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ORIGINAL RESEARCH ARTICLE

Pharmacokinetics of Polyethylene Glycol-Modified Canine Uricase Following Single and Multiple Intravenous Injections in Cynomolgus Monkeys

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Abstract

Background and Objective Polyethylene glycol-modified canine uricase (PEG-UHC) prepared with a lower-molecular-weight (5 kDa) PEG is used to treat gout. This study investigated the comparative pharmacokinetics of single and multiple doses of PEG-UHC administered intravenously and a single dose of uricase (UHC) administered intravenously in cynomolgus monkeys.

Methods A noncompartmental model was used to fit the plasma drug concentration–time curve and calculate the pharmacokinetic parameters of PEG-UHC, which were compared with those obtained for UHC at the equivalent dose (2 mg/kg). To study the pharmacokinetics after multiple dose administration, cynomolgus monkeys were administered five intravenous injections of PEG-UHC (0.5 mg/kg), with one injection performed every 15 days.

Results The area under the curve (AUC) and the maximum plasma concentration (C_{max}) of PEG-UHC were positively correlated with dose, whereas plasma half-life ($t_{1/2}$) and clearance (CL) did not change significantly with increasing dose, suggesting that these pharmacokinetic characteristics are linear. Intravenous PEG-UHC exhibited an average $t_{1/2}$ that was 125.79 times longer and an AUC_{0-t} that was 64.45 times larger than the corresponding values for UHC at the same dose (2 mg/kg), while the CL of PEG-UHC was 1/72.73 times the CL of intravenous UHC. The plasma drug concentration reached a steady state after five injections, and the $t_{1/2}$ values following the first and last drug administration did not differ significantly.

Conclusion Our data show that PEG-UHC is markedly superior to UHC in terms of duration of action, and that the pharmacokinetics of PEG-UHC in cynomolgus monkeys are linear. Sequential administration of PEG-UHC did not accelerate drug clearance. Our findings provide the basis for future clinical studies of PEG-UHC.

Key Points

The pharmacokinetics of a single dose of 5 kDa polyethylene glycol (PEG)-modified canine uricase (PEG-UHC) administered intravenously were superior to those of unmodified UHC in cynomolgus monkeys.

PEGylation increased the half-life and AUC and decreased drug clearance.

Multiple dose administration did not induce rapid drug clearance.

1 Introduction

Gout is a heterogeneous disease resulting from a long-term disorder of purine metabolism and increased blood uric

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acid (UA) levels that can lead to tissue damage. Since the human body lacks functional uricase [1–3], UA cannot be metabolized, rendering it the final product of purine metabolism [4]. Uricase was first marketed in Europe for the treatment of gout in 2001 [5]. This treatment effectively and rapidly reduces UA levels in patients; however, as it is an exogenous protein, uricase readily undergoes enzymatic hydrolysis in the body. In addition, its low stability, short plasma $t_{1/2}$, high antigenicity, tendency to induce hypersensitivity reactions, and short duration of action greatly limit its clinical utility.

Modifying proteins with polyethylene glycol (PEG) is an effective method of addressing such limitations. When PEG with a molecular weight of 5–60 kDa is used to modify drugs, the PEG creates a barrier layer around the modified drug molecules that preserves drug activity, reduces immunogenicity, and prolongs $t_{1/2}$ [6–8]. Pegloticase is a 10 kDa-PEG-modified uricase that was approved by the US Food and Drug Administration (FDA) in October 2010 [5]. However, the results of a phase III clinical trial showed that 58% of the trial subjects experienced a decrease in the pegloticase-induced reduction of UA after repeated use of pegloticase. This is due to the body's production of antipegloticase antibodies after repeated exposure to pegloticase [9], which leads to faster drug clearance. Anti-pegloticase antibodies also cause infusion reactions [10, 11]. Such infusion reactions in response to repeated intravenous administration of pegloticase are common, and corticosteroids are usually administered prophylactically [9, 12]. These reactions tend to lower patient treatment compliance [13, 14] and limit the use of pegloticase in patients with diabetes and glaucoma, in whom corticosteroids are contraindicated.

In our previous study, we found that particle size is an important influence on drug clearance after repeated use of PEG-modified uricase; this effect was mediated by anti-PEG IgM antibodies, and has been widely observed for PEGylated nanomaterials [15–17]. However, after removing urinary enzyme aggregates and PEGylated diol contaminants, rapid drug clearance was not observed after repeated intravenous administration of PEGylated canine uricase (PEG-UHC) prepared with a lower-molecular-weight (5 kDa) PEG reagent in rats [18; further benefits of this approach included a reduced injection site response and fewer anticoupling antibodies. Previous pharmacodynamic studies have shown that 5 kDa PEG-UHC exhibits specific activity at 12 U/mg and has a significant effect on uric acid nephropathy and acute urate arthritis [19]. In another study, Sprague–Dawley (SD) rats and cynomolgus monkeys were treated with a single intravenous dose of 0.9 mg/kg PEG-UHC and 1.0 mg/kg PEG-UHC, respectively. The clearance of PEG-UHC differed significantly between the two species: it was 2.61 mL/h/kg for rats and 0.21 mL/h/kg for monkeys, suggesting that the mechanism for PEG-UHC metabolism differs between rats and cynomolgus monkeys [19]. The mechanism for the metabolism of PEG-UHC in cynomolgus monkeys was found to be closer to that of humans than that of rats [20]. Studying the pharmacokinetic properties of PEG-UHC in cynomolgus monkeys may therefore provide a more reliable reference for drug clinical trial design. Thus, based on previous studies, the present study further investigated the pharmacokinetics of single and multiple doses of 5 kDa PEG-UHC administered intravenously to cynomolgus monkeys in order to provide the foundations for future clinical studies.

2 Materials and Methods

2.1 Animals

A total of 22 healthy adult cynomolgus monkeys (equal numbers of males and nonpregnant females) that were aged 3–4 years and had body weights ranging from 2.75 to 4.80 kg were provided by Suzhou Xishan Zhongke Laboratory Animal Co., Ltd. The animals were housed individually in cages and were fed a standard monkey diet. The temperature and relative humidity of the animal room were maintained at 16–26 °C and 40–70%, respectively. The experiments were carried out in compliance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

2.2 Chemicals and Reagents

PEG-UHC was prepared according to procedures described previously [19], and samples of PEG-UHC (4 mg/mL) were produced and provided by Chongqing Fujin Biomedical Co., Ltd. In the PEG-UHC used in this study, 9.5 5-kDa mPEG chains were coupled to each UHC subunit. A PEG-UHC standard solution (4 mg/mL) and a UHC injection (2 mg/mL) were also provided by Chongqing Fujin Biomedical Co., Ltd. UA (≥ 99.0%) was purchased from Sinopharm Chemical Reagent Co., Ltd.

2.3 Pharmacokinetic Studies

The treatment groups examined in this study are outlined below:

1. *UHC control group* Four animals (two male and two female animals) were administered 2 mg/kg of the control UHC. Blood samples (1.5 mL) were collected at the following intervals: before (− 5 min) drug administration

and at 5 min, 15 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 12 h, and 24 h after drug administration.

2. *Single intravenous PEG-UHC injection group* Eighteen animals were randomly assigned to low-, medium-, and high-dose PEG-UHC groups according to weight. Each group comprised three male and three female animals. The low-, medium, and high-dose groups were intravenously administered PEG-UHC 1, 2, and 4 mg/kg, respectively, at a constant rate (delivered using a microsyringe pump to control the delivery rate). Blood samples (1.5 mL) were subsequently drawn from the various groups at the following intervals: before (− 5 min) drug administration and at 5 min, 1 h, 6 h, 12 h, 24 h, 48 h, 72 h, 144 h, 192 h, 240 h, 288 h, 360 h, 432 h, and 504 h after drug administration.
3. *Multiple intravenous PEG-UHC injection group* Six animals from the single low intravenous dose PEG-UHC group described in (2) above were administered 0.5 mg/kg PEG-UHC at a constant rate (achieved using a microsyringe pump) 40 days after the initial intravenous injection. The drug was administered once every 15 days, with five sequential injections. For the first drug administration, 1.5 mL blood samples were collected at the following intervals: before (− 5 min) drug administration and at 5 min, 1 h, 6 h, 12 h, 24 h, 48 h, 72 h, 144 h, 192 h, 240 h, 288 h, and 360 h after drug administration. For the last (fifth) drug administration, 1.5 mL blood samples were collected at the following intervals: before (− 5 min) drug administration and at 5 min, 1 h, 6 h, 12 h, 24 h, 48 h, 72 h, 144 h, 192 h, 240 h, 288 h, 360 h, and 504 h after drug administration. For the middle three administrations, 1.5 mL blood samples were collected before (− 5 min) drug administration and at 5 min after drug administration; these samples were used to determine the minimum and maximum plasma concentrations (C_{min} and C_{max}), respectively.

Total blood was drawn from the forelimb veins of the animals in all three groups, and 1.5 mL were transferred to vacuum blood collection tubes containing heparin sodium to suppress clotting. The blood was then centrifuged at 3000 rpm for 10 min at 4 °C to isolate the plasma, which was collected and stored at − 80 °C for subsequent determination of plasma drug concentrations.

2.4 Preparation of Quality Control (QC) Samples

PEG-UHC and UHC samples were combined with the appropriate amounts of blank plasma to prepare QC samples for each test drug at concentrations of 2.5, 10, and 20 mg/L, which were then stored at − 80 °C. Each analysis of samples from the PEG-UHC, UHC, and control groups included a total number of PEG-UHC and UHC QC samples, respectively, that was not < 5% of the total number of samples analyzed.

2.5 Analytical Method

Based on the rationale that PEG-UHC catalyzes the oxidation of UA, a validated high-performance liquid chromatography (HPLC) method was used to determine the amount of UA depleted by PEG-UHC or UHC and thus also calculate the PEG-UHC or UHC content [21, 22]. The HPLC system used was a Waters 2695 Alliance with the Empower 2 management system and a Globalsil™ octadecylsilane (ODS)-BP hydrophilic reversed-phase chromatography column (4.6 mm × 250 mm). The mobile phase consisted of methanol:phosphate buffer:water (60:6:934, v/v/v), the flow rate was 1.0 mL/min, and the column temperature was 25 °C. A 2998 photodiode array (PDA) detector was used at a detection wavelength of 293 nm. Frozen plasma samples were retrieved from storage at − 80 °C, allowed to thaw to room temperature, and then 50 µL of each sample were pipetted into a 1.5 mL Eppendorf tube. The samples were preheated in a 37 °C water bath for 10 min and 175 µL (500 µM) of the UA solution that had been preheated under the same conditions were added. Vortexing was performed for 5 s, and then the samples were immediately placed in a water bath at 37 °C to initiate the reaction. After 5 min of incubation, 100 µL of perchloric acid (1 M) were added to terminate the reaction, the sample was centrifuged at 13,000 rpm for 10 min, the supernatant was collected, and then 20 µL were injected into the HPLC for analysis.

The analytical methods used for the PEG-UHC and UHC were validated separately. For the PEG-UHC, the 4 mg/mL PEG-UHC standard stock solution was diluted with blank plasma to prepare calibration standards with final concentrations of 0, 1, 5, 10, 15, and 20 mg/L. The PEG-UHC calibration standards were pretreated using the same method used for the plasma samples, and the peak area of UA was determined after PEG-UHC hydrolysis using HPLC. The difference in peak areas between 0 mg/L PEG-UHC and each of the calibration standards was equivalent to the UA hydrolyzed by PEG-UHC at the corresponding concentration. The PEG-UHC concentration (x-axis) was plotted against the peak area of UA hydrolyzed by PEG-UHC (y-axis), and the weighted least squares ($W = 1/CC$) method was used to conduct a regression analysis. A plot of PEG-UHC concentration versus peak area of UA hydrolyzed by PEG-UHC was fitted using a linear equation in the concentration range 1–20 mg/L. Each point was established from an average of ten determinations. Correlation coefficients (r) were > 0.99% for the calibration curves, $y = 6830.7 + 59{,}978.8x$ ($r = 0.999$). The lower limit of quantitation (LOQ) of PEG-UHC was 1 mg/L. The intraday and interday precisions were

calculated from the RSD of five intraday measurements and the RSD of five interday measurements, respectively, while the accuracy was calculated as the level of agreement between the test value and the true value (expressed as %). Within the concentration range 1–20 mg/L, the intraday precision and accuracy (relative recovery rate) of PEG-UHC measurements ranged from 0.54 to 3.92% and from 93.66 to 104.25%, respectively, while the interday precision and accuracy ranged from 1.76 to 7.51% and from 94.70 to 101.42%, respectively. At the LOQ (1 mg/L), the intraday precision and accuracy were 10.63% and 82.56%, while the interday precision and accuracy were 11.24% and 97.95%, respectively. A plot of UHC concentration versus peak area of the UA hydrolyzed by PEG-UHC was fitted using a linear equation in the concentration range 1–20 mg/L. Each point was established based on an average of ten determinations. Correlation coefficients (r) were > 0.99% for the calibration curves, $y = 28{,}424.40 + 73{,}752.64x$ ($r = 0.997$). The LOQ of UHC was 1 mg/L. The intraday and interday precisions were calculated from the RSD of five intraday measurements and the RSD of five interday measurements, respectively, while the accuracy was expressed as the relative recovery rate. In the concentration range 1–20 mg/L, the intraday precision and accuracy for UHC measurements ranged from 0.89 to 4.05% and from 90.91 to 109.68%, respectively, while the interday precision and accuracy ranged from 0.86 to 2.74% and from 91.39 to 106.95%, respectively. At the LOQ (1 mg/L), the intraday precision and accuracy were 5.08% and 101.93%, respectively, while the interday precision and accuracy were 7.43% and 94.38%, respectively.

2.6 Statistical Analysis

The pharmacokinetic parameters of each animal were calculated based on the noncompartmental model using the DAS3.1.6 statistical software, and the results were subsequently analyzed statistically. Intergroup comparisons were performed using one- or two-sided paired or unpaired t tests as required. The concentrations of the three QC samples (2.5, 10, and 20 mg/L) included in the plasma sample analyses were calculated based on the corresponding standard calibration curves, as mentioned in the previous section. The whole sample dataset was rejected if the measured QC sample concentrations deviated by > 15% for the medium and high concentrations or by > 20% for the low concentration.

3 Results

The average values of the pharmacokinetic parameters of cynomolgus monkeys administered a single intravenous injection of UHC or PEG-UHC are shown in Table 1. The average plasma drug concentration–time curve after a single 2 mg/kg UHC injection in the four control cynomolgus monkeys is shown in Fig. 1. The average plasma drug concentration–time curves of the three groups of cynomolgus monkeys administered a single intravenous injection of PEG-UHC at 1, 2, and 4 mg/kg are shown in Fig. 2. The $t_{1/2}$ of PEG-UHC in cynomolgus monkeys administered a single intravenous injection of PEG-UHC (2 mg/kg) was significantly longer than that of UHC. A comparison of the major pharmacokinetic parameters of cynomolgus monkeys that received an intravenous injection of PEG-UHC (2 mg/kg) with the corresponding parameters of monkeys that received UHC (2 mg/kg) is shown in Table 2.

Table 1 Pharmacokinetic parameters of cynomolgus monkeys after a single intravenous injection of uricase (UHC) at 2 mg/kg or a single intravenous injection of 5 kDa polyethylene glycol (PEG)-modified canine uricase (PEG-UHC) at 1, 2, or 4 mg/kg

Parameter	Unit	UHC (mg/kg)	PEG-UHC (mg/kg)		
		2	1	2	4
AUC_{0-t}	mg·h/L	127.38 ± 25.46	4181.28 ± 519.66	8209.34 ± 797.95	15,689.04 ± 1122.89
$AUC_{0-\infty}$	mg·h/L	130.56 ± 25.84	4982.48 ± 844.20	9163.12 ± 1338.34	17,329.00 ± 1385.49
MRT_{0-t}	h	1.74 ± 0.41	167.52 ± 5.15	152.52 ± 15.32	143.12 ± 16.19
$MRT_{0-\infty}$	h	1.86 ± 0.53	265.04 ± 53.00	211.52 ± 60.67	195.64 ± 60.33
$t_{1/2z}$	h	1.14 ± 0.36	190.24 ± 49.68	143.40 ± 71.90	132.84 ± 67.24
T_{max}	h	0.11 ± 0.084	0.072 ± 0.000	1.008 ± 0.000	1.01 ± 0.000
CL_z	L/h/kg	0.016 ± 0.0033	0.00021 ± 0.000037	0.00022 ± 0.000034	0.00023 ± 0.000023
V_z	L/kg	0.03 ± 0.00	0.06 ± 0.01	0.04 ± 0.02	0.04 ± 0.02
C_{max}	mg/L	60.39 ± 5.89	29.35 ± 4.00	58.58 ± 5.08	120.38 ± 11.30

Data are presented as the mean ± standard deviation (SD)

AUC_{0-t} area under the concentration–time curve from time 0 to time t, $AUC_{0-\infty}$ area under the concentration–time curve from time 0 to infinity, MRT_{0-t} mean residence time from time 0 to time t, $MRT_{0-\infty}$ mean residence time from time 0 to infinity, $t_{1/2z}$ plasma half-life, T_{max} time to maximum plasma drug concentration, CL_z clearance, V_z volume of distribution, C_{max} maximum plasma drug concentration

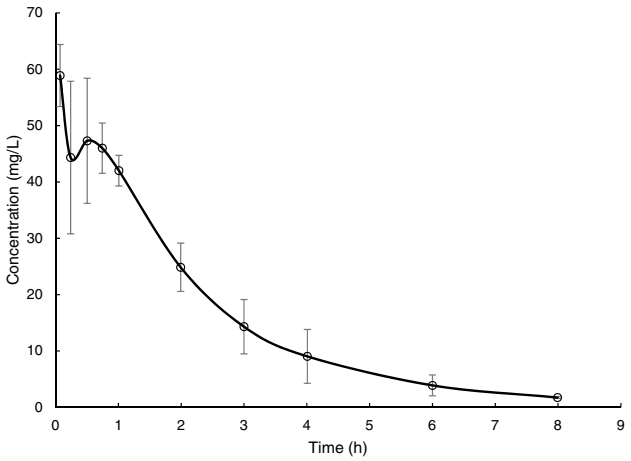

Fig. 1 Mean plasma drug concentration–time curve for cynomolgus monkeys after a single intravenous injection of canine uricase (UHC) at 2 mg/kg. Each *point* represents the mean value and each *bar* depicts the standard deviation

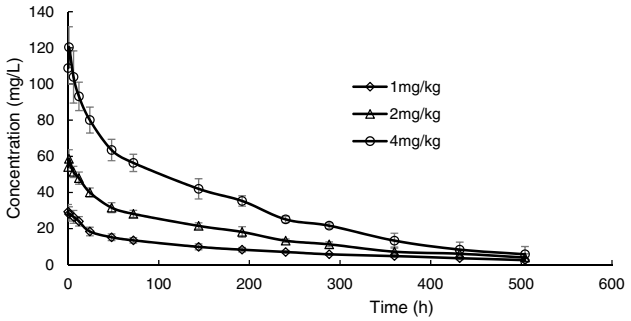

Fig. 2 Mean plasma drug concentration–time curves for cynomolgus monkeys after a single intravenous injection of 5 kDa polyethylene glycol (PEG)-modified canine uricase (PEG-UHC) at 1, 2, or 4 mg/kg. Each *point* represents the mean value and each *bar* depicts the standard deviation

Intravenous injections of the same dose (2 mg/kg) of PEG-UHC and UHC in cynomolgus monkeys showed an average $t_{1/2}$ of 143.40 ± 71.90 and 1.14 ± 0.36 h, respectively, with the former being 125.79 times longer than the latter. In addition, the AUC_{0-t} values of the PEG-UHC and UHC groups were 8209.34 ± 797.95 and 127.38 ± 25.46 mg·h/L, respectively, with the former being 64.45 times larger than the latter. The CL values of the PEG-UHC and UHC groups were 0.00022 ± 0.000034 and 0.016 ± 0.0033 L h/kg, respectively, with the former being 1/72.73 of the latter. The C_{max} values for the PEG-UHC and UHC groups were 58.58 mg/L and 60.39 mg/L, respectively, which were not statistically significantly different. These findings show that modifying UHC with 5 kDa PEG extends its $t_{1/2}$, reduces its CL, and increases the amount of drug circulating in the body, leading to a longer duration of action.

The correlation curve of the AUC versus the dose after a single intravenous injection of PEG-UHC in cynomolgus monkeys is shown in Fig. 3. The correlation curve of C_{max} versus the dose after a single intravenous injection of PEG-UHC in cynomolgus monkeys is shown in Fig. 4. Figures 3 and 4 show that the average area under the concentration–time curve from 0 to 21 days (AUC_{0-21d}) and C_{max} were positively correlated with the dose administered in all three groups ($r^2 > 0.99$). The plasma $t_{1/2}$ and CL values of the three groups were similar (Table 1), with no statistically significant differences, suggesting that these pharmacokinetic characteristics are linear.

Average pharmacokinetic parameter values of cynomolgus monkeys after the first and last intravenous injections of 0.5 mg/kg PEG-UHC are shown in Table 3. There was no statistically significant difference between the plasma $t_{1/2}$ values after the first and last injections (151.44 ± 28.06 h and 190.84 ± 30.66 h, respectively, $p > 0.05$), suggesting that sequential drug administration did not lead to faster drug clearance in the body. The C_{min} values of all five injections are shown in Table 4. A comparison of the five C_{min} values indicates that the C_{min} value of the fifth injection was not statistically significantly different from that of the fourth injection, suggesting that a steady state was reached.

Table 2 Comparison of major pharmacokinetic parameters between cynomolgus monkeys administered a single intravenous injection of uricase (UHC, 2 mg/kg) and monkeys administered a single intravenous injection of 5 kDa polyethylene glycol (PEG)-modified canine uricase (PEG-UHC, 2 mg/kg)

Parameter	Unit	UHC	PEG-UHC	PEG-UHC/UHC	*P* value
AUC_{0-t}	mg·h/L	127.38 ± 25.46	8209.34 ± 797.95	64.45	< 0.01
$t_{1/2z}$	h	1.14 ± 0.36	143.40 ± 71.90	125.79	< 0.01
CL_z	L/h/kg	0.016 ± 0.0033	0.00022 ± 0.000034	0.014	< 0.01
C_{max}	mg/L	60.39 ± 5.89	58.58 ± 5.08	0.97	0.62

Data are presented as the mean ± standard deviation (SD)

AUC_{0-t} area under the curve from time 0 to time *t*, $t_{1/2z}$ plasma half-life, CL_z clearance, C_{max} maximum plasma drug concentration

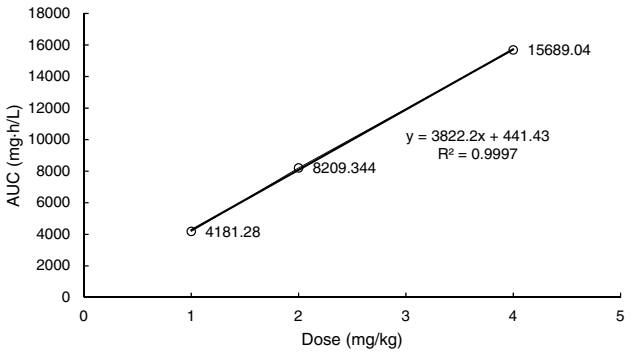

Fig. 3 Correlation curve of the area under the plasma concentration–time curve (AUC) versus the dose after a single intravenous injection of 5 kDa polyethylene glycol (PEG)-modified canine uricase (PEG-UHC) in cynomolgus monkeys

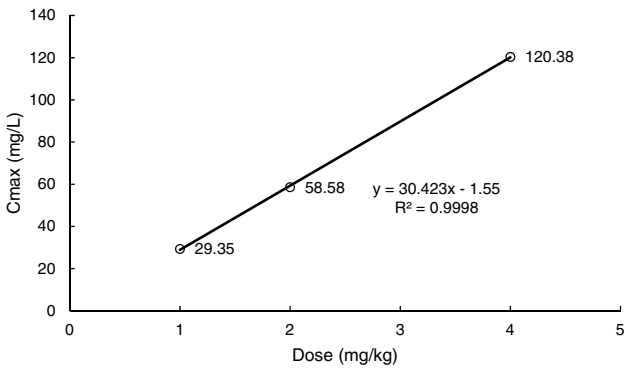

Fig. 4 Correlation curve of the maximum plasma concentration (C_{max}) versus the dose after a single intravenous injection of 5 kDa polyethylene glycol (PEG)-modified canine uricase (PEG-UHC) in cynomolgus monkeys

Table 3 Average pharmacokinetic parameter values of cynomolgus monkeys after the first and last intravenous injections of 5 kDa polyethylene glycol (PEG)-modified canine uricase (PEG-UHC, 0.5 mg/kg)

Parameter	Unit	First injection	Last injection
AUC_{0-t}	mg·h/L	1540.32 ± 192.55	2690.28 ± 401.65
$AUC_{0-\infty}$	mg·h/L	1963.80 ± 310.11	3163.40 ± 403.47
$t_{1/2z}$	h	151.44 ± 28.06	190.84 ± 30.66
T_{max}	h	0.54 ± 0.51	0.384 ± 0.48
C_{max}	mg/L	13.97 ± 2.06	18.00 ± 1.84
CL_z	L/h/kg	0.00026 ± 0.000049	0.00016 ± 0.000017

Data are presented as the mean ± standard deviation (SD), $n = 6$

AUC_{0-t} area under the curve from time 0 to time t, $AUC_{0-\infty}$ area under the concentration–time curve from time 0 to infinity, $t_{1/2z}$ plasma half-life, T_{max} time to maximum plasma drug concentration, C_{max} maximum plasma drug concentration, CL_z clearance

4 Discussion

The main purpose of this study of multiple intravenous injections of PEG-UHC in cynomolgus monkeys was to investigate the effect of the long-term continuous administration of PEG-UHC on its metabolic characteristics. Therefore, the pharmacokinetic characteristics of the first and last doses were of particular interest [23–25]. During the intermediate administration phase, only the peak and valley concentrations of the drug were monitored. This reduced the amount of blood collected, avoiding animal discomfort and reducing the impact on normal drug metabolism.

After analyzing the data in Tables 3 and 4, it is reasonable to assume that the cynomolgus monkeys produced few anti-uricase IgG antibodies after continuous intravenous injection of PEG-UHC (0.5 mg/kg), meaning that the drug was not cleared rapidly. Although PEG-UHC has a longer retention time in the body than UHC, it still requires long-term medication, which will definitely reduce patient compliance. Compared with intravenous injection, subcutaneous injection is safer and more convenient. A phase I clinical trial of pegloticase included subcutaneous administration studies, but subcutaneous administration was found to lead to low bioavailability and more severe infusion reactions than intravenous administration [10]. Continuous intravenous injection of PEG-UHC (0.5 mg/kg) did not cause accelerated drug clearance, which indicates that PEG-UHC has low immunogenicity. Whether PEG-UHC can be used for subcutaneous injection needs to be confirmed. Therefore, further investigation of the bioavailability and pharmacokinetic characteristics of PEG-UHC administered subcutaneously is needed.

5 Conclusion

Compared with that of UHC, the elimination half-life of PEG-UHC in cynomolgus monkeys was found to be significantly prolonged. The pharmacokinetics of PEG-UHC injected into cynomolgus monkeys were linear for doses in the range 1–4 mg/kg. The plasma concentration of PEG-UHC in cynomolgus monkeys reached a steady state after five sequential intravenous injections of PEG-UHC (0.5 mg/kg; one injection every 15 days). Rapid drug clearance was not observed, and PEG-UHC showed low immunogenicity. These findings suggest that clinical trials of PEG-UHC will be worthwhile.

Table 4 Individual and mean (SD) minimum plasma concentration (C_{min}, mg/mL) values for cynomolgus monkeys after multiple intravenous injections of 5 kDa polyethylene glycol (PEG)-modified canine uricase (PEG-UHC, 0.5 mg/kg)

Administration no.	L1 (♀)	L2 (♀)	L3 (♀)	L4 (♂)	L5 (♂)	L6 (♂)	Mean	SD
1st	0	0	0	0	0	0	0.00	0.00
2nd	1.70	1.77	1.20	0.00	1.76	1.73	1.36**	0.70
3rd	2.24	2.56	1.97	0.00	1.15	1.74	1.61*	0.92
4th	2.85	3.55	2.71	1.90	3.44	2.81	2.88**	0.59
5th	3.15	3.85	2.99	3.22	2.62	3.25	3.18*	0.40

$n=6$; *$p>0.05$ and **$p<0.05$ compared with the average C_{min} after the previous injection

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Compliance with Ethical Standards

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Conflict of Interest Haigang Li, JingJing Huo, Dan Sun, Liang Jiang, Chunlan Hu, Yanmin Bai, Xuefeng Ma, Haijuan Zhang, Xiaowei Shi, Zhilong Zhao, Jinchuan Zhou, Yongxin Lu, and Chun Zhang declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving animals were in accordance with the ethical standards of the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. The Ethical Committee of Linyi University approved the experimental protocol (approval record no. 2015L061002).

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