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RESEARCH PAPER

Investigation of the Mechanism of Clearance of AMG 386, a Selective Angiopoietin-1/2 Neutralizing Peptibody, in Splenectomized, Nephrectomized, and FcRn Knockout Rodent Models

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ABSTRACT

Purpose To investigate the mechanisms of clearance of AMG 386, an investigational recombinant peptide-Fc fusion protein (peptibody) that blocks tumor angiogenesis by neutralizing the interaction between angiopoietin-1 and -2 and the Tie2 receptor.

Methods The role of the neonatal Fc receptor (FcRn) in AMG 386 clearance was assessed in wild-type and FcRn-knockout mice; the roles of the spleen and kidneys were assessed in splenectomized and 5/6th nephrectomized rats, respectively, compared with sham-operated rats. Animals were administered AMG 386 as a single intravenous dose of 3 or 10 mg/kg. Blood samples for pharmacokinetic analysis were collected periodically throughout a 504-hour postdose period.

Results Compared with wild-type mice, AMG 386 clearance in FcRn-knockout mice was 18-fold faster at the 3-mg/kg dose (FcRn knockout, 13.2 mL/h/kg; wild-type, 0.728 mL/h/kg) and 14-fold faster at the 10-mg/kg dose (FcRn knockout, 10.7 mL/h/kg; wild-type, 0.777 mL/h/kg). Clearance in nephrectomized rats was slower than in sham-operated rats at both the 3-mg/kg dose (nephrectomized, 1.23 mL/h/kg; sham-operated, 1.75 mL/h/kg) and the 10-mg/kg dose (nephrectomized, 1.14 mL/h/kg; sham-operated, 1.65 mL/h/kg). Splenectomy had no apparent effect on the pharmacokinetics of AMG 386.

Conclusions The FcRn is integral to maintaining circulating levels of AMG 386 in mice. Renal clearance contributed approximately 30% to total AMG 386 clearance in rats.

KEY WORDS AMG 386 · clearance mechanism · neonatal Fc receptor · nephrectomy · peptibody

ABBREVIATIONS

Ang	angiopoietin
AUC	area under the concentration *versus* time curve
$AUC_{0-\infty}$	area under the serum concentration *versus* time curve from time 0 to infinity
AUC_{0-last}	area under the serum concentration *versus* time curve from time 0 to the last quantifiable concentration
AUMC	area under the first moment of the concentration *versus* time curve
C_0	concentration at time 0
CL	clearance
C_{last}	last quantifiable concentration
ELISA	enzyme-linked immunosorbent assay
FcRn	neonatal Fc receptor
IgG	immunoglobulin G
IV	intravenous
kDa	kilodalton
LLOQ	lower limit of quantitation
$t_{½,z}$	estimated terminal elimination half-life
TMDD	target-mediated drug disposition
V_0	initial volume of distribution
V_{ss}	volume of distribution at steady state

INTRODUCTION

Peptibodies are novel fusion proteins that combine an antibody Fc domain and at least one biologically active peptide domain (1). These peptide-Fc fusion proteins have markedly improved pharmacokinetics compared with simple peptides

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(1). They also have significant potential as therapeutic agents, as evidenced by the recent approval of romiplostim for the treatment of immune thrombocytopenic purpura (2). However, the mechanisms of clearance for peptibodies are not well understood. Understanding these mechanisms is a particularly important consideration in the oncology setting, where different therapeutic agents are routinely combined and where there is potential for significant toxicity as a consequence of increased exposure owing to drug-drug interactions (3). Clearance and drug exposure are also important considerations in terminally ill patients, who often have comorbid conditions, such as impaired renal function (4), that can alter the pharmacokinetics of pharmacologic agents. Thus, further investigation of clearance mechanisms for peptibodies intended to treat cancer is important because it may improve the understanding of relationships between dose, pharmacokinetic exposure, and pharmacodynamic response (5).

The clearance of small molecular therapeutics is dependent in large part on their biotransformation (in particular their metabolism by the cytochrome P450 enzymes) (6), but a different set of processes are involved in the clearance of therapeutic proteins (7). For antibodies bearing the immunoglobulin G (IgG) Fc domain, a key determinant of their pharmacokinetic properties is their interaction with the neonatal Fc receptor (FcRn), which allows for their salvage and recycling by the reticuloendothelial system (8–11). Recent evidence has supported the hypothesis that the FcRn also regulates clearance of peptide-Fc fusion proteins bearing an IgG Fc domain (12,13). Tissues that have been demonstrated to be involved in the clearance of IgG include the spleen (14,15) and kidney (16,17). Finally, clearance by high-affinity binding to their target (ie, target-mediated drug disposition [TMDD]) (18) may also contribute to the clearance of peptibodies; TMDD has been reported to account for a significant portion of clearance of romiplostim (13).

AMG 386, a peptide-Fc fusion protein with a molecular weight of 63.5 kilodaltons (kDa), is an investigational peptibody that blocks tumor angiogenesis by selectively neutralizing the interaction between angiopoietin-1 and -2 (Ang1 and Ang2) and their receptor, Tie2 (19,20). In preclinical studies, AMG 386 significantly inhibited vascular endothelial growth factor–stimulated neovascularization in a corneal angiogenesis model and had antitumor activity against established tumor xenografts (19). Importantly, in Colo205 colon cancer tumor xenograft models, inhibition of both Ang1 and Ang2 demonstrated superior tumor growth suppression compared with inhibition of Ang1 or Ang2 alone (21). In phase 1 clinical studies, AMG 386 has shown promising antitumor activity and was well tolerated in patients with advanced solid tumors when administered as a monotherapy (20) or in combination with various chemotherapy regimen (20,22). Among patients with recurrent ovarian cancer, administration of AMG 386 10 mg/kg QW plus weekly paclitaxel resulted in acceptable toxicity and encouraging estimated progression-free survival time compared with placebo plus paclitaxel (23). The pharmacokinetics of AMG 386 are linear between 0.3 and 30 mg/kg (the maximum tested dose in the first-in-human study) when administered as a monotherapy in patients with solid tumors (20). The pharmacokinetics of AMG 386 do not appear to be altered when combined with chemotherapy regimens, including paclitaxel, docetaxel, carboplatin/paclitaxel, and FOLFOX (oxaliplatin, leucovorin, and 5-fluorouracil) (22,23). Moreover, population pharmacokinetic analyses have shown that creatinine clearace is an important covariate affecting AMG 386 total clearance in cancer patients, suggesting that renal clearance may play a key role in AMG 386 disposition (24). Here, we describe results from experiments conducted in rodent models that investigated the mechanisms of clearance of AMG 386. Specifically, we examined the role of the FcRn in AMG 386 recycling and assessed the involvement of the kidneys and spleen in AMG 386 clearance.

MATERIALS AND METHODS

Study Drug

AMG 386 was manufactured by Amgen Inc. (Thousand Oaks, CA). For each experiment, AMG 386 was diluted with vehicle (3.4% [weight/volume] arginine HCI, 10 mM potassium phosphate, 0.004% polysorbate 20; pH 7.0) to achieve the concentrations required for dosing.

Animal Models

All experiments involving animals were performed at Amgen Inc. All study procedures were consistent with the National Resource Council's *Guide for the Care and Use of Laboratory Animals* (25) and the guidelines of the Association for Assessment of Laboratory Animal Care (Frederick, MD), and were approved by the Amgen Animal Care and Use Committee. Animals were maintained in a 12-hour light/dark cycle environment. Food and water were available *ad libitum* throughout the experiments.

FcRn Knockout Mouse Models

Wild-type male mice (C57BL/6J strain), and FcRn-knockout male mice (B6.129P2-B2m^{tm1Unc}/J strain) were purchased from Jackson Laboratories (Bar Harbor, ME). Animals were 6 to 14 weeks of age and weighed between 17 and 40 g on the first day of dosing. AMG 386 was administered as a single intravenous (IV) bolus dose via the lateral tail vein to 78 FcRn-knockout mice and 78

wild-type mice at arbitrarily assigned doses of 3 mg/kg or 10 mg/kg (n=39 per group). The composite blood sampling design used 3 mice per group per time point, and samples were obtained via terminal bleed before dosing and at 0.25, 1, 4, 12, 24, 48, 96, 168, 240, 336, 432, and 504 h postdose. An enzyme-linked immunosorbent assay (ELISA) with a lower limit of quantitation (LLOQ) of 39.1 ng/mL was used to measure AMG 386 concentrations in the serum (see *Assessment of Serum AMG 386 Concentrations*).

Splenectomized and Nephrectomized Rat Models

Male Sprague–Dawley rats (350 to 425 g) were obtained from Charles River Laboratories (Hollister, CA). In each experiment, 16 rats were anesthetized with isoflurane (Abbott Laboratories, North Chicago, IL). Aseptic surgical technique was used in all surgical procedures. For splenectomy experiments, the spleen was removed from 8 rats (splenectomized rats) and gently manipulated in 8 other rats (sham-operated rats) 1 to 2 days before dosing. For subtotal (5/6th) nephrectomy experiments, blood flow to two thirds of the left kidney was blocked by ligating two of the three branches of the renal artery before the kidney was placed back into the abdomen. Following a 1-week postoperative recovery period, the entire right kidney was also removed and the rats underwent an additional 1-week postoperative recovery period. In 8 sham-operated rats, the left and right kidneys were gently manipulated in the same sequence as described for nephrectomized rats. Each experiment also included 8 rats in a control group that did not undergo any surgical procedures. Following postoperative recovery, splenectomized, nephrectomized, or sham-operated animals received a single bolus dose of IV AMG 386 3 mg/kg or 10 mg/kg (n=4 per dose group). Blood samples were collected from the jugular vein or the lateral tail vein predose and at 0.25, 1, 4, 12, 24, 48, 96, 168, 240, 336, 432, and 504 h postdose for pharmacokinetic assessment. For all samples from splenectomized and nephrectomized animals, an ELISA with an LLOQ of 78.1 ng/mL was used to measure AMG 386 concentrations in the serum (see *Assessment of Serum AMG 386 Concentrations*).

Assessment of Serum AMG 386 Concentrations

Quantification of AMG 386 concentrations in mouse and rat serum was performed by ELISA. Human Ang2 was adsorbed onto flat-bottom 96-well polystyrene microtiter plates. Uncoated areas of the plates were blocked with I-Block Buffer (Applied Biosystems, Foster City, CA), and the plates were washed to remove unbound material. Standards, quality controls, and study samples were diluted 1:50 in I-Block Buffer containing 3% bovine serum albumin and dispensed into duplicate wells. After washing with 1× KPL wash buffer (KPL, Inc., Gaithersburg, MD), a horseradish peroxidase–labeled murine anti-human IgG1 (Fc) monoclonal antibody (Amgen Inc.) was added to the wells. A final wash step with 1× KPL wash buffer was performed to remove unbound materials before addition of a substrate solution containing hydrogen peroxide and 3, 3′, 5, 5′-tetramethylbenzidine, which produced a colorimetric signal proportional to the amount of bound AMG 386. The colorimetric reaction was stopped by addition of 2N sulfuric acid, and optical density was measured at 450 nm (OD_{450}) with a reference wavelength of 650 nm. Conversion of OD_{450} to AMG 386 concentration was performed by reference to a concurrently analyzed standard curve using a logistic regression model with a weighting factor of $1/Y^2$ and the Watson data reduction package version 7.0.0.01 (Thermo Fisher Scientific, Pittsburgh, PA).

Pharmacokinetic Analyses

Pharmacokinetic parameters were calculated using standard noncompartmental methods in WinNonlin Professional version 4.1e (Pharsight Inc., Mountain View, CA). The concentration at time zero (C_0) was estimated via linear back-extrapolation to time zero using the first two time points. The area under the serum concentration *versus* time curve (AUC_{0-last}) was calculated from time zero to the last quantifiable concentration (C_{last}). The rate constant of the terminal phase (λ) was calculated via linear regression of the log-linear decay of the terminal phase using at least the last three time points with detectable serum concentrations. Extrapolation of the area under the serum concentration *versus* time curve to infinity ($AUC_{0-\infty}$) was estimated as the sum of corresponding AUC_{0-last} and C_{last}/λ values. Systemic clearance was calculated as the dose divided by $AUC_{0-\infty}$. Terminal half-lives were calculated as $0.693/\lambda$. The initial volume of distribution (V_0) was calculated as $Dose/C_0$. The volume of distribution at steady state (V_{ss}) was calculated as $Dose \bullet AUMC_{0-\infty}/(AUC_{0-\infty})^2$, in which AUMC is the area under the first moment of the concentration *versus* time curve.

SAS version 9.2 was used to perform statistical comparisons of log-transformed pharmacokinetic parameters. Pharmacokinetic parameters from nephrectomized and splenectomized rats were compared with the PK parameters of the respective sham-operated animals. The p value for the comparison between treatment group and sham-operated control as well as the ratio associated with this comparison and its 95% confidence interval are reported. Statistical significance was defined as p values of less than 0.05.

RESULTS

Pharmacokinetics of AMG 386 in FcRn-Knockout *Versus* Wild-Type Mice

In wild-type mice, AMG 386 exhibited a biexponential disposition profile with a fast distribution phase followed by a prolonged elimination phase after a single IV administration of 3 or 10 mg/kg of AMG 386. Using the same doses in FcRn-knockout mice, there was a monoexponential decline in serum AMG 386 concentrations with a markedly faster elimination rate than that occurring in wild-type mice (Fig. 1). The pharmacokinetic parameters of AMG 386 measured in both experiments are summarized in Table I. AMG 386 clearance after 3- and 10-mg/kg doses was markedly faster (approximately 18- and 14-fold, respectively) in the FcRn-knockout mice compared with wild-type animals. Clearance values ranged from 0.728 mL/h/kg (3 mg/kg) to 0.777 mL/h/kg (10 mg/kg) in the wild-type mice and from 13.2 mL/h/kg (3 mg/kg) to 10.7 mL/h/kg (10 mg/kg) in FcRn-knockout mice. The V_{ss} for FcRn-knockout mice was 2- to 3-fold lower than for wild-type animals. The mean half-life was approximately 5 h for knockout mice and approximately 150 h (6 days) for wild-type mice.

Pharmacokinetics of AMG 386 in Splenectomized, Sham-Operated, and Control Rats

The pharmacokinetics of AMG 386 demonstrated a biexponential disposition profile in each of the three groups of animals. In general, the pharmacokinetic parameters of AMG 386 in splenectomized rats were not statistically significantly different from those in control rats (which underwent no surgical procedures) or sham-operated rats ($p > 0.05$). The AMG 386 concentration *versus* time profiles were also similar across animal groups (Fig. 2). Likewise, the serum AMG 386 exposure was comparable in sham-operated, splenectomized, and control rats following administration of 3 or 10 mg/kg of AMG 386 (Table II). Serum exposure appeared to be dose-proportional across the two tested doses of AMG 386 (3 and 10 mg/kg), with 3.4- to 4.4-fold higher C_0 and AUC values observed at the higher dose (Table II). Furthermore, AMG 386 clearance in control rats was similar at both AMG 386 doses, indicating that AMG 386 elimination followed linear kinetics. The estimated half-life for AMG 386 ranged from 78 to 97 h for the 3-mg/kg dose and from 91 to 118 h for the 10-mg/kg dose.

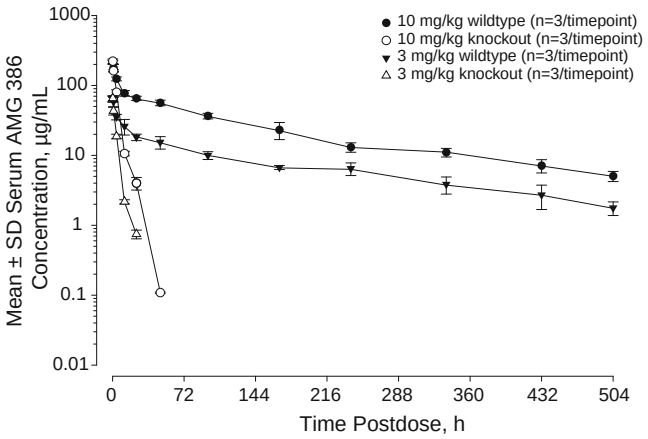

Fig. 1 Pharmacokinetic profile of AMG 386 in wild-type and FcRn-knockout mice following a single intravenous dose of 3 or 10 mg/kg.

Pharmacokinetic Profile of AMG 386 in Nephrectomized, Sham-Operated, and Control Rats

As in the splenectomy experiments, AMG 386 had a biexponential disposition profile with a fast distribution phase and a prolonged elimination phase in each of the three experimental groups. AMG 386 exposure increased in an approximately dose-proportional manner in nephrectomized, sham-operated, and control rats after a single IV dose of 3 or 10 mg/kg of AMG 386 (Fig. 3). At both doses, clearance was approximately 30% lower in the nephrectomized animals compared with those in the control and sham-operated groups (Table III). Nephrectomized rats had higher exposure ($AUC_{0-\infty}$) compared with the control and sham-operated animals. The mean AMG 386 half-life ranged from 86.6 to 88.6 h in nephrectomized rats, compared with a range of 105 to 125 h for sham-operated rats and 112 to 116 h for control rats. Relevant pharmacokinetic parameters (CL and V_{SS}) were statistically significantly different ($p < 0.05$) when comparing data from nephrectomized rats with those obtained from sham-operated animals. Dose linearity appeared to be maintained across both doses, indicating that the contribution of the kidneys to AMG 386 clearance did not change with increasing dose (3 and10 mg/kg).

DISCUSSION

Currently, the mechanisms of clearance of peptibodies are not extensively characterized. Recently, population pharmacokinetic analysis of the investigational peptibody AMG 386, a selective inhibitor of Ang1 and Ang2 (19), using data from patients with advanced solid tumors (20) and patients with recurrent ovarian cancer (23) revealed that clearance of the molecule was significantly associated with creatinine clearance (24). In the present study, we assessed the clearance mechanisms of AMG 386 in three specialized rodent models. In FcRn knockout mice, clearance of AMG 386 was increased 14- to 18-fold compared with wild-type animals. In rats, the kidneys accounted for approximately 30% of AMG 386 clearance, whereas the

Table I Pharmacokinetic Parameters of AMG 386 After a Single Intravenous Dose of 3 or 10 mg/kg of AMG 386 in Wild-Type or FcRn-Knockout Mice

Parameter	Wild-Type (n=39, 3 per time point)		FcRn-Knockout (n=39, 3 per time point)	
	AMG 386 3 mg/kg	AMG 386 10 mg/kg	AMG 386 3 mg/kg	AMG 386 10 mg/kg
C_0, μg/mL	65.1	227	74.9	246
AUC_{0-last}, μg•h/mL	3730	11,800	223	931
$AUC_{0-\infty}$, μg•h/mL	4120	12,900	227	932
$t_{1/2,z}$, h	152	149	4.46	5.31
CL, mL/h/kg	0.728	0.777	13.2	10.7
V_0, mL/kg	46.1	44.1	40.1	40.7
V_{ss}, mL/kg	150	145	61.1	57.1

spleen did not appear to contribute to the total clearance of AMG 386.

There was no evidence of dose-dependent changes in the pharmacokinetic characteristics of AMG 386 when 3- and 10-mg/kg doses of AMG 386 were administered intravenously to wild-type and knockout mice, implying that FcRn is a large reservoir that cannot be easily saturated. Furthermore, the lack of dose dependency at the two tested doses suggests that TMDD does not play an important role in the clearance of AMG 386. This is consistent with the linear pharmacokinetics observed in the first-in-human monotherapy study in which the clearance of AMG 386 was similar (1.17 mL/h/kg) across a wide range of administered AMG 386 doses (0.3 to 30 mg/kg) (20). A lack of TMDD for AMG 386 was not unexpected given that it binds to circulating Ang1 and Ang2. In contrast, romiplostim (which exhibits TMDD) binds to cell surface c-Mpl receptors which may result in internalization and degradation of the protein complex (13). These differences indicate that mechanisms of clearance may vary markedly between peptibodies. Moreover, they underline the importance of studies such as the present one, that allow an early assessment of the clearance mechanisms of novel, investigational peptibodies.

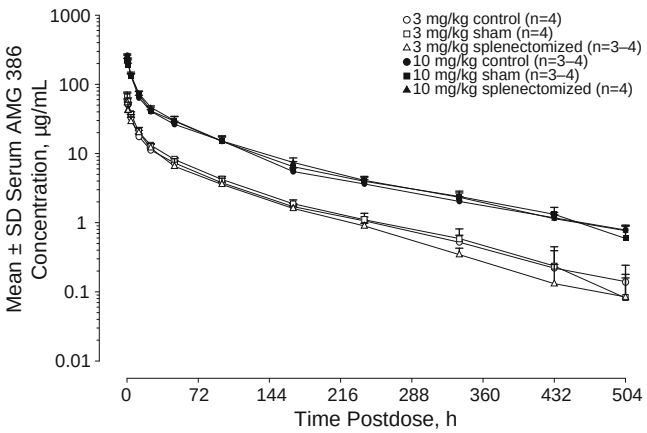

Fig. 2 Pharmacokinetic profile of AMG 386 in control (no surgical procedures), sham-operated, and splenectomized Sprague–Dawley rats following a single intravenous dose of 3 or 10 mg/kg.

The results of our study demonstrate that the FcRn plays a crucial role in the disposition of AMG 386. The Fc domain portion of peptibodies interacts with the FcRn, which presumably prevents them from undergoing lysosomal degradation following cellular pinocytosis, thereby prolonging their circulating half-life (10). Although human FcRn interacts with the Fc domain of IgG in a species-specific manner, residues on FcRn responsible for IgG binding in mice are conserved across species (26), making the murine model appropriate for peptibody-FcRn interaction assessment. The clearance rates of AMG 386 were approximately 18- and 14-times faster in the FcRn-knockout mice compared with those in wild-type animals following single IV doses of 3 and 10 mg/kg of AMG 386, respectively. The magnitude of the clearance increase for AMG 386 between wild-type and FcRn-knockout mice is comparable to that reported in previous studies using IgG or the peptibody romiplostim (approximately 10- to 24-fold increases in clearance) (9,10,13,27,28). Using noncompartmental methods, the estimated V_{ss} was 2- to 3-fold lower in FcRn-knockout mice compared with wild-type animals. However, the true V_{ss} cannot be estimated for FcRn-knockout mice because the fusion protein may be distributed to the tissues and may undergo lysosomal catabolization rather than redistribution to the central compartment. The half-life of AMG 386 in wild-type mice was similar to the reported half-life of IgG1 (6.25 *vs* 5 days) (27), highlighting the advantageousness of retaining the antibody Fc domain in peptibodies.

The pharmacokinetic characteristics of AMG 386 (at doses of 3 or 10 mg/kg) were not altered in splenectomized rats compared with sham-operated or control animals. These results demonstrate that the spleen plays a small role in the clearance of AMG 386 and suggest that the rat spleen contains low levels of FcRn. A study in splenectomized humans reported no splenic involvement in the elimination of ^{123}I-labeled IgG but instead, an increased uptake of IgG by the liver, which compensated for the loss of spleen

Table II Pharmacokinetic Parameters of AMG 386 After a Single Intravenous Dose of 3 or 10 mg/kg of AMG 386 in Control, Sham-Operated, and Splenectomized Sprague–Dawley Rats[a]

Parameter	Control[b] (n=3[c] [3 mg/kg], n=4 [10 mg/kg])		Sham-Operated (n=4)		Splenectomized (n=4)			
	AMG 386 3 mg/kg	AMG 386 10 mg/kg	AMG 386 3 mg/kg	AMG 386 10 mg/kg	AMG 386 3 mg/kg	AMG 386 10 mg/kg	p[d]	Geometric Mean Ratio (95% CI[d])
C_0, μg/mL	60.4 (3.55)	275 (19.2)	69.1 (9.56)	237 (42.4)	59.6 (29.2)	235 (14.8)	0.431	0.88 (0.63–1.23)
AUC_{0-last}, μg•h/mL	1420 (68.2)	5410 (543)	1600 (132)	5470 (830)	1320 (240)	5850 (451)	0.381	0.94 (0.80–1.09)
$AUC_{0-\infty}$, μg•h/mL	1460 (70.0)	5540 (570)	1630 (127)	5620 (770)	1340 (238)	5970 (445)	0.336	0.93 (0.81–1.08)
$t_{1/2,z}$, h	97.4 (14.4)	118 (4.50)	86.0 (13.4)	91.4 (31.8)	77.9 (13.0)	107 (7.79)	0.650	1.05 (0.83–1.33)
CL, mL/h/kg	2.06 (0.095)	1.82 (0.197)	1.85 (0.15)	1.80 (0.244)	2.29 (0.4)	1.68 (0.129)	0.336	1.07 (0.93–1.24)
V_0, mL/kg	49.8 (2.96)	36.5 (2.66)	44.0 (5.61)	43.4 (9.28)	65.9 (45.5)	42.6 (2.63)	0.431	1.14 (0.81–1.59)
V_{ss}, mL/kg	185 (20.2)	170 (10.2)	152 (14.7)	149 (23.8)	175 (40.7)	158 (16.7)	0.222	1.10 (0.94–1.29)

CI confidence interval

[a] All data are mean (SD)

[b] Control animals underwent no surgical procedures

[c] One animal was excluded owing to a suspected error in dose administration

[d] Statistical comparisons of splenectomized *versus* sham-operated groups at 3 and 10 mg/kg of AMG 386; $P < 0.05$ was considered statistically significant

function (29). Because of the general lack of cytochrome P450 enzyme involvement in protein metabolism, the contribution of the liver to the elimination of AMG 386 was not evaluated. Additionally, because the incidence of anti-AMG 386 antibody formation in humans is low (20), it was not anticipated that immunogenicity would play a significant role in the elimination of the molecule.

In the absence of TMDD and spleen involvement, the kidneys can play an important role in elimination of Fc-containing macromolecules, secondary to FcRn recycling (16,17). In the present study, we found that the clearance of AMG 386 in nephrectomized rats was decreased by approximately 30% compared with control and sham-operated rats, indicating that the kidneys contributed approximately 30% to catabolism and/or elimination of the total administered dose of AMG 386. In similar studies using the peptibody romiplostim, exposure was increased by 26% to 80% in nephrectomized rats compared with control animals (13). Although extrapolation of these data from rats to humans has inherent limitations, it should be noted that the pharmacokinetic profile of AMG 386 in the rat is closer to that of humans than any other species tested (Amgen Inc., data on file). The V_{ss} values of nephrectomized rats were also stastistically significantly lower ($p < 0.05$) than the values obtained from sham-operated rats. However, the noncompartmental approach assumes that the drug elimination occurs only from the central compartment, which conflicts with the accepted finding that antibodies are catabolized in extravascular tissues (30). Thus, without directly measuring tissue concentrations, the interpretation of V_{ss} for protein therapeutics is usually limited.

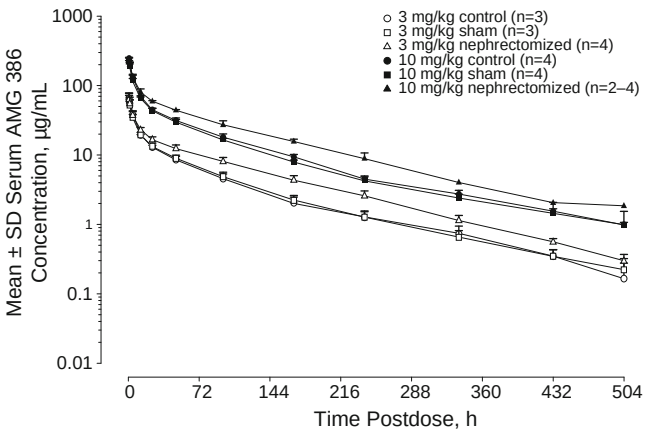

Fig. 3 Pharmacokinetic profile of AMG 386 in control (no surgical procedures), sham-operated, and nephrectomized Sprague–Dawley rats following a single intravenous dose of 3 or 10 mg/kg.

The glomerular sieving coefficient (GSC), an indicator of the rate of renal filtration of a molecule, is likely to be relatively low for peptibodies. The GSC is affected by numerous biochemical properties including molecular weight, size, charge, lipophilicity, and affinity with opsonization factors (31). The molecular weight of AMG 386 is slightly lower than that of albumin (63.5 *vs* 69 kDa), which has a GSC of 0.001 (32). Therefore, it is reasonable to speculate that fusion proteins are also filtered by the kidneys, although not efficiently. Nonetheless, given the high perfusion rate of kidneys and the long half-life of AMG 386, renal clearance may still play an important role in the overall clearance of AMG 386.

Table III Pharmacokinetic Parameters of AMG 386 After a Single Intravenous Dose of 3 or 10 mg/kg of AMG 386 in Control, Sham-Operated, or Nephrectomized Sprague–Dawley Rats[a]

Parameter	Control[b] (n=3[c])		Sham-Operated (n=3[c] [3 mg/kg], n=4 [10 mg/kg])		Nephrectomized (n=4[c])			
	AMG 386 3 mg/kg	AMG 386 10 mg/kg	AMG 386 3 mg/kg	AMG 386 10 mg/kg	AMG 386 3 mg/kg	AMG 386 10 mg/kg	p[d]	Geometric Mean Ratio (95% CI[d])
C_0, μg/mL	68.9 (6.82)	258 (15.1)	65.3 (16.3)	241 (23.9)	66.2 (16.2)	226 (11.8)	0.7662	0.97 (0.80–1.18)
AUC_{0-last}, μg•h/mL	1670 (204)	6290 (489)	1700 (238)	5880 (287)	2440 (319)	8530 (665)	<0.0001	1.39 (1.22–1.59)
$AUC_{0-\infty}$, μg•h/mL	1730 (230)	6480 (530)	1730 (246)	6060 (285)	2480 (308)	8830 (659)	<0.0001	1.39 (1.22–1.58)
$t_{1/2,z}$, h	112 (9.26)	116 (39.2)	105 (4.72)	125 (7.84)	88.6 (15.6)	86.6 (28.0)	0.012	0.68 (0.51–0.91)
CL, mL/h/kg	1.77 (0.242)	1.55 (0.119)	1.75 (0.241)	1.65 (0.0799)	1.23 (0.174)	1.14 (0.0876)	<0.0001	0.72 (0.63–0.82)
V_0, mL/kg	43.8 (4.51)	38.9 (2.29)	48.2 (13.4)	41.8 (4.58)	48.1 (15.1)	44.2 (2.34)	0.7662	1.03 (0.84–1.25)
V_{ss}, mL/kg	171 (9.64)	164 (22.8)	167 (18.1)	175 (11.3)	136 (27.3)	120 (22.7)	0.0003	0.72 (0.62–0.84)

CI confidence interval

[a] All data are mean (SD)

[b] Control animals underwent no surgical procedures

[c] One animal was excluded owing to a suspected error in dose administration

[d] Statistical comparisons of nephrectomized *versus* sham-operated groups at 3 and 10 mg/kg of AMG 386; $P < 0.05$ was considered statistically significant

The excretion of AMG 386 in the urine was not directly measured because of assay limitations. A biodistribution study of ^{125}I-labeled AMG 386 in cynomolgus monkeys showed that, although most of the administered radioactivity was collected in the urine and cage rinse, <10% of it was incorporated in intact protein based on trichloroacetic acid precipitation assays (Amgen Inc., unpublished data). Presently, it is unknown what proportion of AMG 386 is excreted intact via the kidney. A clinical study in cancer patients with either normal or impaired renal function has been initiated to evaluate the effect of differences in estimated glomerular filtration rates on the pharmacokinetics of AMG 386 (ClinicalTrials.gov, NCT01331941).

Based on the data collected in the experiments with FcRn-knockout mice, the main determinant of AMG 386 disposition appears to be FcRn recycling. Furthermore, Sarav *et al.* (17) have shown that FcRn is expressed on podocytes and the brush border of the proximal tubular epithelium in murine kidneys, and that renal FcRn plays a role in the metabolism of albumin and IgG. This observation is consistent with a previously proposed mechanism of FcRn protection and clearance for IgG molecules, including monoclonal antibodies (10,30). However, the exact FcRn association and dissociation characteristics of various types of protein therapeutics are presently unknown. Future exploration to elucidate the rate-limiting factors that could contribute to a prolonged half-life would be of interest.

While the experiments described herein show that renal clearance contributed to approximately 30% of the total clearance of AMG 386 in rats, other clearance mechanisms must account for the remainder. Although a comprehensive understanding of all pathways of clearance for biologic therapeutics requires future research, it is generally accepted that most of them undergo nonspecific catabolism, similar to endogenous and dietary proteins (33). This includes nonspecific proteolytic catabolism following endocytosis by endothelial cells and hepatic protein metabolism (34). Although the cytochrome P450 system does not play a role in metabolism of biologic therapeutics, alternative pathways that allow efficient uptake and catabolism of exogenous proteins are present in hepatocytes (34,35). Additionally, because FcRn plays a central role in regulating the half-life of peptibodies, variation in FcRn binding affinity could potentially affect the clearance of these molecules as has been demonstrated for other IgG1 molecules (36). Development of immunogenicity may also influence the clearance of protein therapeutics. Even when a recombinant human protein molecule has nearly identical sequence to an endogenous protein, anti-drug antibodies have been shown to influence the clearance of peptibodies including those containing sequences nearly identical to endogenous proteins (30,37). However, in clinical studies, the incidence of immunogenicity to AMG 386, assessed using a highly specific assay, was low (20,22,38,39); thus it appears unlikely that immunogenicity contributes substantially to the clearance of AMG 386. In summary, although we have explored the involvement of FcRn, spleen, and kidneys in the clearance of AMG 386, understanding the exact contributions of other rate-limiting factors to the clearance of AMG 386 and other peptibodies will require further investigation.

CONCLUSIONS

In summary, the peptibody AMG 386 demonstrated linear pharmacokinetic properties after single-dose administration as monotherapy in mice and rats, and no evidence of TMDD was apparent. The FcRn appeared to be a key contributor to the long half-life of AMG 386. In rats, no involvement of the spleen in AMG 386 elimination was observed, but the kidneys accounted for approximately 30% of AMG 386 clearance. Although the GSC for AMG 386 is expected to be low, the kidney may still play an important role in AMG 386 clearance because of the long half-life of the drug and the kidneys' high perfusion rate. Renal drug-drug interaction at the transporter level is not expected, but renal impairment may play a role in fusion protein clearance. Currently, the US Food and Drug Administration recommends that renal impairment studies be conducted for drugs with substantial renal elimination (ie, ≥30% of agent excreted in urine unaltered) (40). A clinical study to evaluate the pharmacokinetics of AMG 386 in cancer patients with varying levels of renal function has been initiated (ClinicalTrials.gov, NCT01331941). Overall, the results of the study described herein provide valuable insight into the pharmacokinetic characteristics of peptibodies, a group of molecules that has yet to be studied in detail.

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