NOTE

Polyethylene Glycol Modification of Filgrastim Results in Decreased Renal Clearance of the Protein in Rats

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ABSTRACT: This report provides the evidence that pegfilgrastim, which is produced by covalently binding a 20-kDa polyethylene glycol molecule to filgrastim, has decreased renal clearance compared with the native protein, filgrastim. After intravenous administration, the area under the plasma concentration versus time curve values for pegfilgrastim were significantly higher than those for filgrastim, indicating that the clearance was slower for pegfilgrastim. The concentration-time profiles of pegfilgrastim were similar between sham-operated and bilateral nephrectomized rats, suggesting that the kidney had an insignificant role in the elimination of pegfilgrastim. In contrast, bilateral nephrectomy resulted in decreased clearance of filgrastim by 60–75%. These data are consistent with the current knowledge that pegylation of proteins decreases the renal clearance of these conjugated proteins. © 2004 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 93:1367–1373, 2004

Keywords: pegfilgrastim; filgrastim; pegylation; renal clearance

INTRODUCTION

Pegfilgrastim is a sustained-duration form of filgrastim, which is a recombinant methionyl human granulocyte colony-stimulating factor (r-metHuG-CSF) that stimulates the proliferation of bone marrow precursor cells and their differentiation into granulocyte colonies leading to an increase in the number of neutrophils in the circulation.[1] Filgrastim must be administered daily to maintain effective drug levels because of its short circulating half-life (2–4 h).[2] In contrast, a single subcutaneous injection of pegfilgrastim per chemotherapy cycle produces efficacy and safety results comparable to daily subcutaneous injections of filgrastim in patients with nonsmall-cell lung cancer,[3] breast cancer,[4,5] and Hodgkin and non-Hodgkin lymphoma.[6]

Pegfilgrastim is produced by covalently attaching a 20-kDa polyethylene glycol (PEG) molecule to the methionine residue at the amino terminus of filgrastim. The identity, purity, and mechanism of action of filgrastim are not altered by pegylation.[7] A number of pegylated proteins have demonstrated an increase in the *in vivo* activity compared with native proteins.[8,9] One of the factors contributing to the increased *in vivo* activity is increased systemic exposure, secondary to a decrease in renal clearance of the pegylated protein. An inverse relationship between urinary clearance and molecular weight was observed after intravenous

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administration of PEGs with different molecular weights.[10] This study was conducted to examine the role of the kidney in the elimination of pegfilgrastim.

EXPERIMENTAL

Study Drugs

Filgrastim (0.3 mg protein/mL) and pegfilgrastim (10 mg protein/mL) were manufactured by Amgen Inc. (Thousand Oaks, CA). Two doses of the drugs were tested in this study to examine the differential contribution of renal clearance to the total clearance. The lower dose (5 μg/kg) is the clinical dose of filgrastim and the higher dose (100 μg/kg) matches the clinical dose of pegfilgrastim.

Surgical Procedures

One day before dosing, male Sprague-Dawley® rats (316–400 g) from Charles River Laboratories (Raleigh, NC and Portage, MI) underwent surgeries. One silastic cannula was inserted into the right femoral vein for intravenous dosing and another into the right jugular vein for blood collection. On the day of dosing, rats were randomized to receive a sham or bilateral nephrectomy surgery. For the nephrectomy procedure, the blood vessels to and from the kidneys and ureters were ligated, and then both kidneys were removed. For the sham-operated procedure, the kidneys were gently manipulated.

Pharmacokinetic Studies

Within 2 h after surgery, rats were randomized to receive a single intravenous bolus dose of 5 or 100 μg protein/kg of filgrastim or pegfilgrastim (four rats/dose/procedure). The stock solutions for filgrastim and pegfilgrastim were diluted with a placebo to 50 μg protein/mL for the 5-μg/kg groups or 300 μg protein/mL for the 100-μg/kg groups on the day of drug administration. Plasma samples were collected predose and at timepoints up to 18 h postdose. Samples were stored frozen at approximately −70°C until shipped on dry ice to Covance Laboratories, Inc. (Vienna, VA) for sample analysis.

Sample Analysis

Plasma concentrations of filgrastim and pegfilgrastim were analyzed with a commercial enzyme-linked immunosorbent assay (Quantikine™ Human G-CSF immunoassay kit; R&D Systems Inc., Minneapolis, MN). This enzyme-linked immunosorbent assay does not distinguish pegfilgrastim from filgrastim or endogenous G-CSF. The analytical range was 0.156–8.991 ng/mL for pegfilgrastim and 0.063–4.0 ng/mL for filgrastim in 100% plasma matrix. The lower limit of quantification was approximately 0.187–0.373 ng/mL for pegfilgrastim and 0.075–0.149 ng/mL for filgrastim in 100% plasma matrix. The raw data were reduced using a log-log regression mode: $\log(Y) = A + B * \log(X)$, where A is the intercept, B is the slope, X is the concentration, and Y is the optical density. For the pegfilgrastim assay, the accuracy (%AR of the QCs) ranged from 96 to 118% between assays and the precision (%CV of the QCs) ranged from 4 to 12% between assays. For the filgrastim assay, the accuracy ranged from 101 to 125% between assays and the precision ranged from 6 to 15% between assays.

Noncompartmental Pharmacokinetic Analysis

Individual pharmacokinetic parameter values were estimated by noncompartmental analysis using WinNonlin Professional (Pharsight, Inc., Mountain View, CA). The concentration at time 0 was set to the first valid concentration value postdose (5 min) before data analysis. The maximum concentration, C_{max}, and the time it occurred, T_{max}, after dosing were recorded as observed. The initial volume of distribution, $V_{initial}$, was calculated as dose divided by C_{max}. The terminal half-life, $T_{\frac{1}{2}}$, was estimated as ln(2) divided by λ_z, where λ_z was the first-order terminal rate constant. Area under the plasma concentration versus time curve, $AUC_{(0-last)}$, was estimated using the linear trapezoidal method from time 0 to last, the time of the last quantifiable concentration, C_{last}. AUC calculated from time 0 to infinity, $AUC_{(0-\infty)}$, was estimated as the summation of $AUC_{(0-last)}$ and the result of C_{last} divided by λ_z. Plasma clearance, CL, was calculated as dose divided by $AUC_{(0-\infty)}$.

Compartmental Pharmacokinetic Analysis

Compartmental pharmacokinetic analysis was conducted to estimate the contribution of the renal clearance to the total clearance of the protein. A two-compartmental (central and peripheral compartments) disposition model was used to describe the concentration-time profile after intravenous administration. The elimination was by parallel

nonlinear (Michaelis-Menten) and linear pathways as follows.

For the sham-operated groups,

$$k_{(0,1)} = \left(\frac{V_{\max}}{k_m + [C_p]} + CL_{neph} + CL_{res}\right)/V_c.$$

For the nephrectomized groups,

$$k_{(0,1)} = \left(\frac{V_{\max}}{k_m + [C_p]} + CL_{res}\right)/V_c.$$

$k_{(0,1)}$ is the elimination rate constant from the central compartment, V_{max} is the maximal elimination rate by the Michaelis-Menten pathway, k_m is the Michaelis constant, $[C_p]$ is the plasma concentration of the protein, CL_{neph} is the renal clearance (linear pathway), CL_{res} represents the residual clearance (linear pathway), and V_c is the volume of distribution at the central compartment. The movement of drug between the central (1) and the peripheral (2) compartments is described by the rate constants, $k_{(1,2)}$ and $k_{(2,1)}$. The contribution of the renal clearance to the total plasma clearance when the Michaelis-Menten pathway operates under linear kinetics is estimated as: CL_{neph} divided by the summation of CL_{res}, CL_{neph}, and V_{max}/k_m.

The compartmental modeling was performed simultaneously on the mean data from all four groups receiving the same drug (sham-operated and nephrectomized; 5 and 100 μg/kg). Compartmental models were optimized to plasma profiles using a constant covariance (10%) and relative weighting scheme, based on model-predicted concentrations and a Rosenbrock integrator with relative error of 0.001. Computational settings of the optimizer included a minimum of 2100 calculations and a convergence criterion of 0.0001. The compartmental analysis was performed using SAAM II (SAAM Institute, University of Washington, Seattle, WA).

Statistical Analysis

A student's t test was used to compare pharmacokinetic parameter values between sham and nephrectomy groups receiving the same test material at the same dose and between the filgrastim and pegfilgrastim groups. A p value < 0.05 was considered significant. Statistical analysis was conducted using JMP (SAS Institute Inc., Cary, NC).

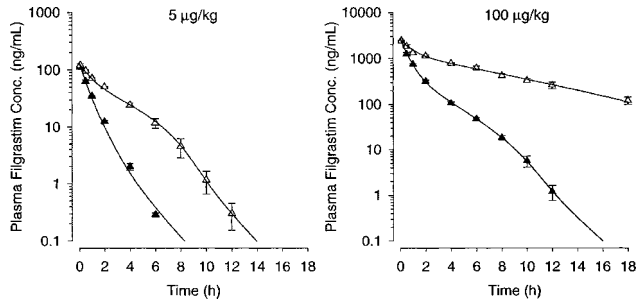

Figure 1. Observed (symbols) and modeled (solid line) filgrastim concentration-time profiles after intravenous administration of filgrastim in sham-operated (closed triangle) and bilateral nephrectomized (open triangle) rats ($n = 4$/group, except for $n = 3$ for the nephrectomized group at 100 μg/kg). Data shown are mean ± SE.

RESULTS AND DISCUSSION

Plasma concentrations of filgrastim declined rapidly after intravenous administration because of a small volume of distribution and moderate systemic clearance (Fig. 1). The pharmacokinetic parameter values estimated from this study are comparable to those reported previously (Table 1).[2] Plasma clearance of filgrastim was reported to decrease with increasing dose and was described by parallel linear and nonlinear pathways. The linear pathway is attributed to the renal clearance; the dose-dependent, nonlinear elimination is attributed to saturation of neutrophil-mediated clearance (NMC), presumably mediated by G-CSF receptors, at high concentrations of filgrastim.[11] In this study, in addition to the two clearance pathways mentioned above, a third residual linear clearance was also detected by compartmental analysis. The modeled profiles demonstrate that the two-compatment model used in this study adequately describe the observed profiles (Fig. 1).

Renal clearance has been demonstrated to be an important pathway for filgrastim elimination in rats; unilateral and bilateral nephrectomy caused a 46 and 79% reduction, respectively, in the total clearance after intravenous administration of 10 μg/kg recombinant human G-CSF.[12] Results from this study not only confirm that the kidney has a significant role in the elimination of filgrastim but also demonstrate that the contribution of the renal clearance is dose dependent. The clearance of filgrastim for the bilateral nephrectomized rats decreased by 62% at 5 μg/kg and by 75% at 100 μg/kg compared with the sham-operated rats. The higher contribution of the renal pathway

Table 1. Mean (SD) Pharmacokinetic Parameters in Rats after Intravenous Administration of Filgrastim and Pegfilgrastim

	Filgrastim		Pegfilgrastim	
Parameter	5 μg/kg	100 μg/kg	5 μg/kg	100 μg/kg
Sham-operated rats				
n	4	4	4	4
C_{max} (ng/mL)	112 (6)	2470 (130)[a]	106 (20)	2140 (110)
T_{max} (h)	0.08 (0.00)	0.08 (0.00)	0.08 (0.00)	0.08 (0.00)
$V_{initial}$ (mL/kg)	44.6 (2.2)	40.6 (2.0)[a]	48.6 (10.1)	46.9 (2.6)
$AUC_{(0-last)}$ (ng · h/mL)	111 (6)[a]	2670 (250)[a]	447 (75)	17,400 (1900)
CL (mL/h/kg)	45.1 (2.4)[a]	37.7 (3.8)	11.4 (1.8)	NE
$T_{½}$ (h)	0.71 (0.05)[a]	1.22 (0.18)	1.22 (0.21)	NE
Bilateral nephrectomized rats				
n	4	3[b]	4	4
C_{max} (ng/mL)	118 (16)	2390 (240)	104 (5)	2110 (270)
T_{max} (h)	0.19 (0.21)	0.08 (0.00)	0.19 (0.21)	0.08 (0.00)
$V_{initial}$ (mL/kg)	42.9 (5.2)	42.2 (4.4)	48.1 (2.5)	47.9 (6.1)
$AUC_{(0-last)}$ (ng · h/mL)	293 (27)[a,c]	10,000 (1300)[c]	574 (160)	16,000 (4300)
CL (mL/h/kg)	17.2 (1.7)[a,c]	9.28 (1.50)[c]	9.16 (2.74)	NE
$T_{½}$ (h)	1.19 (0.25)[c]	5.23 (0.73)[c]	2.40 (1.05)	NE

NE, not estimated because a significant amount of pegfilgrastim remained in the body at 18 h postdose.
[a]Significantly different from pegfilgrastim at the same dose level and after the same surgery procedure (t test, $p < 0.05$).
[b]One rat assigned to this group accidentally received the drug subcutaneously; thus, data collected from this rat were excluded from the data analysis.
[c]Significantly different from the sham-operated rats receiving the same test material at the same dose (t test, $p < 0.05$).

in the elimination of filgrastim at the higher dose group is consistent with the clearance mechanism of filgrastim. When the nonlinear elimination pathway, NMC, is saturated, the contribution of nonsaturable and high-capacity clearance pathways (such as the renal pathway) to the total clearance of the drug increases. When NMC operates in the linear range, that is, when the plasma concentration of filgrastim is lower than the Michaelis constant (k_m), approximately 20% of filgrastim is cleared by the renal pathway (Table 2). In comparison, the residual clearance accounts for approximately 5% of the total clearance under the linear kinetic range.

Compared with those for filgrastim, the mean pegfilgrastim concentrations are higher for the sham-operated rats (Fig. 2), whereas the initial volume of distribution was similar between pegfilgrastim and filgrastim groups and approximated the plasma volume (Table 1). The mean $AUC_{(0-last)}$ values for pegfilgrastim were 300% higher at 5 μg/kg and 550% higher at 100 μg/kg than those for filgrastim, indicating that the clearance of pegfilgrastim was markedly slower than that of filgrastim.

In addition to pegfilgrastim, different versions of pegylated recombinant human G-CSF have been produced.[13–16] All have exhibited sustained circulating drug concentrations and longer duration of action relative to native recombinant human G-CSF. A decrease in renal clearance is one of the reasons why pegylated proteins have slower clearance than native proteins; an increase in the molecular size due to pegylation prevents glomerular filtration of the conjugated protein. Results from this study show that the systemic exposures of pegfilgrastim for sham-operated and bilateral nephrectomized rats are not distinguishable at 100 μg/kg (Fig. 3), suggesting that the

Table 2. Estimates and Precisions of Filgrastim Pharmacokinetic Parameters Predicted from Compartmental Modeling

Parameter	Mean	SD	95% Confidence Interval
CL_{neph} (mL/h/kg)	29.5	0.6	28.4–30.6
CL_{res} (mL/h/kg)	8.60	0.18	8.23–8.98
V_{max} (ng/h/kg), sham-operated	355	27	300–410
V_{max} (ng/h/kg), nephrectomized	312	11	289–335
k_m (ng/mL)	3.00	0.29	2.40–3.59
V_c (mL/kg)	38.5	1.1	36.3–40.7
$k_{(1,2)}$ (1/h)	0.638	0.031	0.574–0.701
$k_{(2,1)}$ (1/h)	0.442	0.039	0.361–0.523

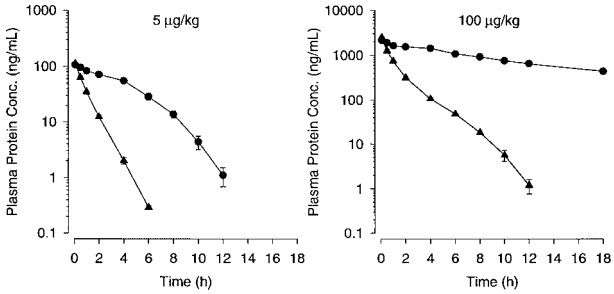

Figure 2. Protein concentration-time profiles after intravenous administration of filgrastim (closed triangle) and pegfilgrastim (closed circle) in sham-operated rats ($n = 4$/group). Data shown are mean ± SE.

kidney has an insignificant role in the elimination of pegfilgrastim. At 5 μg/kg, lower pegfilgrastim concentrations were observed in nephrectomized rats than in sham-operated rats when pegfilgrastim concentrations decreased below 10 ng/mL; however, this difference did not result in a significant difference in AUC values. In the compartmental analysis, renal clearance for pegfilgrastim could not be detected (Table 3).

The cut-off molecular weight for glomerular filtration is approximately 60 kDa.[11] However, in addition to molecular weight, the shape, charge, and hydrodynamic radius of molecules are important factors in glomerular filtration.[17] Although the molecular weight for pegfilgrastim (38.8 kDa) is only approximately twice that of filgrastim (18.8 kDa), the hydrodynamic radius, measured by a dynamic light-scattering technique, for pegfilgrastim (120 Å) is approximately 4.5-fold that of filgrastim (26.5 Å) (G-M Wu, data on file at Amgen). The reason for the greater difference in

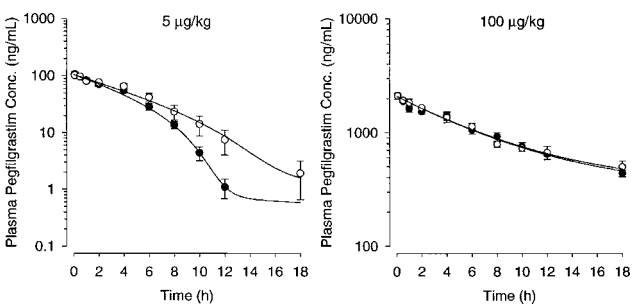

Figure 3. Observed (symbols) and modeled (solid line) pegfilgrastim concentration-time profiles after intravenous administration of pegfilgrastim in sham-operated (closed circle) and bilateral nephrectomized (open circle) rats ($n = 4$/group). Data shown are mean ± SE.

Table 3. Estimates and Precisions of Pegfilgrastim Pharmacokinetic Parameters Predicted from Compartmental Modeling

Parameter	Mean	SD	95% Confidence Interval
CL_{neph} (mL/h/kg)	ND	—	—
CL_{res} (mL/h/kg)	4.03	0.18	3.79–4.27
V_{max} (ng/h/kg), sham-operated	368	21	325–410
V_{max} (ng/h/kg), nephrectomized	218	19	181–256
k_m (ng/mL)	5.45	1.24	2.92–7.98
V_c (mL/kg)	48.2	1.6	44.9–51.4
$k_{(1,2)}$ (1/h)	0.394	0.042	0.309–0.479
$k_{(2,1)}$ (1/h)	0.068	0.027	0.013–0.124

ND, not detectable.

hydrodynamic radius is that the PEG moiety is highly hydrated in aqueous solution.[9] Yamasaki et al.[16] showed that the molecular size, measured by gel-permeation chromatography of pegylated G-CSF, increased more than proportionally with increasing numbers of 5-kDa PEG attached to the native protein. Therefore, it is reasonable to suggest that the larger hydrodynamic radius of pegfilgrastim prevents its clearance by the kidney.

For the bilateral-nephrectomized rats, the mean $AUC_{(0-last)}$ values for pegfilgrastim were approximately 100% higher at 5 μg/kg and 60% higher at 100 μg/kg than those for filgrastim, suggesting that the clearance of pegfilgrastim was slower than that of filgrastim when both kidneys were removed. Pegylation not only can reduce the renal clearance of proteins, but also can protect proteins from proteolysis because of steric hindrance.[8] In this study, the residual clearance, which could represent the proteolytic clearance, was lower for pegfilgrastim (4.03 mL/h/kg) than for filgrastim (8.60 mL/h/kg).

In addition to reduced proteolytic clearance, NMC appeared to be slightly lower for pegfilgrastim than for filgrastim in this study. The k_m value was higher for pegfilgrastim (5.45 ng/mL) than for filgrastim (3.00 ng/mL). In nephrectomized rats, the V_{max} value was lower for pegfilgrastim (218 ng/h/kg) than for filgrastim (312 ng/h/kg); however, the V_{max} values were similar in sham-operated control rats (355 ng/h/kg for filgrastim versus 368 ng/h/kg for pegfilgrastim). Based on the compartmental modeling, V_{max} was found to be lower in the nephrectomized rats. The severe acute renal failure model used in this animal study may

have reduced NMC; this could also be the reason for the difference observed in the concentration-time profiles between sham-operated and nephrectomized rats for pegfilgrastim at 5 μg/kg (Fig. 3).

It is important to note that, although there seemed to be a difference in NMC between pegfilgrastim and filgrastim, the contribution of NMC to the total clearance is higher for pegfilgrastim because of a 90% reduction in the linear, neutrophil-independent clearance pathways. The linear clearance ($CL_{neph} + CL_{res}$) was 4.03 mL/h/kg for pegfilgrastim compared with 38.1 mL/h/kg for filgrastim. Therefore, the clearance of pegfilgrastim should be more efficiently regulated by NMC. That is, pegfilgrastim stimulates the production of neutrophils that, in turn, clear it from the circulation. During chemotherapy-induced neutropenia, clearance of pegfilgrastim is significantly reduced. Consequently, a single subcutaneous injection of pegfilgrastim resulted in an efficacy that was clinically and statistically similar to that observed after daily injections of filgrastim.[4,5]

In conclusion, the contribution of the kidney to the clearance of pegfilgrastim is insignificant as determined by a bilateral nephrectomy study in rats. Results from compartmental analysis suggest that NMC is the primary pathway for pegfilgrastim elimination.

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