Published in final edited form as:

Curr Pharm Des. 2013 ; 19(42): 7479–7485.

Phase 1 Safety, Tolerability and Pharmacokinetics of 3K3A-APC in Healthy Adult Volunteers

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Abstract

Background and Purpose—Activated Protein C (APC) stimulates multiple cytoprotective pathways via the protease activated receptor-1 (PAR-1) and promotes anticoagulation. 3K3A-APC was designed for preserved activity at PAR-1 with reduced anticoagulation. This Phase 1 trial characterized pharmacokinetics and anticoagulation effects of 3K3A-APC.

Methods—Subjects (n=64) were randomly assigned to receive 3K3A-APC (n=4) at 6, 30, 90, 180, 360, 540 or 720 g/kg or placebo (n=6) and were observed for 24 hr. After safety review additional subjects received drug every 12 hr for 5 doses (n=6 per group) at 90, 180, 360, or 540 g/kg or placebo (n=8) and were observed for 24 hr.

Results—All subjects returned for safety assessments at 72 hours and 15 days. We found few adverse events in all groups. Systolic blood pressure increased in both active and placebo groups. Moderately severe headache, nausea and vomiting were reported in one of two subjects treated with 720 g/kg so 540 g/kg was considered the highest tolerated dose. Mean plasma concentrations

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CONFLICTS OF INTEREST

B.V.Z. is the scientific founder of ZZ Biotech, a biotechnology company with a focus to develop APC and its functional mutants for stroke and other neurological disorders. T.P.D. and J.H.G. are members of the Scientific Advisory Board of ZZ Biotech LLC. P.D.L. is a consultant to ZZ Biotech. KP and SW are employees of ZZ Biotech, LLC. HL was a paid consultant to ZZ Biotech with respect to the study design and safety monitoring of this study. William G. Kramer was a paid consultant to ZZ Biotech with respect to the work done on this study.

increased in proportion to dose. Clearance ranged from $11,693 \pm 807$ to $18,701 \pm 4,797$ mL/hr, volume of distribution ranged from $4,873 \pm 828$ to $6,971 \pm 1,169$ mL, and elimination half-life ranged from 0.211 ± 0.097 to 0.294 ± 0.054 hours. Elevations in aPTT were minimal.

Conclusions—3K3A-APC was well tolerated at multiple doses as high as 540 g/kg. These results should be confirmed in stroke patients with relevant co-morbidities. Clinical Trial Registration-Url: <http://www.clinicaltrials.gov>. Unique identifier: NCT01660230

Keywords

Protein C; coagulation; Activated protein C; PAR1; pharmaco-kinetics; clinical trials; humans

INTRODUCTION

Activated protein C (APC) is an endogenous serine protease with systemic anti-inflammatory, anti-apoptotic, and anticoagulant properties [1, 2]. APC's cytoprotective effects are independent of its anticoagulant effect and significant protection in the central nervous system (CNS) has been demonstrated in multiple models of acute and chronic CNS injury [3, 4]. APC anticoagulant activity is mediated by irreversible proteolysis and inactivation of clotting factors Va and VIIIa, whereas its cytoprotective effects are mediated via activation of the protease activated receptor 1 (PAR-1) [1, 2, 5-7], and transactivation of sphingosine-1-phosphate receptor 1 (S1P1) [8, 9]. Neuronal protection by APC requires PAR3 in addition to PAR1 [10].

Pharmacologic 3K3A-APC for human use is a 405-residue protein produced via recombinant technology in Chinese hamster ovary (CHO) cells. Its amino acid sequence differs from that of the wild-type (wt) human APC and the commercial wt-APC product, drotrecogin alfa [activated] (DrotAA), in that 3 sequential lysine residues have been replaced with 3 sequential alanine residues; the amino acid substitutions are K191A-K192A-K193A. These 3 particular amino acids were changed to alter a major factor Va binding exosite in APC (reducing anticoagulation) without affecting APC's exosites that recognize PAR-1 and endothelial protein C receptor (EPCR). 3K3A-APC retains the full cytoprotective activities of wt-APC in vitro with < 10% of its anticoagulant activity [11]. This decrease in the anticoagulant activity of 3K3A-APC relative to DrotAA should reduce the risk of bleeding in subjects, which was a serious side effect of DrotAA [12]. In animal models of stroke [13-15], traumatic brain injury [16] and amyotrophic lateral sclerosis [17], 3K3A-APC exerted beneficial effects that were equivalent to, and sometimes greater than, the recombinant wt-APC [3].

In pre-clinical models, 3K3A-APC exhibits synergistic efficacy and reduces hemorrhagic complications when combined with rt-PA. Combination treatment with 3K3A-APC and tPA was effective, even when administration was delayed up to 4 hr after ischemia using middle cerebral artery occlusion (MCAo) in mice and embolic stroke in rats [15].

The mechanism of 3K3A-APC neuroprotection is partly elucidated [13, 18]. In animals and humans, PAR-1 activation by APC exerts protective effects throughout the neurovascular unit because the PAR-1 receptor functions on neurons, glia and endothelial cells. APC

signaling via PAR-1 differs remarkably from that caused by thrombin in that thrombin's actions are G-protein mediated whereas APC-induced signaling is mediated by beta-arrestin; this phenomenon for PAR-1 signaling is termed "biased agonism." [19] Moreover, thrombin cleaves PAR-1 at Arg41 whereas APC cleaves PAR-1 at Arg46 with the net effect that APC initiates cytoprotective activities by its unique Arg46 cleavage [20]. Thus, we hypothesize that 3K3A-APC mediated activation of PAR-1 represents a novel strategy targeted at the entire neurovascular unit comprising distinct subsets of cells at the blood brain barrier.

The primary objectives of this Phase 1 study were to evaluate the safety and pharmacokinetics of single and multiple ascending intravenous (IV) doses of 3K3A-APC with its reduced anticoagulant activity in healthy adult subjects.

METHODS

Study Design and Objectives

The protocol was reviewed and approved by the Austrian Agency for Health and Food Safety (EudraCT # 2011-000793-60) and the Ethics Committee of the Medical University of Graz and conducted in compliance with Good Clinical Practice (GCP) and listed on clinicaltrials.gov (NCT01660230). The primary objectives of the study were to evaluate the safety and pharmacokinetics of single and multiple ascending doses of 3K3A-APC. This was a sequential-cohort, randomized, double-blind, placebo-controlled, ascending single- and multiple-dose study. Eligible adult subjects were assigned sequentially to cohorts at successively higher single doses, followed by successively higher multiple doses. Single-dose cohorts consisted of subjects randomized 4:1 to receive active drug (n=4 at 6, 30, 90, 180, 360, and 720 g/kg (reduced to 540 g/kg for subsequent subjects) or matching placebo (n=6). Multiple-dose cohorts consisted of subjects randomized 6:2 to receive active drug (n=6 at 90, 180, 360, and 540 g/kg) or matching placebo (n=8) every 12 hr for 5 doses.

The starting dose of 3K3A-APC in this trial was 6 g/kg, which is 1/10th of the human equivalent dose (HED) of the "no adverse event level" (NOAEL) established in a cynomolgus monkey 14-day IV toxicology study [21]. The NOAEL was 0.2 mg/kg, which translates to an HED of 60 g/kg. HED calculations used species conversion factors outlined in the FDA guidance for estimating the maximum safe starting dose in initial clinical trials (FDA Center for Drug Evaluation and Research, July 2005). The planned dose range of 3K3A-APC in this Phase 1 study included HEDs that resulted in improved outcomes in mouse stroke models [13]. In murine efficacy models, murine wt-APC and 3K3A-APC were equally effective in reducing infarct volume and edema and in improving neurological function at IV doses of 40 to 200 g/kg with maximal protective effects achieved at approximately 200 g/kg, although there are species differences between murine and human 3K3A-APC [18].

A Safety Review Committee (SRC), comprised of the clinical site investigator, independent data safety monitors (DSM), sponsor appointed physicians and sponsor representatives, reviewed the blinded safety data following each cohort. The SRC could alter the dose escalation scheme and time points for safety evaluations, pharmacokinetics, and coagulation parameters based on review of blinded safety data and PK results. If needed for evaluation

of safety, the protocol allowed unblinded safety committee members to unmask individual subjects after a consensus from the SRC. Unmasking occurred in 6 subjects.

Safety Evaluation and Study Procedures

Subjects were admitted to a Phase I clinical study unit after a rigorous pre-screening assessment that included vital signs, comprehensive blood and urine assessment, screen for recreational substances, and a serum pregnancy test in females. All Subjects were free of disorders that could predispose to stroke or coagulopathy. The inclusion and exclusion criteria are listed in Table 1. No more than 2 subjects were to be dosed on the first day of a cohort. As soon as subjects were enrolled to complete one dose tier, the SRC met to review exam findings, vital signs, serum chemistries, blood counts, coagulation studies, and urinalysis. If a potential dose-limiting toxicity was identified, based on pre-specified definitions and stopping rules, the SRC could stop the trial or require additional subjects at that dose tier before approving escalation to the next level.

Subjects agreed to abstain from alcohol and cigarettes until Study Day 15. Subjects arrived at the study unit the night before dosing and underwent repeat physical examination, fasting chemistries, blood counts, coagulation studies, urinalysis and anti-3K3A-APC antibody specimen draw. Once all procedures were completed, subjects received a standard dinner and remained confined overnight. Prior to dosing, subjects had an IV catheter placed in each arm, one for blood sampling and a second for study drug infusion. Prior to study drug infusion blood was drawn for pre-infusion PK and coagulation studies. During and following study drug infusion subjects were confined for 24 hr after dosing for vital signs, PK specimens and adverse events. Blood pressure, heart rate, respirations, and temperature were obtained at baseline and frequently during and after the infusion. Plasma samples were collected immediately prior to infusion of study drug, at multiple time points after the infusion in the single-dose cohorts. The number of blood samples was adjusted in subsequent cohorts after the PK data from earlier cohorts were analyzed. Coagulation studies—prothrombin time (PT), International normalized ratio (INR), activated partial thromboplastin time (aPTT), fibrinogen—were drawn for coagulation studies at baseline and 1, 2, and 4 hr after the end of the infusion. A 12-lead ECG (supine for 5 min) was performed 1 hour after the end of infusion and assessment for AEs continued for 24 hr after dosing completion. Before discharge from the unit and at each follow up visit each subject underwent a physical examination, vital signs (after supine for 3 min), fasting (8 hr) serum chemistries, blood count, coagulation studies, and urinalysis. Procedures were the same during the multiple dosing phases except that drug was administered at 0 and 12 hr (Day 1), at 24 and 36 hr (Day 2), and at 48 hr (Day 3). All procedures described above were performed during and following study drug infusion and subjects were confined until 24 hr after the last infusion. At the 15-day follow up visit, an ECG was performed, anti-3K3A-APC antibody specimen was drawn, and women of child bearing potential also underwent a serum HCG test. Subjects were contacted by telephone 28 days from their last administration of study drug to assess for adverse events.

Pharmacokinetic and Pharmacodynamic Assessments

Blood (4 mL) was collected into sodium citrate collection tubes preloaded with the trypsin-like serine protease inhibitor benzamidine. After blood collection, samples were held at room temperature and centrifuged within approximately 10 min at 3000 rpm for 10 min. The plasma was removed, split into 2 equal aliquots, and stored in 2 mL Cryotube™ containers at -70°C until shipped on dry ice to the central laboratory.

Plasma concentrations of enzymatically active 3K3A-APC were measured with a validated enzyme capture assay using an immobilized murine monoclonal antibody to APC followed by a direct assay of APC activity of the captured drug with a lower limit of quantitation of 75 ng/mL [22].

Pharmacokinetic Analysis

Pharmacokinetic parameters were determined by compartmental analysis of the single and multiple dose data. Examination of semi-logarithmic graphs of the individual subject data indicated that the data appeared to be consistent with an open, one compartment IV infusion model. The primary PK parameters, those that were estimated in fitting the model to the data, were total plasma clearance (CL) and volume of distribution (V). The secondary PK parameters — maximum plasma concentration (C_{max}), area under the curve to infinity [AUC (inf)], and the elimination rate constant (z) and half-life (t) — were estimated from the primary parameters. All compartmental modeling was done using Phoenix WinNonlin Version 6.2 (Pharsight Corporation, Sunnyvale, CA). Because this was an exploratory study, no formal hypothesis testing was performed. Descriptive statistics for continuous data included number of subjects, arithmetic mean, standard error of the mean, standard deviation, median, minimum value, and maximum value. Descriptive statistics for categorical data included frequency and/or percent.

Publication Policy and Role of the Sponsor

The Coordinating Investigator drafted the primary manuscript based on full access to all study data and the ability, if needed, to confirm any statistical calculations. Initial drafts were circulated by the investigator among key ZZ Biotech personnel, and co-authors. The Coordinating Investigator was responsible for finalizing the manuscript and had final control of each draft including the final draft.

RESULTS

Of the 67 subjects who were screened for this study and entered into the clinical database, 64 were randomized. One screened subject was excluded for an elevated aPTT; one for elevated liver function tests; and one subject was screened as a ‘back up’ should one of the enrolled subjects drop out— this did not happen. All subjects completed all follow up visits and assessments. A total of 64 subjects received placebo or 3K3A-APC (range, 6 – 720 g/kg). The mean age was 27.0± 7.4 years; 53% were male. No subject suffered an adverse event sufficiently severe to require termination of the dose infusion. Based on adverse events, the highest tolerated dose evaluated was 540 g/kg in both single- and multiple-dose groups. A single-dose of 720 g/kg was evaluated in two subjects and deemed intolerable

after one subject experienced dose-related moderate headache associated with nausea, vomiting and vertigo. The Safety Review Committee chose to explore an intermediate single- dose of 540 g/kg, which was tolerated with transient, treatment- related headache, in 4 out of 5 subjects (mild in 3; moderate in 1). The most common 3K3A-APC related adverse events reported were headache, nausea, and vomiting, all mild or moderate, re- ported in 54%, 8%, and 4% of the treated subjects respectively. Only three post-infusion ECGs showed abnormalities: two subjects had prolonged QTc considered not clinically significant (single dose 6 g/kg Day 1 post infusion; and multiple dose 90 g/kg at Day 15); one placebo subject had sinus bradycardia on Day 1.

Blood samples for coagulation testing were obtained pre-dose and 1, 2 and 4 hr following each dose of 3K3A-APC. As illustrated in (Fig. 1), mild dose-related elevations in aPTT (up to 7 seconds on average) were seen 1 hour following 3K3A-APC at single or multiple doses of 180, 360, 540 and 720 g/kg. The highest recorded aPTT in a single patient was 60.4 sec following infusion #4 of 360 g/kg, representing a 22.7 sec increase from baseline in that patient. The longest prolongation of the aPTT in a patient was 23.0 sec (to 58.9 sec) following infusion #3 at 540 g/kg. Other infusions at the same dose in this subject had shorter prolongations. Neither of these increased aPTT was considered to be clinically significant. The majority of aPTT prolongations returned to normal by 2 hr following the infusion (Fig. 1). This relatively mild and transient effect, suggests that 3K3A-APC acts on factors Va and VIIIa and not the precursors V and VIII. No clinical manifestations of bleeding or bruising were observed in any subject. No subjects developed anti-bodies to 3K3A-APC.

After administration of single 6 g/kg doses (Cohort 1), all plasma 3K3A-APC concentrations were below the limit of quantitation (75 ng/mL) and this cohort was excluded from the pharmacokinetic analysis. The analysis population for pharmacokinetics was therefore comprised of 22 subjects in the single dose phase (Cohorts 2 through 6) and 24 subjects in the multiple doses phase (Cohorts 7 through 10). There was good agreement between the observed and model-predicted plasma 3K3A-APC concentrations after single and multiple doses indicating that the one-compartment model was concordant with the data. There was a dose-related and dose-proportional increase in the mean plasma concentrations of 3K3A-APC after IV infusion of single 30 to 720 g/kg doses (Fig. 2). As shown in (Fig. 3), a log-log plot of the individual subject values for the model-predicted AUC (inf) versus the total dose was linear with a slope of 1.03, demonstrating linear pharmacokinetics. The mean values for CL and Vz were independent of dose across the 6 single and 4 multiple doses (Table 2). The mean elimination half-life ranged from 0.211 h (13 min) to 0.294 h (18 min) and was not dependent on dose (Table 2). The data demonstrate that the pharmacokinetics of 3K3A-APC are linear after IV infusion of single doses ranging from 30 to 720 g/kg and multiple doses ranging from 90 to 540 g/kg Q12H 5 doses.

DISCUSSION

We established tolerability and safety of human 3K3A-APC in healthy volunteers up to a maximum dose of 540 g/kg. At higher doses, one subject suffered headache, nausea and vomiting such that—while the infusion did not need to be terminated—the safety review

committee felt the dose was not well tolerated. No evidence of severe or moderate coagulopathy was seen (Fig. 1). The drug exhibits linear pharmacokinetics over the range of single and multiple doses tested and has an elimination half-life of about 16 min (Fig. 2 and Table 2). Antibodies developed in no subjects.

3K3A-APC was engineered to exhibit less coagulopathy than wt-APC and our data confirm a minimal elevation of aPTT. The absence of significant coagulopathy is important because neuroprotective doses of both wt-APC and 3K3A-APC in rodents require doses that alter coagulation during treatment with wt-APC. While anti-coagulants were long promoted for stroke treatment, no clinical trial of any anticoagulant has demonstrated neuroprotection, including heparin, low-molecular weight heparin, and abciximab, among others [23, 24]. On the other hand, the neuroprotective effects of wtAPC and 3K3AAPC have been demonstrated clearly, and are apparently less dependent on anticoagulant properties [3]. Data collected in this study with 3K3A-APC are not directly comparable with the anticoagulant properties of DrotAA (wild type APC) due to differences in dosing regimens, but in clinical trials of severe sepsis, significant elevations in aPTT were seen at doses of 24 g/kg/hr DrotAA for 96 hr continuous infusion, and serious bleeding events were reported in 3.5% vs. 2.0% in controls, including intracranial hemorrhages in approximately 1% [12]. Presumably, 3K3A-APC could show neuroprotection in stroke subjects with far less risk of bleeding side effects, compared to DrotAA. By comparison, infusions of the direct thrombin inhibitor argatroban sufficient to elevate PTT to 1.5x baseline during rt-PA treatment for acute stroke caused no increase in cerebral bleeding [31].

Translating pre-clinical efficacy to successful human clinical trials has proven difficult in stroke [25]. Many schemes have been proposed for improving the likelihood that pre-clinical development will yield a success in final clinical testing [26-28]. To date, pre-clinical development of 3K3A-APC includes demonstration of efficacy in rats and mice, in multiple laboratories, using behavioral, histological, and biomarker endpoints. The drug shows efficacy out to clinically relevant delay times [15]. Importantly, 3K3A-APC shows synergistic efficacy in combination with rt-PA: hemorrhage rates were reduced following rt-PA treatment in the presence of 3K3A-APC [15, 29, 30]. When used in combination, 3k3a-APC shows no effect on rt-PA lytic effect [32]. While all of these data are reassuring, there remains uncertainty that the drug will work in humans, because there no valid guidance exists to translate effective serum concentrations in rodents to dosing in humans. In the past, many neuroprotectants have failed partly because serum concentrations were significantly lower in subjects than in the successful animal studies. Thus it is reassuring that the maximally tolerated dose in healthy volunteers is well above the dose (200 g/kg) shown to be maximally protective in animals [6, 13, 18]. Nevertheless, Phase 2 dose finding studies in stroke subjects will be necessary.

Another important source of uncertainty is the duration of treatment needed in stroke subjects. While spontaneously hypertensive or aged laboratory animals subject to temporary occlusion of the MCA may need only brief treatment periods, it is unknown whether stroke subjects—with attendant comorbidities— might require longer treatment periods. There exists a practical limit on acute treatment during stroke—stays longer than 3 days are not

common in the US and Europe. For these practical reasons, a 3 day limit on treatment duration may be imposed on clinical development of 3K3A-APC.

In summary, the 3K3A-APC was engineered to show minimal coagulopathy and we have confirmed this property of the molecule. Non-serious side effects limited tolerability of the drug to a maximum dose of 540 g/kg. The drug was tolerated twice daily for 3 days. Further clinical development should include a tolerability study in stroke subjects.

Acknowledgments

This work was supported by the National Institutes of Health grants HL63290 (BZ), HL031950 (JHG) and HL052246 (JHG).

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Fig. (1).

Adjusted mean aPTT. For each subject, the baseline aPTT was calculated as the average of all pre-treatment aPTT values: pre-screening, arrival, and pre-first dose. Then, each aPTT was adjusted for the average baseline and reported as a ratio. Each cohort is shown as the average of all subjects in the cohort, without standard error limits for clarity. Elevations were observed one hour after the infusion and returned to baseline within 2 hr for most time points. The 90µg/kg cohort and the placebo group did not exhibit elevations.

Fig. (2).

Arithmetic mean $\pm$ standard error plasma concentrations of 3K3A APC after IV infusion of single 30 to 720 µg/kg doses to healthy subjects — linear axes.

Fig. (3).

Relationship between the compartmental model-predicted AUC (inf) of 3K3A-APC and dose after IV infusion of single 30 to 720µg/kg doses to healthy subjects.

Table 1

Main inclusion and exclusion criteria for the trial.

Inclusion Criteria
1. Healthy males or non-pregnant, non-lactating females
2. Agreement by men and women of child-bearing potential to use contraception
3. Age 18 to 55 years, inclusive
4. Body Mass Index (BMI) of 19 to 30, inclusive
5. Willing and able to complete all study visits
6. Agreement to abstain from smoking and drinking alcoholic beverages from 48 hr prior to randomization through last Study Day (15)
7. Signed informed consent form (ICF)
Exclusion Criteria
1. Any medical problem for which the subject is being evaluated and/or treated
2. Activated partial thromboplastin time (aPTT) greater than upper limit of normal (ULN)
3. Platelet count < 125,000 cells/mm ³
4. International Normalized Ratio (INR) > 1.3
5. Any other clinically significant abnormalities in laboratory values (chemistries, hematology, coagulation studies, and urinalysis)
6. Clinically significant abnormalities on electrocardiogram (ECG)
7. Positive serum β HCG pregnancy test at screening or on Study Day -1 (for women of child-bearing potential)
8. Positive urine drug screen at screening or on Study Day -1
9. Positive blood test for hepatitis B surface antigen, hepatitis C antibody, or HIV antibody
10. Known family history of bleeding or blood clotting disorders
11. History of bleeding diathesis
12. History of liver disease with ongoing coagulopathy
13. Use of any prescription or non-prescription medications or supplements within 8 days prior to Study Day 1, including oral contraceptives
14. Use of anticoagulant medication within 14 days prior to Study Day -1
15. Major surgery within 60 days prior to Study Day -1
16. Receipt of an investigational drug within 30 days prior to Study Day -1
17. Donation of blood or plasma within 30 days prior to Study Day -1
18. Any other condition that in the opinion of the Site Investigator may adversely affect the safety of the subject, the subject's ability to complete the study, or the outcome of the study

Table 2

Summary of compartmental pharmacokinetic parameters for 3K3A-APC after IV infusion of single 30 to 720 µg/kg doses and multiple 90 to 540 µg/kg Q12H × 5 doses to healthy subjects. * Arithmetic mean ± standard deviation (N) except Tmax for which the median (N) is reported. † The initial 2 subjects in Cohort 6 received 720 µg/kg. Due to tolerability issues, the dose was reduced to 540 µg/kg for subsequent subjects. ‡ Secondary parameters were calculated from the primary parameters.

Cohort	Dose (µg/kg)	Parameters*						
		Primary†			Secondary‡			
		CL (mL/h)	V (mL)	Cmax (ng/mL)	AUC(inf) (hTng/mL)	λz (1/h)	t _{1/2} (h)	
Single Dose								
2	30	17,017 ± 4,163 (4)	5,967 ± 1,236 (4)	249 ± 592 (4)	123 ± 32.0 (4)	2.85 ± 0.30 (4)	0246 ± 0.027 (4)	
3	90	18,016 ± 1,695 (4)	6,831 ± 1,231 (4)	741 ± 502 (4)	381 ± 29.6 (4)	2.69 ± 0.49 (4)	0263 ± 0.043 (4)	
4	180	17,981 ± 2,321 (4)	5,931 ± 0,726 (4)	1300 ± 111 (4)	623 ± 131 (4)	3.08 ± 0.65 (4)	0232 ± 0.046 (4)	
5	360	17372 ± 1,700 (4)	5,970 ± 1,194 (4)	3374 ± 490 (4)	1,615 ± 142 (4)	2.96 ± 0.33 (4)	0237 ± 0.029 (4)	
6	540	16,665 ± 1,555 (4)	6,359 ± 1277 (4)	4,489 ± 654 (4)	2309 ± 191 (4)	2.68 ± 0.49 (4)	0264 ± 0.041 (4)	
	720	11,693 ± 807 (2)	4,922 ± 574 (2)	5,715 ± 306 (2)	3,195 ± 263 (2)	2.40 ± 0.44 (2)	0294 ± 0.054 (2)	
Multiple Dose								
7	90	18,701 ± 4,797 (6)	5,151 ± 982 (6)	810 ± 118 (6)	370 ± 145 (6)	3.87 ± 58 (6)	0211 ± 0.097 (6)	
8	180	17,177 ± 2,593 (6)	6,971 ± 1,169 (6)	1,447 ± 135 (6)	794 ± 148 (6)	2.50 ± 0.46 (6)	0284 ± 0.044 (6)	
9	360	14,231 ± 2,536 (6)	5,732 ± 645 (6)	2,990 ± 608 (6)	1,634 ± 365 (6)	2.47 ± 0.20 (6)	0282 ± 0.022 (6)	
10	540	13,647 ± 1,584 (6)	4,873 ± 828 (6)	5,577 ± 1,134 (6)	2,750 ± 512 (6)	2.83 ± 0.25 (6)	0247 ± 0.022 (6)	

* Arithmetic mean ± standard deviation (N).

† Primary parameters were used to fit the model to the data.

‡ Secondary parameters were calculated from the primary parameters.