Interspecies Pharmacokinetics of a Novel Hematoregulatory Peptide (SK&F 107647) in Rats, Dogs, and Oncologic Patients

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Purpose. To study the pharmacokinetics of SK&F 107647, a novel hematoregulatory agent, in rats, dogs, and patients with non-lymphoid solid tumor malignancy.

Methods. Sprague Dawley rats and beagle dogs (n = 6 each; 3 M, 3 F) were given 25 mg/kg of SK&F 107467 as an *iv* bolus injection, and patients (n = 6; 4 M, 2 F) received 100 ng/kg as a 2 hour *iv* infusion. Plasma samples were assayed for drug using either HPLC (rat and dog) or RIA (human).

Results. In each species the plasma clearance (CL) of SK&F 107647 was low in relation to hepatic blood flow, and the volume of distribution (Vd_{ss}) was reflective of distribution to extracellular body water. The plasma CL in humans was near that of average glomerular filtration rate. Using allometric equations for interspecies scaling ($Y = a \cdot W^b$), body-weight normalized human pharmacokinetic data were reasonably predicted using either the body weight normalized rat or the dog data. The allometric exponents (b) for CL, Vd_{ss}, and $T_{1/2}$ of SK&F 107647 were 0.63, 0.94, and 0.29, respectively.

Conclusions. Use of a limited pool of available animal data allowed for reasonable predictions of human pharmacokinetics of SK&F 107647.

KEY WORDS: allometric scaling; peptide; pharmacokinetics; hematology; infection.

INTRODUCTION

The concept of interspecies scaling of pharmacokinetics can be a useful tool in drug development, as it may allow for the prediction of the pharmacokinetics of a drug in humans using data obtained from preclinical animal species (1, 2). The utility of this approach is particularly well suited for extrapolations involving compounds in which a high proportion of the total body clearance is attributable to physical processes, such as biliary and renal excretion of unchanged drug (3). On this basis, small peptides are ideal candidates for predictive interspecies extrapolation of pharmacokinetics, since renal excretion is often a major determinant in their elimination from the body (4–6). In order to prove the utility of interspecies scaling, most studies have included three or more species (≥3) to test the concept (1–3, 7, 8). In actuality, however, appropriate animal data may be available from only two species prior to first administration of a new compound to humans. For example, in toxicological evaluations in which definitive toxicokinetic data may be obtained, often only two species are utilized, one rodent (usually rat), and the other non-rodent (eg. dog or monkey).

SK&F 107647 {HN-40123; (S)-5-oxo-L-prolyl-L-α-glutamyl-L-α-aspartyl-N8-(5-amino-1-carboxypentyl)-8-oxo-N7-[N-[N-(5-oxo-L-prolyl)-L-α-glutamyl]-L-α-aspartyl]-L-*threo*-2,7,8-triaminooctanoyl-L-lysine} is a novel hematoregulatory pentapeptide dimer which enhances myelopoiesis and causes an accentuation of host defense mechanisms in preclinical species (9). These pharmacological properties support its development for use as a host defense modifier in patients at an increased risk of serious infectious complications. Because SK&F 107647 possesses a peptide structure, it is a potentially ideal candidate for the use of interspecies scaling to predict its pharmacokinetics in humans.

For the purposes of toxicokinetic evaluation of the compound, an HPLC assay was developed which utilized a unique post-column derivatization reaction with fluorescence detection (10). This assay allowed for characterization of the toxicokinetics of the compound in rats and dogs, thus permitting an opportunity to prospectively estimate the pharmacokinetics of the drug in humans, and to predict levels of human exposure at doses which were to be used in Phase I trials. The following report describes the toxicokinetic results, and relates them to the pharmacokinetic results obtained in the first Phase I evaluation of the peptide in patients with cancer.

MATERIALS AND METHODS

Toxicokinetic Studies

For the toxicokinetic evaluations, six Sprague Dawley rats (3 M, 3 F; mean ± SD wt, 0.238 ± 0.044 kg) and six purebred Beagle dogs (3 M, 3 F; mean ± SD wt, 11.6 ± 1.14 kg) were studied. These rat and dog experiments were conducted at Nycomed Hafslund Pharma and SmithKline Beecham, respectively, and adhered to the NIH Principles of laboratory animal care (NIH publication #85-23). The acclimatization period was at least 11 days for the Sprague-Dawley rats, and 7 days for the purebred Beagle dogs. Animals were allowed food *ad libitum* over the course of the experiments.

On the morning of the day of dosing, a predose blood sample was obtained, followed by the bolus intravenous injection of 25 mg/kg of SK&F 107647 to the tail vain of rats and the jugular vein of dogs. Following the injection, blood samples (0.2–0.4 ml each) were obtained from the orbital vein plexus of the rats at 5, 15, and 30 minutes, and at 1.5 and 2 hour postdose. In the dogs, blood samples (5 ml each) were drawn from the jugular vein at 5, 15, and 30 minutes, and at 1, 2, 4, and 8 hours postdose. All blood samples were collected into heparinized tubes, and centrifuged at >2500 g for 10 minutes. The resultant plasma was kept frozen at approximately −70°C until analyzed for SK&F 107647.

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Pharmacokinetic Study in Cancer Patients

The pharmacokinetics of SK&F 107647 were evaluated in two female and four male patients (mean ± SD age, 62 ± 8.3 y, wt 80.1 ± 29.7 kg) with a variety of non-lymphoid solid tumor malignancies who were either refractory to standard chemotherapy, or for whom no standard chemotherapy existed. These patients were studied as part of a single blind, placebo controlled, intravenous dose-rising study. SK&F 107647 was administered to all patients as a 2 hour infusion. Although doses ranging from 0.01 to 100 ng/kg were administered, the pharmacokinetic evaluation could only be performed in six patient volunteers (4 M, 2 F) at the highest dose of 100 ng/kg, due to limitations in assay sensitivity. The study was conducted at Mother Francis Hospital, Tyler, Texas and Methodist Hospital, Dallas, Texas, after approval by the local Institutional Review Boards. The study was conducted according to the provisions of the Declaration of Helsinki and its amendments, and patients were enrolled after signed, informed consent was given. The renal function of the patients, assessed using creatinine clearance (CLcr), was calculated from the patients age, weight, and serum creatinine concentration (11).

Serial blood samples were collected into lithium heparin polypropylene tubes. A pre-dose blood sample was obtained. After the start of the 2 hour intravenous infusion, blood samples (5 ml) were collected at 1, 2, 2.083, 2.25, 2.5, 3, 4, 5, 6, 8, 10, 12, 16, and 26 hours relative to the start of the infusion. Aliquots of urine were collected at timed intervals and assayed for SK&F 107647 immunoreactive material using the RIA assay method.

Blood was centrifuged at 4°C at ~2500 g for 20 minutes, and the resultant plasma was stored frozen at approximately −20°C until assayed for drug.

Sample Assay

Rat and Dog Samples

For the analysis of rat and dog plasma samples, a reversed-phase HPLC assay was utilized (10). The lower limit of quantitation was 20 ng/ml based on 0.25 ml of dog or rat plasma.

Human Samples

Because of the much lower dose administered to humans, a more sensitive radio-immunoassay (RIA) assay was developed for the analysis of SK&F 107647 in human plasma. The method involved incubation of 120 µl of human plasma with antiserum, which was raised in sheep to SK&F 107647 immunogen, and radio-iodinated tracer. The immunogen was prepared by conjugating SK&F 107647 to ovalbumin with glutaraldehyde. The radio-iodinated tracer was produced by chloramine-T-oxidation of the di-tyrosine derivative of SK&F 107647 and elemental ^{125}I. The RIA reaction mixture was incubated for 16–24 hours at 4°C and bound radiolabeled SK&F 107647 was separated from free radiolabeled tracer by polyethylene glycol precipitation. The plasma concentration range of the assay was 0.040 ng/ml to 0.500 ng/ml of plasma and the lower limit of quantification was 0.040 ng/ml. A three-run validation was performed to verify the precision and accuracy of the assay. The within-run precision for the assay at nominal concentrations of 0.040, 0.080, 0.200, and 0.500 ng/ml was 9.99, 4.72, 4.78, and 3.91%, respectively. Between-run precision at these concentrations was 4.39, 7.76, 8.43, and 6.68%, respectively, and mean accuracy was 98, 100, 97, and 94%, respectively.

Pharmacokinetic Analysis

For the bolus dosing experiments involving rats and dogs, the value of the plasma concentration immediately after completion of the injection was estimated by fitting the plasma concentration vs time data to a two-compartment model, using the curve-fitting program program MODFIT Version 5 (12). The pharmacokinetic parameters of plasma clearance (CL), volume of distribution at steady-state (Vd_{ss}), and terminal elimination half-life ($T_{1/2}$) of SK&F 107647 were then estimated using non-compartmental methods.

To allow a prospective comparison of the human data to those values predicted based on pharmacokinetic data obtained from preclinical animal species, the allometric scaling equation was used (human predicted parameter = $a \cdot W^b$) where a is the value of the parameter in the animal species, W is the ratio of human to animal body weight, and b is the allometric exponent. For predictive purposes, the allometric exponents for CL (ml/min/kg), Vd_{ss} (ml/kg), and $T_{1/2}$ (min) were set to values of −0.3, 0, and 0.3, respectively (3).

The allometric exponents derived by power regression analysis of the individual SK&F 107647 pharmacokinetic parameters vs. those of the individual body weights were also determined retrospectively using Microsoft Excel version 5, and the results compared to corresponding literature values of other molecules.

RESULTS

In all species, the plasma concentrations appeared to decline in a biexponential manner (Figure 1). Additionally, in

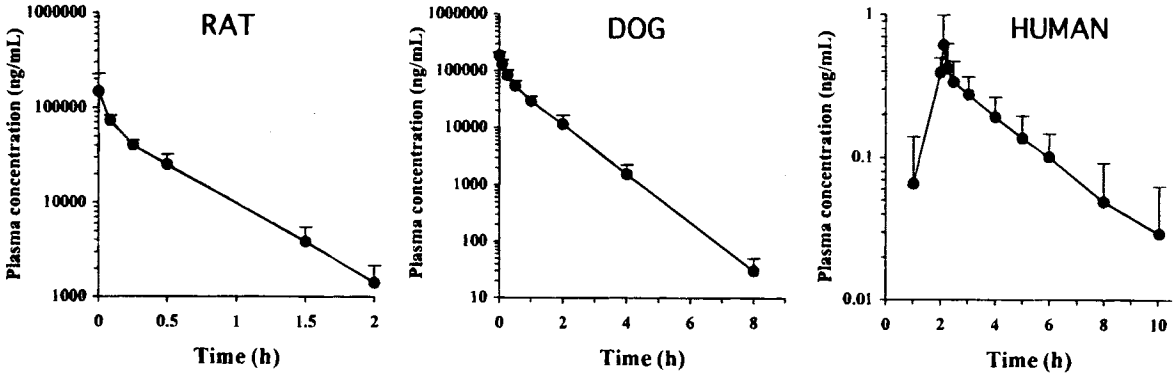

Fig. 1. Plasma concentration vs time profiles.

Table I. SK&F 107647 Pharmacokinetic Data from Rat, Dog, and Oncologic Patients, and Corresponding Human Values Predicted Using the Rat and Dog Data and Interspecies Scaling Equations (3) for Body-weight Normalized Data

Parameter	Actual mean ± SD (range) values			Predicted mean human values from animal species[a]		
	Rat	Dog	Human	Rat	Dog	Mean[b]
Plasma CL (mL/h/kg)	645 ± 79.7 (555 – 757)	263 ± 54.2 (196 – 322)	65.0 ± 18.3 (45.0 – 89.3)	119	146	133
Vd_{ss} (mL/kg)	297 ± 50.7 (219 – 350)	222 ± 35.2 (178 – 271)	244 ± 133 (95.1 – 465)	297	221	259
$T_{1/2}$ (h)	0.359 ± 0.053 (0.303 – 0.452)	0.743 ± 0.064 (0.675 – 0.853)	2.44 ± 1.07 (1.20 – 4.07)	2.03	1.34	1.69

[a] Assuming average weights of 0.25, 10, and 70 kg for rat, dog, and human, respectively.
[b] Average of predictions made from rat and dog.

all species the CL of SK&F 107647 was relatively low, and the Vd_{ss} approximated that of mean extracellular body water (13). The variability in the determined pharmacokinetic parameters was relatively low in all three species (Table I), although the variability in the cancer patients was higher than in the rats and dogs. There was no apparent relationship between the level of renal function, as assessed by calculated CLcr (110.1 ± 59.8 mL/min), and CL of SK&F 107647. The individual data reflected no clear gender-related differences in the pharmacokinetics of SK&F 107647.

Despite the wide range in dose between animals and humans, the interspecies scaling predictions based on body-weight normalized equations were similar to the actual values obtained in the study patients (Table 1). The allometric exponents derived from retrospective power regression analysis (Figure 2) of the relationship between pharmacokinetic parameter and body weight were 0.63, 0.94, and 0.29 for CL, Vd_{ss}, and $T_{1/2}$, respectively.

In the patients, analysis of the predose urine samples for SK&F 107647 showed a lack of immunoreactive material. After dosing substantial amounts of immunoreactive material were detectable in the urine samples. However, the cumulative amount of measured immunoreactive material in urine far exceeded the administered dose of SK&F 107647, indicating a lack of assay specificity for drug in that medium after dosing.

DISCUSSION

From a pharmacokinetic perspective, SK&F 107647 behaved similarly in all three species. Similar to other small, polar peptide molecules, the pharmacokinetic properties were suggestive of a drug with a relatively restricted distribution to tissues. Although SK&F 107647 had a short terminal phase $T_{1/2}$ in each of the species studied, its plasma CL was nevertheless relatively low in comparison to hepatic blood flow (13). Using literature values for the hepatic blood flow and hematocrit for the three species (13), and assuming that the drug is not bound to blood cells, the maximum blood CL of SK&F 107647 would be approximately 36%, 25%, and 11% of hepatic blood flow in the rat, dog, and human, respectively. Hence, its short $T_{1/2}$ in plasma can largely be attributed to a combination of low plasma clearance and a restricted tissue distribution.

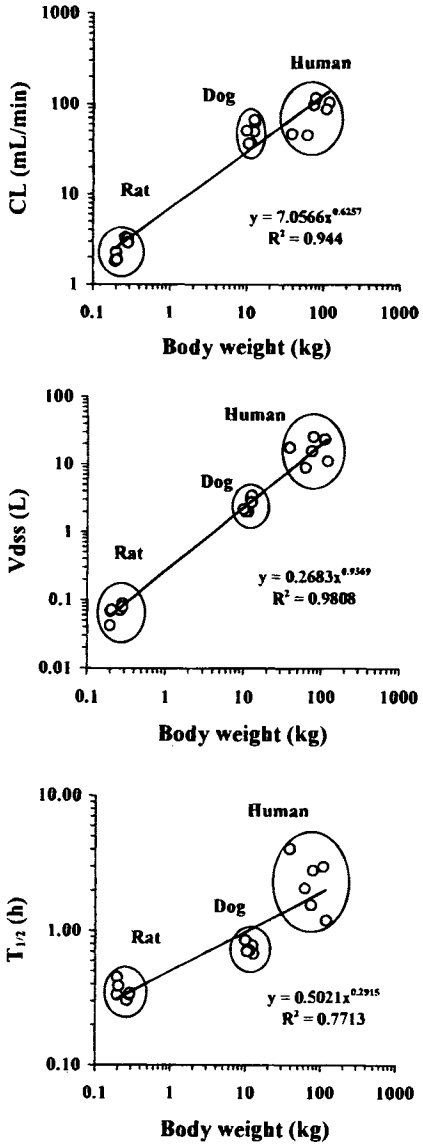

Fig. 2. Log pharmacokinetic parameters vs log body weight relationships.

Compared to data from preclinical species, the dose-normalized pharmacokinetic data from patients were in agreement with the values prospectively estimated (Table 1) using allometric scaling (3). Specifically, the predicted values for mean CL, Vd_{ss}, and $T_{1/2}$ based on compiled rat and dog data were approximately 2.0-fold, 1.1-fold, and 0.69-fold different, respectively, than the corresponding actual values (Table 1). Despite the great difference in dose between animals and humans, the actual values were within a factor of two from those predicted (Table 1). These results may indicate that the pharmacokinetics of SK&F 107647 are linear over a wide range of plasma concentrations in animals, and perhaps in humans.

Some factors can be identified which could have influenced the prediction of CL. A fixed exponent value of −0.3 was used in the allometric scaling of body-weight normalized data, whereas in actuality this value may differ for SK&F 107647. Furthermore, patients rather than healthy volunteers were studied, which might have affected the experimentally-derived values to some degree due to clinical factors (eg., pathophysiological changes, concomitant medications). There were also sizable differences in administered dose between animals and humans, and the variability in body weight amongst the patients was higher than that in the animal groups studied, which might have caused the prediction to deviate from the actual value (Table 1). Mechanistic differences might also exist in the manner of excretion of SK&F 107647, as possibly indicated by the inter-species differences in the ratio of plasma CL to hepatic blood flow.

When the comparative interspecies pharmacokinetics of SK&F 107647 were assessed without normalization to body weight, the exponents determined from regression analysis of the log parameter vs log body weight relationships were consistent with those identified previously for small organic molecules, proteins and peptides (3). The range in allometric exponents for CL, Vd_{ss}, and $T_{1/2}$ derived for small organic molecules have generally been found to be [0.6–0.8], [0.8–1.0], and [0.2–0.4], respectively (3). In a study in which allometric scaling was applied to five therapeutic proteins, ranges in exponents of CL and Vd_{ss} were [0.65–0.84] and [0.84–1.02], respectively (3). In comparison, for SK&F 107647, allometric exponents of CL, Vd_{ss}, and $T_{1/2}$ were 0.63, 0.94, and 0.29, respectively (Figure 2).

Although it is not a naturally occurring molecule, SK&F 107647 is a peptide analogue, and has a low MW of approximately 1200. Small peptide molecules of MW < 5000 are usually filtered unchanged through the glomerulus at concentrations equivalent to those of the unbound species in plasma (4, 5). This factor might explain why, despite the huge disparity in dose level between animals and humans, the interspecies scaling predictions were so near the actual values, as involvement of a saturable process would not be anticipated. Once in the renal tubules, the excreted peptides are normally extensively metabolized in the tubular cells themselves or by the brush border membrane into smaller peptide fragments or amino acids, which are then reabsorbed into the systemic circulation (5, 6). Consequently, for many small peptides, little unchanged species is excreted into the urine (4–6). This may explain why specificity of the RIA assay for SK&F 107647 was lost in the urine samples.

In relation to renal function, the mean body-weight uncorrected plasma CL of SK&F 107647 in the patients was 87 mL/min, which is approximately 69% of the average glomerular filtration rate in humans of 125 mL/min (14), and 79% of the mean value of CLcr calculated in the study patients. It must be noted that the population physiological values used for comparison with CL of SK&F 107647 are derived primarily from subjects with normal renal and hepatic function, whereas the patients enrolled in the present study were afflicted with systemic disease, and were receiving other medications, both of which may have interfered with excretory organ functions. Another consideration is binding to plasma proteins, which would cause a reduction in the plasma CL compared to the glomerular filtration rate.

In conclusion, the pharmacokinetics of SK&F 107647 were qualitatively similar in rat, dog, and human, and were characterized by a relatively low plasma CL and limited Vd_{ss}. The study provides a working example of the ability of allometric scaling to make reasonable predictions of the pharmacokinetics of a peptide drug in humans in a prospective manner, using only a limited pool of available preclinical data.

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