FGF-10.² Among the various fibroblast growth factors, KGF-2 is most closely related to FGF-7 (KGF-1), with which it shares 45% to 57% identity.^{1,3} KGF-2 is highly conserved across species, with mouse and rat homologs having 92%³ and 96%⁴ identity, respectively, to the human form.^{2,5} Under normal physiological conditions, KGF-2 is believed to display a paracrine-type activity on epithelial cell proliferation and differentiation, because it is synthesized and secreted by stromal (mesenchymal) cells adjacent to epithelial cells expressing the appropriate FGF receptors.⁶ KGF-2 plays an important role in organogenesis during embryonic development. Specifically, mouse KGF-2 is critical to embryonic lung and limb bud development, and deletion of this gene is lethal.⁷⁻⁹ KGF-2 stimulates fetal rat skin keratinocyte proliferation but has essentially no mitogenic activity for cultured fibroblasts.⁵

In preclinical studies, it has been shown that repifermin exerts its effects through a splice variant of FGF receptor type-2 (FGFR2iib), also referred to in the literature as KGFR.^{2,10} Additionally, repifermin stimulates proliferation of BaF3 cells transfected with a splice variant of type-1 FGF receptor, FGFR1iib, albeit at concentrations approximately 10 times higher than those required for FGFR2iib-mediated activation of the same cells.¹⁰ In addition to promoting re-epithelialization by a direct effect on keratinocytes via proliferation and migration, repifermin may stimulate granulation tissue formation by a direct chemotactic effect on fibroblasts.¹⁰ The specificity of repifermin for keratinocytes and other epithelial cells is in contrast to other members of the FGF family, such as FGF-2, which regulate the proliferation and/or the differentiation of several cell types (e.g., fibroblasts, smooth muscle, endothelial cells) and exhibit potent angiogenic and neurotrophic properties.¹¹ Since KGF-2 appears to have a receptor binding profile different from the other members of the FGF family, it is speculated that repifermin may exhibit a distinct pharmacological profile.

In other preclinical studies, repifermin stimulated the proliferation of epithelial cells in the liver, kidney, pancreas, and urinary bladder and increased the numbers of intestinal goblet cells in the gastrointestinal tract when administered systemically to rats.¹⁰ Repifermin provided protection against intestinal injury in

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a murine model of dextran sulfate sodium-induced colitis¹² and promoted healing of indomethacin-induced jejunal ulceration in rats.¹³ Repifermin was found to be more effective than the vehicle control at closing the interstices of a human meshed skin graft explanted to athymic nude rats.¹⁴ In light of this pharmacological profile, repifermin is being developed as a potential therapeutic agent for chemotherapy- or radiotherapy-induced mucositis, ulcerative colitis, and cutaneous wounds. Mucositis (or stomatitis) is a toxic inflammatory reaction affecting epithelial cells lining the alimentary tract¹⁵ for which there are no generally accepted specific agents for prevention or treatment. Similarly, ulcerative colitis and chronic wounds, such as venous ulcers, involve epithelial cell injury and tend to be refractory in nature without satisfactory therapies. This report describes the effects of repifermin treatment on epithelial and nonepithelial tissues in the normal cynomolgus monkey and compares the pharmacokinetic and immunogenicity profiles of monkeys and healthy human subjects.

MATERIALS AND METHODS

Repifermin was provided as a sterile, single-use, lyophilized material. Upon reconstitution with 1.2 mL water for injection, each vial contained 4 mg/mL repifermin. The placebo for repifermin was also supplied as a sterile, single-use, lyophilized material containing the same excipients as the active drug when reconstituted.

Four-Week Dosing and Recovery Study in Cynomolgus Monkeys

A study was carried out in normal cynomolgus monkeys (*Macaca fascicularis*) in which repifermin was administered IV (20, 75, or 200 µg/kg/d) or SC (750 µg/kg/d) once daily for 29 days. IV and subcutaneous (SC) routes of administration were chosen since both are potential routes of administration in humans. A group of animals that were administered placebo (vehicle) by the IV route served as controls for both the IV and SC repifermin-treated monkeys. A subgroup of animals was included in each of the groups treated with either placebo, high-dose repifermin IV (200 µg/kg/d), or repifermin SC (750 µg/kg/d) for which the treatment phase was followed by a 4-week treatment-free (recovery) period. The study design is summarized in Table 1. The cynomolgus monkey was chosen for this study because it is a species that was sensitive to the pharmacologic effects of repifermin in pilot studies. The study was carried out at the facilities of Sierra Biomedical, Inc (Sparks, NV) in accordance with Good Laboratory Practice, the provisions of the US Animal Welfare Act (1966 and amendments) as described in the

Guide for Care and Use of Laboratory Animals (ILAR Commission on Life Sciences), and under the auspices of AAALAC-International guidelines.¹⁶ Forty naive adult cynomolgus monkeys (20 males, 20 females) were assigned to the study and observed twice daily for routine clinical evaluation, beginning at least 5 days prior to the first day of dosing and continuing throughout the study. Food consumption was assessed daily. Body weights were measured prior to the first administration of repifermin and weekly thereafter. After either 29 or 57 days, monkeys underwent a complete necropsy. Various tissues were obtained including those from the tongue, buccal mucosa, esophagus, stomach, small intestine, and colon. Other samples were obtained, including nonepithelial tissues to evaluate the specificity of repifermin. Tissues were fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin/eosin.

For pharmacokinetic analysis, blood samples were drawn on day 1 and day 29; prior to dosing (0 minutes); and at 5, 15, and 30 minutes and at 1, 2, 4, and 6 hours after dosing for IV administration. The sampling schedule following SC administration was 5 and 30 minutes; 1, 2, 4, 8, and 24 hours after dosing. Serum was obtained from the blood samples, and serum repifermin levels were determined by a solid-phase enzyme-linked immunosorbent assay (ELISA), as described below. Compartmental and noncompartmental pharmacokinetic analyses were performed on individual serum repifermin concentration curves using WinNonlin® (Pharsight Corporation, Mountain View, CA). The area-under-the-curve (AUC) in the noncompartmental analysis was calculated using the log-linear trapezoidal rule over the period with detectable levels and extrapolating to infinity by addition of the last observed concentration divided by the terminal rate constant. Pharmacokinetic parameters derived for individual monkeys were averaged for each dose group. To determine repifermin-reactive antibody titers, approximately 1 mL of blood was collected before the study and before dosing on days 14, 29, and 56 (recovery animals) into tubes without anticoagulant. Serum samples were stored frozen at -20°C until analyzed for human subjects, as described below.

Interspecies Scaling of Doses

The clearance of many therapeutic proteins follows allometric scaling with an allometric constant ranging from 0.65 to 0.8.¹⁷ An allometric constant of 0.66 is equivalent to the use of body surface area as the metric for normalizing doses and is a common method for scaling doses across species.¹⁸ Based on body surface area scaling, doses of 20, 75, and 200 µg/kg IV in a 3-kg monkey correspond to 7, 26, and 70 µg/kg, respectively, in a 70-kg human. The phase 1 clinical study of this human growth factor employed IV doses of 1, 5, 25, and

Table 1. Study Design of a 4-Week Dosing, 4-Week Recovery Study in Cynomolgus Monkeys

Group	No. Animals (M/F)	Route	Dosage ($\mu\text{g/kg}$)	No. Euthanized	
				Day 29	Day 57
I	4/4	IV	0	3/3	1/1
II	3/3	IV	20	3/3	-
III	3/3	IV	75	3/3	-
IV	5/5	IV	200	3/3	2/2
V	5/5	SC	750	3/3	2/2

50 $\mu\text{g/kg}$, given either as a single injection or as 7 daily injections.

Human Pharmacokinetics

The pharmacokinetic profile of repifermin was evaluated as part of a phase-1 trial in healthy humans. Forty-seven subjects (ages 18-65 years) were enrolled. Subjects were administered either single IV bolus injections ($n = 15$) or 7 daily IV bolus injections ($n = 24$) of repifermin at doses of 1, 5, 25, or 50 $\mu\text{g/kg}$. Eight subjects were administered 7 daily doses of placebo. The pharmacokinetic portion of the study was designed to investigate whether repifermin exhibits dose-dependent pharmacokinetics and whether the pharmacokinetic profile changes after administration of multiple doses. For both single- and multiple-dose cohorts, blood samples were withdrawn on day 1, prior to administration of the first dose; at 5, 15, and 30 minutes; and at 1, 2, 4, and 6 hours after the first dose on that day. For subjects in the multiple-dose cohort, blood samples were also collected at these same time points on day 7. In addition, predose samples were collected on day 2 through day 6 from these subjects, and a final sample from this cohort was taken 24 hours after the last dose on day 8. Subjects in the 50 $\mu\text{g/kg}$ repifermin multiple-dose group also had 2-hour postdose samples collected on days 2 through 6. Compartmental and noncompartmental pharmacokinetic analyses were performed using WinNonlin. As in the monkey study, the AUC in the noncompartmental analysis was calculated using the log-linear trapezoidal rule. Pharmacokinetic parameters derived from individual subjects were used to calculate a mean value for the various dosage groups. The change in AUC from day 1 to day 7, normalized to the AUC on day 1, was calculated for each subject. The mean of the differences was tested against the null hypothesis using a 2-tailed Student *t*-test. Statistical

significance was set at the 95% level. For those subjects who were administered multiple injections of 50 $\mu\text{g/kg}$ and had serum samples taken 2 hours after dosing, a 1-way ANOVA procedure was used to determine whether day of injection was a significant factor.

Repifermin ELISA

Serum samples were analyzed for repifermin content by an ELISA that employed a polyclonal goat antirepifermin antibody (HGS) for capture and a biotinylated polyclonal chicken antirepifermin antibody (HGS) for detection, horseradish peroxidase/streptavidin conjugate (Vector Labs, Burlingame, CA) and tetramethylbenzidine. The assay had a lower limit of detection of 4 ng/mL for monkey-serum samples and 1 ng/mL for human-serum samples. The assay used a 4-parameter fit for the standard curve. Samples falling above the upper end of the linear range of the standard curve were retested at appropriate dilutions to achieve an absorbance in the linear range. In each validation run, 2 positive controls (5 ng/mL and 1 ng/mL) and 1 negative control were included. The following criteria were used for acceptance of an analytical run: the standard curve composed of 7 different concentrations must have an $r^2 > 0.98$, the absorbance from the highest concentration must be > 1.3 , the positive controls must be detected within $\pm 20\%$ of the expected values, the absorbance from the negative control must be < 0.3 , and the coefficient of variation for each point must be $< 20\%$.

Immunogenicity

The presence of repifermin-reactive antibodies was determined in 100-fold diluted sera from both monkeys and human subjects by an ELISA procedure employing capture with repifermin and detection with biotinylated goat antihuman immunoglobulin (Ig) G, antihuman IgA,

and antihuman IgM antisera followed by streptavidin-horseradish peroxidase, hydrogen peroxide, and tetramethylbenzidine reagents. The criteria for a positive antibody titer were based on recommendations set forth at the AAPS-sponsored meeting "Bioanalytical Methods Validation for Macromolecules" (Crystal City, VA, March 2000). A sample was considered positive for repifermin-reactive antibodies if the absorbance value at 450 nm for the postdose sample was at least 0.5 and at least twice that for the predose sample. This represents a level approximately 2-fold above the mean value determined for a panel of 100 normal individuals. Inhibition of binding of a positive control sample by addition of soluble repifermin was checked. Any clinical sample found to be positive by these criteria was further characterized by determining specificity against a panel of related and unrelated chemokines and by isotyping with individual biotinylated goat anti-IgG, anti-IgA, and anti-IgM antisera. Antibody profiles were compared between repifermin-treated and placebo-treated groups.

RESULTS

Preclinical Pharmacologic Profile

Over 4 weeks, repeated daily administration of repifermin at doses of 0 (vehicle), 20, 75, and 200 $\mu\text{g/kg}$ IV and 750 $\mu\text{g/kg}$ SC was well tolerated in cynomolgus monkeys. Gross findings at necropsy related to repifermin treatment were limited to the high-dose IV group (200 $\mu\text{g/kg}$) and showed thickening of the esophageal mucosa only. The associated histologic findings were consistent with the known pharmacologic activity of repifermin as a stimulator of epithelial proliferation. Mucosal thickening of the esophagus was evident, microscopically, in all repifermin-treated groups. The incidence and degree of mucosal thickening increased with increasing IV dose. This change was also noted in monkeys injected SC with repifermin, but to a lesser degree. Other tissues (and associated cell types) showing repifermin-related epithelial proliferative changes, at all IV and SC dose levels, included tongue epithelium, oral mucosa (cheek), nasal passages (epithelium, goblet cells, and submucosal glands), and intestinal tract (mucosal epithelium and goblet cells). Figure 1 illustrates representative dose-related histologic changes observed in the oral (buccal) and intestinal (jejunum) mucosae. No changes in mucosal thickness were observed in the stomach. There was an increase in the number of well-differentiated goblet cells that were uniformly arranged over the mucosal surface of the small intestine and colon. It is important to point out that the histologic changes in these epithelial tissues were morphologically normal and, in general, were more pronounced in the high-dose IV group (200 $\mu\text{g/kg}$). The histologic effects were either minimally evident or not evident in those animals terminated at the end of the 4-

week recovery period, suggesting reversibility. The only repifermin-treatment-related effect that could not be readily explained by an action on epithelial tissue was a decrease in the weight of the thymus glands. Thymus weights were decreased in monkeys receiving doses of 75 $\mu\text{g/kg}$ and 200 $\mu\text{g/kg}$ IV and 750 $\mu\text{g/kg}$ SC after 29 days of dosing, but not in monkeys at the end of the recovery period. Microscopically, thymic lymphoid atrophy was present only at the 200 $\mu\text{g/kg}$ IV dose. The mechanism underlying these thymic changes is not known.

Figure 1. Photomicrographs of the effects of vehicle or different repifermin doses (20, 75, or 200 $\mu\text{g/kg}$ injected IV) once daily for 29 days on representative alimentary tract tissues: buccal mucosa (A-D) and jejunum (E-G); H&E stain, 4Xobj. Note repifermin-induced mucosal thickening in each tissue.

Preclinical Immunogenicity

None of the monkeys injected with repifermin by the IV route developed elevated antirepifermin antibody titers in serum relative to predose levels or compared with serum from vehicle-treated monkeys. In animals injected 750 µg/kg SC, 8 of 10 monkeys had a weak elevation in antirepifermin antibody titers (1:100 to 1:500), which peaked at day 29. The phenomenon of a higher incidence of antibody titers produced by SC injection compared with IV or intramuscular (IM) injection is well known and has been observed with other protein/polypeptide therapeutics, such as interferon-beta.¹⁹

Clinical Immunogenicity

None of the monkeys injected with repifermin by the IV route developed elevated antirepifermin antibody titers in serum relative to predose levels or compared with serum from vehicle-treated monkeys. In animals injected 750 µg/kg SC, 8 of 10 monkeys had a weak elevation in antirepifermin antibody titers (1:100 to 1:500), which peaked at day 29. The phenomenon of a higher incidence of antibody titers produced by SC injection compared with IV or intramuscular (IM) injection is well known and has been observed with other protein/polypeptide therapeutics, such as interferon-beta. In the human study, serum samples were taken before dosing (day 1) and at follow-up on days 8 and 29 for the single-dose cohorts and on days 14 and 35 for the multiple-dose cohorts. Of the 47 subjects tested, 1 subject was found to have a weak immunologic response. This subject, in the 25 µg/kg repifermin multiple-dose group, exhibited an approximately 3-fold increase in titer to about 1:150 over 35 days. In comparison, the titer for specific polyclonal chicken antirepifermin antiserum was about 1:150 000. This subject had preexisting antibody reactive with FGF-1, FGF-2, and heparin-binding epidermal growth factor (HB-EGF), but not repifermin, epidermal growth factor, or myeloid progenitor inhibitory factor-1 (MPLF-1). The day 35 sample had a positive signal to repifermin, but reactivity to the other chemokines was unchanged. The isotypes of the antirepifermin antibodies generated in this patient represented all 3 major isotypes with IgA > IgG > IgM.

Monkey Pharmacokinetics

Figure 2 shows mean serum concentrations of repifermin in cynomolgus monkeys following IV injection of 20, 75, or 200 µg/kg repifermin on day 1. The most complete serum concentration profiles were obtained at 200 µg/kg and appear to be biphasic. Serum repifermin concentration data from the 200 µg/kg IV dose group on day 1 were fitted to a 2-compartment model, and fits were obtained in 7 monkeys. Average PK parameters

are summarized in Table 2. The mean AUC was 5026 min·ng/mL with the 200 µg/kg IV dose. The alpha phase accounted for approximately 49% of the total AUC. The alpha- and beta-phase half-lives (mean ± SEM) were 4.08 ± 0.82 minutes and 77.8 ± 15.5 minutes, respectively, and the mean residence time was 64.3 ± 16.6 minutes. Clearance (mean ± SEM) was 48.5 ± 8.5 mL/min/kg. This value of clearance is approximately 22 times the glomerular filtration rate for cynomolgus monkeys (2.2 mL/min/kg),²⁰ suggesting that additional clearance pathways contribute substantially to the elimination of repifermin. The initial and steady-state volumes of distribution (mean ± SEM) were 611 ± 162 mL/kg and 3107 ± 804 mL/kg, respectively, considerably greater than plasma or total body water volumes.²¹ The dose-dependence of the pharmacokinetics of repifermin in cynomolgus monkeys was assessed by normalizing the serum concentrations by the dose administered, and plotting the data against time. The curves for the 3 IV doses are nearly superimposable (not shown), suggesting that the pharmacokinetics of repifermin are linear over the range of doses tested.

Figure 2. Serum repifermin concentrations following a single IV bolus injection of repifermin (20, 75, or 200 µg/kg) in cynomolgus monkeys. In those cases in which the concentrations of repifermin in all samples at a particular time point were below the detection limit (BDL) of the assay (4 ng/mL), no means were calculated. Where some samples had detectable levels and others were BDL, a nominal value of 50% of the detection limit was assigned to each of the BDL samples before calculating mean values, for the purpose of graphing only.

Mean serum concentrations of repifermin in cynomolgus monkeys on days 1 and 29 are shown in Figure 3 for the 3 IV doses (20, 75, and 200 µg/kg) and the SC dose (750 µg/kg). It was difficult to assess the SC bioavailability of repifermin on day 1 due to the limited data, but on day 29 the bioavailability was approximately 10%. This low bioavailability indicates that the IV route is preferable to the SC route for systemic therapy. Serum

Table 2. Two-Compartment Pharmacokinetic Parameters (Mean $\pm$ SEM) for Repifermin Following a Single Intravenous Bolus Injection in Cynomolgus Monkeys or Healthy Humans

Parameters	Cynomolgus Monkeys	Healthy Humans
Dose ($\mu\text{g/kg}$)	200	50
A (ng/mL)	640.2 $\pm$ 302	212.3 $\pm$ 22
B (ng/mL)	23.7 $\pm$ 3.8	10.0 $\pm$ 2.7
α (min ⁻¹)	0.208 $\pm$ 0.035	0.1072 $\pm$ 0.0149
β (min ⁻¹)	0.011 $\pm$ 0.002	0.0067 $\pm$ 0.0020
AUC (min $\cdot$ ng/mL)	5026 $\pm$ 971	3720 $\pm$ 291
% AUC $_{\alpha}$	49.1% $\pm$ 5.1%	58.8% $\pm$ 5.2%
C ₀ (ng/mL)	664 $\pm$ 306	222 $\pm$ 23.1
t _{1/2α} (min)	4.08 $\pm$ 0.82	7.35 $\pm$ 0.83
t _{1/2β} (min)	77.8 $\pm$ 15.5	157.1 $\pm$ 29.9
CL (mL/min/kg)	48.5 $\pm$ 8.5	14.2 $\pm$ 1.3
V _i (mL/kg)	611 $\pm$ 162	244 $\pm$ 24
V _{ss} (mL/kg)	3107 $\pm$ 804	1277 $\pm$ 186
MRT (min)	64.3 $\pm$ 16.6	94.5 $\pm$ 14.7

*A, B, α , β indicate pre-exponential and exponential constants in bi-exponential equation: $C = A \exp(-\alpha t) + B \exp(-\beta t)$; AUC, area under the curve; % AUC $_{\alpha}$, percentage of AUC in the alpha-phase; C₀, maximum concentration by extrapolation to time 0; t_{1/2 α} , half-life of alpha-phase; t_{1/2 β} , half-life of beta-phase; CL, clearance; V_i, initial volume of distribution; V_{ss}, steady-state volume of distribution; MRT, mean residence time; and SEM, standard error of the mean.

concentrations were higher on day 29 compared with day 1, indicating slower clearance of repifermin following 29 consecutive days of dosing, by either the IV or SC routes, at all doses tested (Figure 3). At 200 $\mu\text{g/kg}$ IV, clearance determined by noncompartmental analysis was 64.4 $\pm$ 13.0 (mean $\pm$ SEM) mL/min/kg on day 1 and 31.3 $\pm$ 7.8 mL/min/kg on day 29, the steady-state volume of distribution (V_{ss}) was 2765 $\pm$ 779 mL/kg on day 1 and 2082 $\pm$ 360 mL/kg on day 29, and the terminal half-lives were 72 $\pm$ 33 minutes and 91 $\pm$ 15 minutes, on days 1 and 29, respectively. Even with the change in half-life, dosing on a once-daily basis would not lead to drug accumulation. Because repifermin was not immunogenic following IV dosing and only slightly immunogenic following SC dosing, the cause of the change in pharmacokinetic parameters is unclear.

Human Pharmacokinetics

Figure 4 shows mean serum concentrations of repifermin in healthy human subjects following IV bolus injection of 1, 5, 25, or 50 $\mu\text{g/kg}$ repifermin on day 1. As with the monkey, the serum profiles were biphasic. The most complete serum concentration profiles were obtained at a dose of 50 $\mu\text{g/kg}$. A 2-compartment model was used to analyze the pharmacokinetic profiles of

subjects at this dose. The pharmacokinetic parameters derived from each curve-fitting procedure ($n = 9$) were averaged. Mean ($\pm$ SEM) values are provided in Table 2. Approximately 60% of the AUC occurred in the alpha phase. The alpha- and beta-phase half-lives (mean $\pm$ SEM) were 7.35 $\pm$ 0.83 minutes and 157 $\pm$ 29.9 minutes, respectively. The mean residence time was 94.5 $\pm$ 14.7 minutes. The clearance of repifermin in humans (mean $\pm$ SEM) was 14.2 $\pm$ 1.3 mL/min/kg, or 994 mL/min for a 70-kg human. This high clearance rate, relative to a typical normal human glomerular filtration rate of approximately 125 mL/min, suggests that metabolism of repifermin and/or other clearance mechanisms make a major contribution to the removal of the drug from the circulation. The initial and steady-state volumes of distribution (mean $\pm$ SEM) were 244 mL/kg and 1277 mL/kg, respectively. The high distribution volumes of repifermin compared with normal plasma volume and total body water volume (40 mL/kg and 580 mL/kg, respectively) are indicative of a high degree of binding to sites rapidly accessible to circulating repifermin and to other sites throughout the body. The dependence on dose of the pharmacokinetics of repifermin in humans was assessed by normalizing the serum concentrations by the dose administered and plotting the data against time (data not shown). The 4 curves are almost superimposable up to 1 hour after injection, indicating

Figure 3. Serum repifermin concentrations on day 1 and day 29 following daily IV bolus (20, 75, or 200 µg/kg) or SC (750 µg/kg) injections of repifermin in cynomolgus monkeys.

that the pharmacokinetics of repifermin show dose proportionality over the range of doses tested, as was also seen in the monkeys. In addition, the mean concentration at 5 minutes across the 4 dose levels was linearly proportional to dose ($r^2=0.995$).

The finding in monkeys that serum concentrations were higher after 29 days of dosing led to careful examination of the pharmacokinetics of repifermin following the first and last (7th) daily dose in humans. Three approaches were taken: 1) Average serum concentrations for each dose level (1, 5, 25, and 50 µg/kg) were plotted against time for study day 1 and day 7. As shown in Figure 5, the pairs of curves were similar for the 2 days; 2) AUC values on day 1 and day 7 were compared for subjects in the groups receiving 25 µg/kg and 50 µg/kg IV repifermin who had evaluable curves with detectable drug levels on both days. Subjects who received repifermin doses of 1 µg/kg or 5 µg/kg were not included in the analysis because of the small number of time points at which there were detectable drug levels in the serum. The difference between the mean AUC values

Figure 4. Serum repifermin concentrations on day 1 and day 7 with daily IV bolus injections of repifermin (1, 5, 25, or 50 µg/kg) in healthy human subjects.

Figure 5. Serum repifermin concentrations on day 1 and day 7 with daily IV bolus injections of repifermin (1, 5, 25, or 50 µg/kg) in healthy human subjects.

for day 1 and day 7, normalized to the day 1 AUC, were not significantly different from zero (Student *t*-test; $P > .05$); 3) One-way ANOVA of repifermin concentrations in 2-hour samples collected daily from subjects injected with the highest dose (50 µg/kg) showed no correlation with day of injection. Taken together, these results show that, in healthy human subjects, repifermin pharmacokinetics are not altered following 7 days of daily injection.

Due to the limited number of time points with detectable serum concentrations at doses other than the highest dose in either the monkey or human study, compartmental analysis at lower doses was not feasible. Noncompartmental pharmacokinetic analysis of the 2 highest doses from both studies allows comparison of basic pharmacokinetic characteristics of repifermin across doses and species (Table 3). Values for dose-normalized AUC (AUC/dose) were similar at each of the 2 high doses in monkeys, indicating that the pharmacokinetics of repifermin are linear over this dose range. Likewise, the 2 highest doses in humans had comparable AUC/dose. As predicted by interspecies scaling by body surface area from monkeys to humans, the AUC at 75 µg/kg IV in the monkeys is comparable to the AUC at 25 µg/kg IV in humans; while the AUC at 200 µg/kg IV in the monkeys is approximately 25% higher

than the AUC at 50 µg/kg IV in humans. The maximum serum concentration, C_{max} , at 75 µg/kg IV in the monkeys is similar to C_{max} at 50 µg/kg IV in humans.

DISCUSSION AND CONCLUSIONS

Daily administration of repifermin at 20, 75, or 200 µg/kg by the IV route or 750 µg/kg by the SC route was well tolerated in cynomolgus monkeys. Other than the effects of repifermin on thymus gland weight, the observed effects were attributable to the pharmacologic effects of repifermin predicted from previous preclinical studies. Effects were more pronounced at higher doses and were reversed after a 4-week treatment-free period. Salient repifermin-related changes included gross esophageal mucosal thickening and associated microscopic epithelial proliferation observed in tissues of the oral cavity, tongue, nasal passages, esophagus, and intestinal tract, but not the stomach. Of particular note was the increase in the number of well-differentiated goblet cells that were uniformly arranged over the mucosal surface of the small intestine and colon. Goblet cells are thought to play a role in the protection of epithelium by releasing mucus and in the repair of damaged mucosal surfaces by secreting trefoil peptide.²²

Table 3. Comparison of Noncompartmental Pharmacokinetic Parameters for Repifermin (Mean $\pm$ SEM) Following a Single Intravenous Bolus Injection in Cynomolgus Monkeys or Healthy Humans

Parameters	Cynomolgus Monkeys		Healthy Humans	
Dose ($\mu\text{g/kg}$)	75	200	25	50
n*	3	10	11	9
AUC (min $\cdot$ ng/mL)	1756 $\pm$ 239	4216 $\pm$ 771	1574 $\pm$ 202	3405 $\pm$ 314
AUC/dose	23.4 $\pm$ 3.2	21.1 $\pm$ 3.9	63.0 $\pm$ 8.1	68.1 $\pm$ 6.3
$t_{1/2, \text{term}}$ (min)	11.0 $\pm$ 4.9	72.1 $\pm$ 33.9	22.7 $\pm$ 12.5	109 $\pm$ 18.1
CL (mL/min/kg)	44.2 $\pm$ 5.6	64.4 $\pm$ 13.0	19.0 $\pm$ 2.8	16.3 $\pm$ 6.8
C_{max} (ng/mL)	195 $\pm$ 48	462 $\pm$ 146	104 $\pm$ 20	200 $\pm$ 30
Vss (mL/kg)	400 $\pm$ 50	2765 $\pm$ 779	336 $\pm$ 73	1063 $\pm$ 145
MRT (min)	9.4 $\pm$ 2.0	62.6 $\pm$ 29.4	23.0 $\pm$ 9.0	72.5 $\pm$ 11.9

*Animals with fewer than 3 time points above detectable levels were not included in the analysis.

†AUC indicates area under the curve; $t_{1/2, \text{term}}$, terminal phase half-life; CL, clearance; C_{max} , maximum concentration; Vss, steady-state volume of distribution; MRT, mean residence time; and SEM, standard error of the mean.

Significantly, with respect to the specificity of repifermin, nonepithelial cells were not affected where epithelial changes were observed in the same tissue. Mild immunogenicity was seen in monkeys injected SC with repifermin, but titers were not elevated following IV administration.

The pharmacokinetics of repifermin following IV bolus injection in both monkeys and humans were biphasic, and serum concentrations were dose-proportional. The mean clearance was 48 mL/min/kg in monkeys and 14 mL/min/kg in humans. Scaling of clearance from monkeys using a range of allometric constants of 0.65 to 0.8 typical for proteins¹⁷ would predict a clearance in humans of 16 to 26 mL/min/kg. Thus the observed human clearance is slightly lower than predicted from interspecies scaling. However, clearance values for both the monkeys and human subjects were well above the glomerular filtration rate of the respective species (2.2 and 1.8 mL/min/kg, respectively), suggesting that additional clearance pathways contribute substantially to the elimination of repifermin. The mean steady-state volume of distribution was 3.1 L/kg in monkeys and 1.3 L/kg in humans. Volumes of distribution for proteins typically are proportional to body weight¹⁷; hence, the volume of distribution in humans was lower than expected on the basis of the monkey value. Nonetheless, the large distribution volumes in both monkeys and humans in comparison to plasma and total body water volumes are indicative of a high degree of binding to tissue sites. The observed high rates of clearance and large volumes of distribution are consistent with tissue binding followed by rapid internalization and degradation of repifermin. A close

homolog of repifermin, FGF-7 (KGF), undergoes 50% internalization within 20 minutes at 37°C after binding to KGFR positive cells.²³ The large volume of distribution may be explained in part by the heparin binding property of KGF-2,² since heparin-like molecules, such as heparan sulfate proteoglycans are widely and abundantly present in tissues and vasculature throughout the body.

In the cynomolgus monkeys, there was a decrease in clearance following 29 daily doses. Since repifermin was not markedly immunogenic in monkeys, it is not clear what caused this change in the clearance. Two possibilities are down-modulation of an elimination pathway or the production of a soluble repifermin-binding protein that is not an antibody. In humans, however, there were no apparent changes in the pharmacokinetics of repifermin after 7 daily doses.

Serum repifermin concentrations in human subjects receiving doses of 25 $\mu\text{g/kg}$ and 50 $\mu\text{g/kg}$ attained levels comparable to those obtained in monkeys that produced significant pharmacologic effects in target epithelial tissues. In addition, the qualitative pharmacokinetic characteristics of repifermin were similar in monkeys and humans, and repifermin was not markedly immunogenic following multiple IV doses in either species. These findings have helped to establish a dose range in which pharmacologic responses might be expected in diseased patients, and they support the continuing development of repifermin for the treatment of chemotherapy- or radiotherapy-induced mucositis, ulcerative colitis, and cutaneous wound healing.

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