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A New Practical System for Evaluating the Pharmacological Properties of Uricase as a Potential Drug for Hyperuricemia

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The use of uricase-deficient mammals to screen formulations of engineered uricases as potential drugs for hyperuricemia involves heavy costs and presents a technical bottleneck. Herein, a new practical system was investigated to evaluate the pharmacological significance of a bacterial uricase based on its ability to eliminate uric acid in plasma *in vitro*, its pharmacokinetics *in vivo* in healthy rats, and the modeled pharmacodynamics *in vivo*. This uricase, before and after modification with the monomethyl ether of poly(ethylene glycol)-5000, effectively eliminated uric acid *in vitro* in rabbit plasma, but its action was susceptible to xanthine inhibition. After intravenous injection of the modified uricase without purification, a bi-exponential model fit well to uricase activities *in vivo* in the plasma of healthy rats; the half-life of the modified uricase was estimated without interference from the unmodified uricase leftover in the sample and was nearly 100-fold longer than that of the unmodified uricase. Using a model of the elimination of uric acid *in vivo* taking into account of uricase pharmacokinetics and xanthine inhibition, modeled pharmacodynamics supported that the half-life of uricase and its susceptibility to xanthine are crucial for the pharmacological significance of uricase. Hence, this practical system is desirable for doing preliminary screening of formulations of engineered uricases as potential drugs for hyperuricemia.

Key words: Hyperuricemia, Uricase, Pharmacokinetics, Pharmacodynamics, Half-life time, Mathematic modeling

INTRODUCTION

The human body lacks uricase, and uric acid of lower solubility (the end-product of purine degradation) is eliminated mainly through the kidneys (Ramazzina et al., 2006). Increase in uric acid production and/or decreases in uric acid excretion cause(s) elevated levels of uric acid in plasma, i.e., hyperuricemia. Hyperuricemia is usually associated with gout and tumor lysis syndrome. Hyperuricemia aggravates many diseases including gout, kidney dysfunction and cardiovascular diseases (Davidson et al., 2004; Kang and Nakagawa, 2005; Feig et al., 2008; Mene and Punzo, 2008; Bandukwala et al., 2009). To handle hyperuricemia, inhibitors of xanthine oxidase and/or those of uric acid reabsorption are widely used but are unsatisfactory (Bomalaski and Clark, 2004; Chohan and Becker, 2009), and they are inapplicable to refractory gout (Sundy et al., 2007; Sherman et al., 2008). Nonetheless, uricase is able to convert uric acid into allantoin, which has higher solubility and no obvious adverse actions. Thus, uricase becomes a promising drug for handling hyperuricemia (Bomalaski and Clark, 2004; Jeha et al., 2005; Sundy et al., 2007; Bertrand et al., 2008; Biggers and Scheinfeld, 2008; Sherman et al., 2008).

The principal determinants of the pharmacological significance of a uricase as a drug include its ability to eliminate uric acid from plasma, its half-life, and its

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immunogenicity, all *in vivo* (Jeha et al., 2005; Sundy et al., 2007; Bertrand et al., 2008; Biggers and Scheinfeld, 2008; Sherman et al., 2008; Yue et al., 2008). Uricases usually have an optimum pH over 8.5, and these enzymes thus invariably exhibit low activities in plasma of neutral pH (Bomalaski et al., 2002; Zhao et al., 2006). Hyperuricemia is associated with elevated plasma levels of xanthine, a potent competitive inhibitor of most uricases (Baum et al., 1956; Fridovich, 1965; Sedor and Sander, 1977; Hande et al., 1979; Liao et al., 2005). Protein drugs require modification with hydrophilic polymers to diminish their immunoreactivity and prolong their half-life *in vivo*, but uricases show different sensitivity to such modifications (Bomalaski et al., 2002; Zhang et al., 2010). Therefore, molecular engineering of uricases is desired to enhance their pharmacological significance, and efficient evaluations of the pharmacological properties of formulations of engineered uricases as potential drugs for hyperuricemia are required.

Currently, uricase-deficient mammals are usually used for the preclinical *in vivo* evaluation of uricases as potential drugs for hyperuricemia, but both the high cost and the technical bottleneck limit their routine use (Wu et al., 1994; Kelly et al., 2001). Classical hyperuricemic mammals have been developed using potent uricase inhibitors and used for the evaluation of drugs for hyperuricemia (Johnson et al., 1969; Stavric and Nera, 1978; Nguyen et al., 2005). However, uricases are sensitive to such inhibitors in classical hyperuricemic mammalian models (Baum et al., 1956; Fridovich, 1965; Johnson et al., 1969; Sedor and Sander, 1977; Stavric and Nera, 1978; Hande et al., 1979; Nguyen et al., 2005), which theoretically invalidates the use of classical hyperuricemic mammalian animals for evaluating the pharmacological significance *in vivo* of uricases as potential drugs. Therefore, new strategies are needed for the efficient determination of the pharmacological significance of formulations of engineered uricases for handling hyperuricemia.

Herein, we developed a new practical system for evaluating the pharmacological significance of a bacterial uricase modified with monomethyl polyglycol-ethylene glycol 5000 (mPEG5k). The evaluation is based on (1) the ability of the test drug to eliminate uric acid *in vitro* in rabbit plasma, (2) its pharmacokinetics *in vivo* in healthy rats, and (3) the modeled pharmacodynamics *in vivo*. Our results support the view that this practical system is desirable for screening formulations of engineered uricases as potential drugs for hyperuricemia.

MATERIALS AND METHODS

Animals

New Zealand rabbit and male SD rats were obtained from the animal center of our university. Animals were maintained in air-conditioned housing. All experiments were approved by the Bioethics Commission of our University.

Chemicals

Uric acid and diethyltriaminepentaacetic acid (DETAPA) were from Alfa Aesar. DEAE-cellulose was from Whatman (Kent, ME14 2LE). Monomethyl ether of poly(ethylene glycol)-5000 (mPEG5k) was from Sigma-Aldrich. N-hydroxylsuccinimide (NHS), dicyclohexylcarbodiimide (DCC) and other chemicals were domestically obtained analytical reagents.

Determination of uricase activity

Sodium borate buffer (100.0 mM) plus 0.10 mM DETAPA (pH 9.2) were pre-incubated at 25°C (this buffer was used throughout unless stated otherwise) and the final uric acid concentration was 75.0 μM (Zhao et al., 2006). After addition of a uricase sample of 150 μL to initiate the reaction, absorbance at 293 nm of a reaction mixture in 1.20 mL was recorded at 5-s intervals on a spectrophotometer linked to a computer (Liao et al., 2009). The classical initial rate was the averaged rate of absorbance decrease from 50 to 180 s after the addition of a uricase sample. One unit of uricase was defined as the amount of enzyme activity that was capable of oxidizing one micromole of uric acid per min at 25°C (calculated using an absorptivity of 11.5 $mM^{-1}\cdot cm^{-1}$ for uric acid). The ratio of the maximal reaction rate (V_m) to the Michaelis-Menten constant (K_m), V_m/K_m, indexed enzyme activity independent of substrate concentrations (Liao et al., 2001). For estimating V_m/K_m, uric acid concentrations were converted into absorbance with the preset absorptivity, and K_m was fixed at 0.22 mM in the integrated Michaelis-Menten rate equation for kinetic analyses of the elimination process of plasma uric acid *in vitro* (Liao et al., 2005, 2006; Zhao et al., 2006; Liu et al., 2009a). An executable subprogram written in Visual Basic 6.0 was used for kinetic analyses of the elimination of uric acid from plasma (Supplementary Materials).

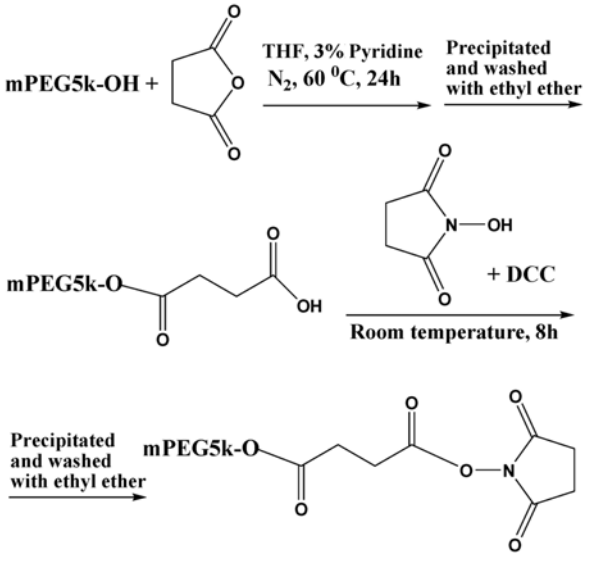
Preparation of uricase from *Bacillus fastidious*

For assaying uric acid, intracellular uricase from *Bacillus fastidious* A.T.C.C 29604 was purified by DEAE-Cellulose chromatography with 50 mM Tris-HCl buffer at pH 8.0 (Zhao et al., 2006). Eluted frac-

tions with specific activities over 5.0 $U \cdot mg^{-1}$ protein were pooled, and lyophilized after dialysis against sodium borate buffer plus DETAPA at pH 9.2. For the evaluation of its pharmaceutical properties, the uricase was purified via two consecutive DEAE-Cellulose chromatography columns, and eluted fractions with specific activities over 8.0 $U \cdot mg^{-1}$ protein were pooled, dialyzed against sodium borate buffer minus DETAPA, and lyophilized; this caused negligible contamination of proteins analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Activation of mPEG5000 and modification of uricase

mPEG5k (3 g, 0.6 mM) was reacted with succinic anhydride (0.12 g) in 100 mL tetrahydrofuran (THF) plus 3 mL pyridine under nitrogen atmosphere at 60°C for 24 h (Scheme 1). After removal of THF under reduced pressure, the product mPEG5k-succinic acid was repetitively precipitated and washed with large volumes of ethyl ether. Then the purified mPEG5k-succinic acid was dissolved in 50 mL THF followed by the consecutive addition of 0.173 g NHS and 0.31 g DCC. The resultant mixture was stirred at room temperature for 8 h before removal of insoluble materials. Then, the NHS ester of mPEG5k-succinic acid in THF was purified by repetitive precipitation after the wash with large volumes of ethyl ether, and then quantified via TLC using titration with N-(1-naphthyl)-ethylenediamine (yield was about 75%) (Kynclova et al., 1996; Zhang et al., 2010). A stock solution of the NHS ester of mPEG5k-succinic acid of 20 mM was prepared in THF. The NHS ester of mPEG5k-succinic acid (200 molar equivalents) was reacted with uricase (the molar equivalent was calculated using a molecular weight of 36 kDa for a subunit containing 17 lysines and one N-terminus residue, Genebank accession no. FJ393559) in 50 mM sodium borate at pH 9.2, followed by gentle stirring at 4°C for 30 min. Then, the mPEG5k-modified uricase was thoroughly dialyzed against 50 mM Tris-HCl buffer at pH 8.0 plus 100 mM NaCl (Zhao et al., 2009a). Estimating from protein bands by SDS-PAGE analysis, about 25% of the unmodified uricase was leftover in the preparation. This preparation, without purification, was used as the sample of the modified uricase throughout all following experiments.



Scheme 1. Activation of mPEG5k for linkage to amino groups on uricase

Measurement of uric acid

A kinetic uricase method based on the prediction of the end-point absorbance at 293 nm of the uricase reaction mixture was used (Liao et al., 2006; Zhao et al., 2006, 2009b). The final activity of uricase in the reaction mixtures was 0.04 $U \cdot L^{-1}$ at 25°C, which had negligible absorbance at 293 nm. To measure uric acid, sodium borate buffer was pre-incubated at 25°C. An aliquot of the reaction mixture (1.195 mL containing 100 μL neutralized sample) was transferred into a quartz cell having a 10 mm light path to measure the initial absorbance at 293 nm. Then, 5 μL of a concentrated solution of uricase was added into the quartz cell and mixed thoroughly just before recording the absorbance at 293 nm (done within 7.0 min at 5-s intervals). The end-point absorbance at 293 nm was predicted by kinetic analyses of the uricase reaction process with K_m fixed at 0.22 mM (Liao et al., 2006). The difference between the initial absorbance (the dilution effect for adding uricase solution was corrected) and the end-point absorbance indexed the net absorbance of uric acid in the reaction mixture. The subprogram to predict the end-point absorbance was incorporated into the software used above (Supplementary Material) (Liao et al., 2006; Liu et al., 2009a; Zhao et al., 2009b).

Uric acid-eliminating activity in rabbit plasma *in vitro*

Heparinized rabbit blood was collected from the heart to routinely prepare plasma. The ability of formulations of the bacterial uricase to eliminate uric acid *in vitro* in rabbit plasma was determined as follows. To 4.4 mL rabbit plasma at 37°C containing a given amount of a formulation of uricase, plus a uricase inhibitor as indicated, 0.60 mL of a suspension of uric acid in physiological saline at 37°C (over 20 mmol/L) was added for a final concentration of uric acid > 0.4 mM. To de-

termine residual uric acid at an indicated time, an aliquot of 0.2 mL plasma was withdrawn and the uricase reaction was terminated by precipitation of proteins with 5% perchloric acid. After centrifugation to remove the precipitates, the solution was neutralized with saturated potassium carbonate. Finally, after centrifugation at 4°C again to remove the precipitates, residual uric acid in the neutralized supernatant was determined as described above. To check the continued actions of uricases, 0.6 mL uric acid suspension was added to the leftover plasma again and samples were collected and processed as described above.

Pharmacokinetics of uricase in healthy rat

Male rats (230 ± 10 g) were used. Uricase formulations after removal of bacteria were administrated via intravenous injection. Five minutes after injection, blood from the rat orbital vein was taken at the indicated times and centrifuged at 3000 × *g* for 10 min at 4°C to obtain plasma, which was kept at 4°C for the assay of uricase activity within 2 h. The half-life of the unmodified uricase (t_1) was determined by kinetic analysis of the change of its activity in plasma *in vivo* using a single exponential model. The half-life of the uricase modified with mPEG5k (t_2) was estimated by kinetic analysis of the change of the total uricase activity in plasma *in vivo* with a bi-exponential model as Eq. (1) (Guo et al., 2007).

$$V_m(t) = V_{m1} \times \mathrm{Exp}(-\ln(2) \times t/t_1) + V_{m2} \times \mathrm{Exp}(-\ln(2) \times t/t_2) \quad (1)$$

At the beginning point of the analysis, the reaction time (t) was converted to zero. The maximal activity of the modified uricase (V_{m2}) plus the maximal activity of the unmodified uricase (V_{m1}) should be a constant determined experimentally. This left only t_1, t_2 and either V_{m1} or V_{m2} in Eq. (1) as parameters. The modified and unmodified uricases showed a consistent K_m (Zhang et al., 2010), and a constant was used to convert the classical initial rates into V_m. Therefore, the classical initial rates (V_i) were used directly as the maximal activities for kinetic analyses of the pharmacokinetics of uricase formulations. In Eq. (1), t_1 was permitted to fluctuate within ± 30% of the value that had been independently determined. During nonlinear adjustments of the three parameters in Eq. (1), a set of such parameters was accepted after the determination coefficient was > 0.999. The apparent distribution volume of uricase was calculated from the total activity of the uricase administrated, and the activity of uricase in plasma *in vivo* at the moment just after the injection, which was obtained via extrapolation according to Eq. (1). The subprogram for kinetic analyses of the change of uricase activities *in vivo* was combined into the executable program used above (Supplementary Materials) (Guo et al., 2007; Liu et al., 2009a; Zhao et al., 2009b).

Simulated pharmacodynamics *in vivo*

It was assumed that within 24 h, the levels of uric acid in human plasma *in vivo* were steady if no uricase was administrated, i.e., the rate of production of uric acid was equal to the rate of excretion of uric acid by the kidney. Most uricase inhibitors were competitive and uricase followed Michaelis-Menten kinetics (Fridovich, 1965; Bomalaski et al., 2002; Liao et al., 2005; Zhao et al., 2006). Therefore, the ability of uricase to lower plasma uric acid levels was simulated with Eq. (2), in which S was the instantaneous concentrations of plasma uric acid.

$$-\frac{dS}{dt} = (V_{m1} \times \mathrm{Exp}(-\ln(2) \times t/t_1) + V_{m2} \times \mathrm{Exp}(-\ln(2) \times t/t_2)) \times \frac{S}{K_m \times (1 + C_i/K_i) + S} \quad (2)$$

The K_m of uricase was competitively altered by xanthine (Fridovich, 1965; Liao et al., 2005; Zhao et al., 2006). Because of the complexity of Eq. (2), iterative numerical integration was used with an integration step of 0.5 min. Namely, uricase activities were calculated as Eq. (1) using the integration step of 0.5 min. Uricase activity was assumed to be a constant in order to calculate the plasma uric acid level according to Eq. (2) after such an integration step. To simulate the pharmacodynamics of the modified uricase after purification, V_{m1} was preset to zero. The subprogram for simulation was incorporated into the executable program used above (Supplementary Materials).

Protein quantification

The Bradford method was used to quantify protein concentrations using bovine serum albumin as the reference (Bradford, 1976).

Statistic analysis

Results are presented as mean ± S.D. Results were compared via Student's t-test with $p < 0.05$ indicating statistical significance.

RESULTS

Actions of uricase in plasma *in vitro*

After incubation in borate buffer at pH 9.2 for 6 h at 37°C, the unmodified uricase retained about 90% of its original activity, and the mPEG5k-modified uricase retained more than 90% of its original activity. The half-life was (45 ± 3) h (n = 3) for the unmodified uri-

case, and (92 ± 6) h (n = 3) for the modified uricase.

During repetitive addition of uric acid in batch mode to mimic the situation *in vivo*, the unmodified uricase and the mPEG5k-modified uricase continuously eliminated uric acid from rabbit plasma *in vitro* at 37°C (Fig. 1A and B). The addition of xanthine or oxonate significantly reduced the rates to eliminate plasma uric acid *in vitro* (Fig. 1C and D).

By kinetic analysis of the elimination process of uric acid *in vitro* in rabbit plasma, activities of the modified and unmodified uricase *in vitro* in rabbit plasma, indexed by V_m/K_m, were found to be consistent with those for the same amounts of the two uricase formulations determined at pH 7.4. After continued action at 37°C for 6 h in rabbit plasma *in vitro*, the decreases in V_m/K_m of the unmodified and modified uricase were basically consistent with those estimated using the half-life at 37°C (Fig. 1A and B).

Pharmacokinetics of uricase in healthy rat *in vivo*

In plasma of healthy rats *in vivo*, uricase activity was undetectable and the levels of uricase-inhibiting substances were steady over two days at an amount close to 10 μM oxonate (data not shown). Determined independently in healthy rats, the half-life of the unmodified uricase *in vivo* was 0.26 ± 0.05 h (n = 5) by kinetic analysis using a single exponential model (Fig. 2A). The half-life was less than 1% of that at 37°C *in vitro* and clearly supported roles for some biological mechanisms for fast clearance of the unmodified uricase *in vivo* in rat. The fitting of the bi-exponential model to activities of the mPEG5k-modified uricase (without purification) in rat plasma gave a half-life of 26 ± 10 h (n = 5) for the modified uricase *in vivo*, and a half-life of (0.32 ± 0.09) h (n = 5) for the unmodified uricase *in vivo*. This was consistent with values determined independently (Fig. 2B). After the contribution of the unmodified uricase left in the sample of the modified uricase was corrected according to its pharmacokinetics, kinetic analysis of the decrease of uricase activities in plasma with a single exponential model gave a consistent half-life for the modified uricase *in vivo*.

Based on the total activity of uricase administrated

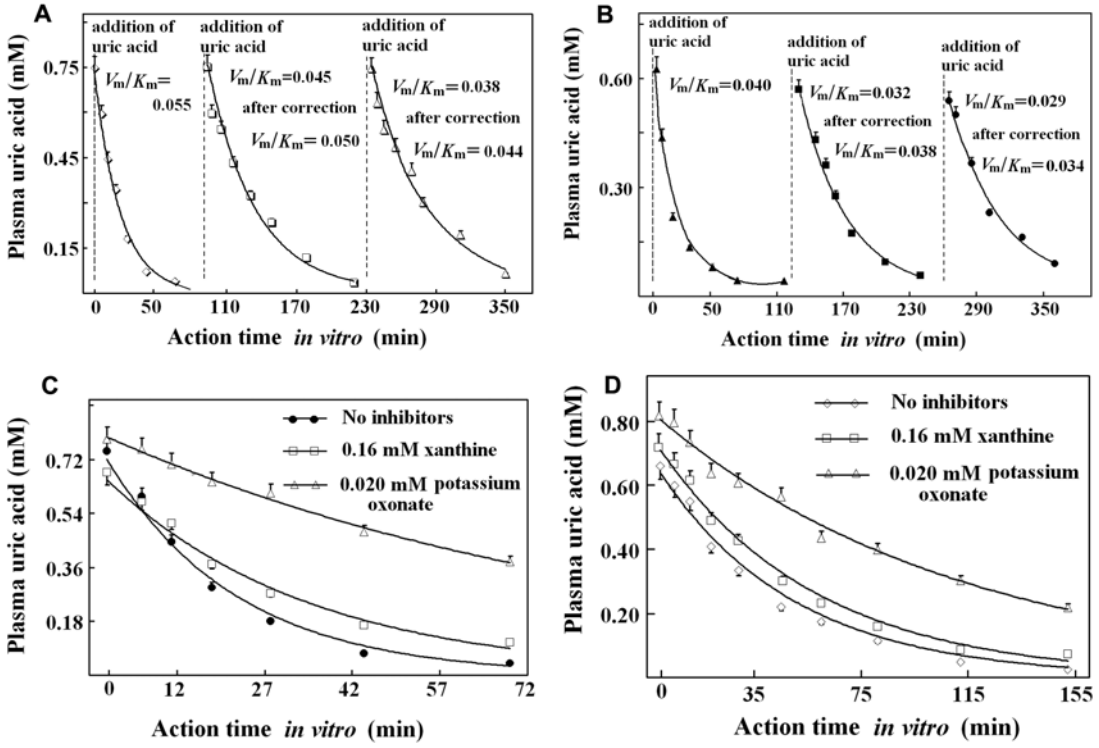


Fig. 1. Elimination of uric acid *in vitro* in rabbit plasma by uricase. (**A**) Efficacy of the unmodified uricase during repetitive addition of uric acid. Dilution effects of the addition of uric acid suspension on the estimation of V_m/K_m (min^{-1}) were corrected. (**B**) Efficacy of mPEG5k-modified uricase during repetitive addition of uric acid. Dilution effects of the addition of uric acid suspension on the estimation of V_m/K_m (min^{-1}) were corrected. (**C**) Efficacy of the unmodified uricase in the presence of 0.16 mM xanthine (prepared in rabbit plasma) or 0.020 mM potassium oxonate. The final activity of the uricase was about 120 $U \cdot L^{-1}$ at pH 9.2, and was about 20 $U \cdot L^{-1}$ at pH 7.4 in plasma. (**D**) Efficacy of the mPEG5k-modified uricase in the presence of 0.16 mM xanthine (prepared in rabbit plasma) or 0.020 mM potassium oxonate. The final activity of the uricase was about 120 $U \cdot L^{-1}$ at pH 9.2, and about 20 $U \cdot L^{-1}$ at pH 7.4 in plasma.

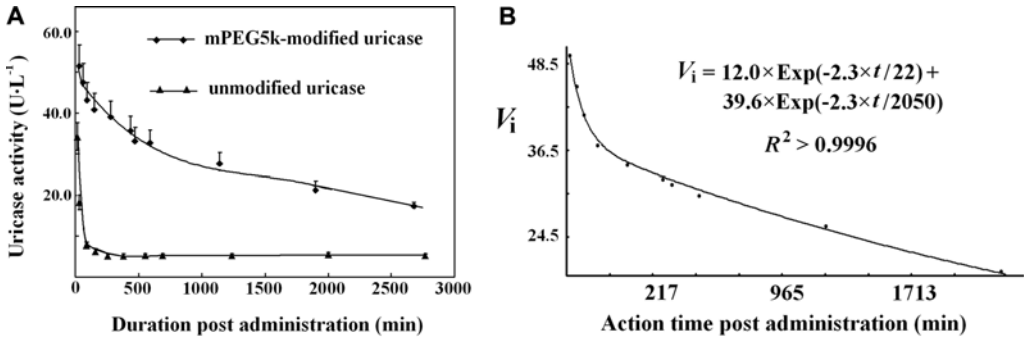


Fig. 2. Pharmacokinetics of uricases in healthy rats. Pharmacokinetics of uricases in plasma of healthy rats *in vivo*. Data are the mean from five healthy rats; vertical bars index standard deviations. Fitting of a bi-exponential model to activities in a rat *in vivo* of plasma uricase after injection of mPEG5k-modified uricase.

via intravenous injection and the activity of uricase in plasma calculated by extrapolation using the bi-exponential model, the apparent distribution volume of the modified uricase *in vivo* was 10 ± 1 mL (n = 5), which was consistent with expectations for a protein drug in plasma.

Modeled pharmacodynamics of uricase

Using a specified model for the pharmacokinetics of uricase formulations in plasma *in vivo*, the dynamic changes of plasma uric acid under the action of a uricase formulation were easily simulated by numerical integration. The use of a uricase at higher activities caused faster clearance of plasma uric acid. The efficacy of a uricase was stronger if it had a smaller K_m and a stronger resistance to xanthine (Fig. 3). There were negligible actions of most eukaryotic uricases in classical hyperuricemic rats at oxonate concentrations of about 0.10 mM because the inhibition constant of oxonate on this bacterial uricase was 8 μM, but was below 1 μM for most fungal uricases (data not shown).

To reduce plasma uric acid from 0.6 mM to 0.1 mM, any uricase with a smaller K_m was preferred for the same therapeutic efficacy. Eukaryotic uricases usually exhibited a lower K_m but a higher sensitivity to xanthine (Baum et al., 1956; Fridovich, 1965; Sedor and Sander, 1977; Hande et al., 1979; Liao et al., 2005). In the presence of plasma xanthine levels close to 0.16 mM, which was close to the saturated concentration of xanthine in plasma *in vivo*, eukaryotic and prokaryotic uricases showed comparable therapeutic efficacy as long as their maximum activities in plasma were

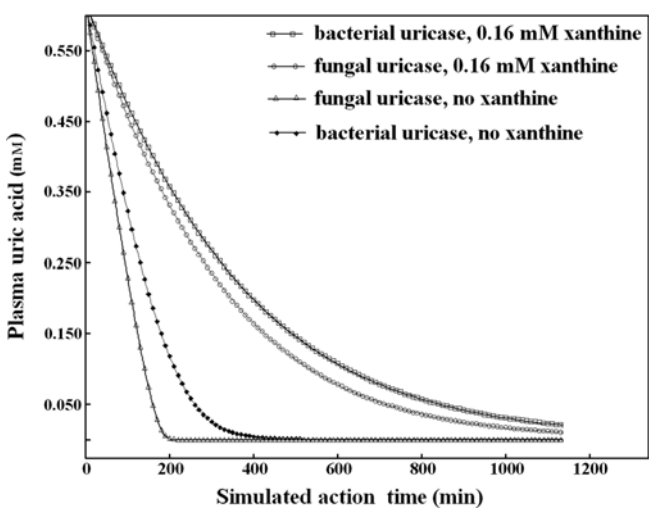


Fig. 3. Modeled pharmacodynamics of uricase after a single injection. The fungal uricase was assigned a K_m of 35 μM and the inhibition constant of xanthine was preset at 6.0 μM (Liao et al., 2005). The bacterial uricase was assigned a K_m of 0.220 mM and the inhibition constant of xanthine was preset at 40.0 μM (Zhao et al., 2006). The maximal activities of these two uricases were fixed at 5.0 U·L^{-1} in plasma at pH 7.4.

Table I. Simulated effects of xanthine on minimal action durations to reduce plasma uric acid from 0.60 mM to 0.10 mM by a bacterial uricase (K_m was 0.220 mM; the inhibition constant for xanthine was 40.0 μM) and a fungal uricase (K_m was 35 μM; the inhibition constant for xanthine was 6.0 μM) (Liao et al., 2005; Zhao et al., 2006). The maximum activities of the two uricases were fixed at 5.0 U·L^{-1} in plasma. Xanthine in plasma was 0.16 mM and the variation of the estimated minimal duration was within 10 min.

condition	K_m (mM)	Half-life (min)	Minimal duration (min)
No xanthine	0.220	2160	220
	0.035	2160	140
	0.220	600	250
	0.035	600	140
Xanthine (0.16 mM)	1.10	2160	630
	0.92	2160	560
	1.10	600	900
	0.92	600	730

comparable and the inhibition of xanthine as well as their pharmacokinetics were considered (Table I).

DISCUSSION

The actions of uricase and the potential effects of any uricase inhibitor (xanthine or oxonate) on the elimination of plasma uric acid were easily characterized *in vitro* in rabbit plasma. Obviously, the stronger inhibition on uricase by oxonate than that by xanthine resulted in a slower elimination rate of plasma uric acid *in vitro* in the presence of oxonate. To evaluate the pharmacokinetics of a protein drug *in vivo*, the monitoring of protein levels via either immunoreactivity or radioactive isotope labels was unreliable in cases where there was loss of protein function but no concomitant loss of its immunoreactivity or of the isotope labels. Because there were no detectable uricase activities in plasma from healthy rats, it was simple to determine the half-life time of a uricase formulation *in vivo* in healthy rats by monitoring changes of its activities in plasma. A mammalian uricase after modification with poly(ethylene glycol) (PEG) followed one-compartment pharmacokinetics in human plasma *in vivo* (Sundy et al., 2007; Yue et al., 2008). It is possible that there was a large difference in the half-life time of uricase before and after modification with mPEG5k. Results supported the view that the half-life of the modified uricase in the sample, after modification but without purification, was reliably estimated by kinetic analyses with the bi-exponential model. This model potentially has some advantages for evaluating a lot of uricase formulations because no laborious purification of the modified uricase is needed.

The half-life of the modified uricase *in vivo* was much longer than that of the unmodified uricase, but was still shorter than that at 37°C *in vitro*, supporting roles of some biological mechanisms in its clearance *in vivo*. The clearance of the unmodified uricase and the modified uricase *in vivo* in healthy rats should be due principally to nonspecific biological mechanisms such as scavenging by macrophages, hydrolysis by proteases, and filtration via the kidneys *in vivo*; it should involve few specific immune responses because these healthy rats were not immunized before experimentation. After repetitive injection of uricase formulations, specific immune responses may play important roles in their clearance *in vivo*. A mammalian uricase after modification with a long-chain mPEG still showed a half-life of about 3 d *in vivo* in human body after the first administration (Sundy et al., 2007; Yue et al., 2008). Usually the modification of a protein with long-chain mPEG can produce a longer half-life *in vivo*. Therefore, the half-life of this bacterial uricase *in vivo* was expected to be further increased after optimization of its modification with hydrophilic polymers, but the maximum of its half-life was expected to be its thermo-inactivation half-life at 37°C in plasma *in vitro*.

Modeled pharmacodynamics of uricase formulations supported the conclusion that the maximum activity in plasma, the resistance to xanthine, and the half-life *in vivo* of a uricase formulation are crucial for its pharmacological actions. Obviously, the continuous production of uric acid in patients *in vivo* made the half-life of different uricase formulations *in vivo* more crucial in maintaining the desirable low levels of plasma uric acid. Common eukaryotic uricases in plasma have retained about 50% of their maximum activities at optimum reaction pH (Bomalaski et al., 2002), but they exhibited low thermostability at 37°C and high sensitivity to xanthine (Conley and Priest, 1980; Liu et al., 2009b). The administration of uricase formulations at high doses resulted in an acute increase in hydrogen peroxide in plasma *in vivo* and caused deleterious side effects (Ducros et al., 1991; Browning and Kruse, 2005; Kizer et al., 2006; Borinstein et al., 2008). This property supports the use of uricase formulations with longer half-lives *in vivo* rather than higher activity for hyperuricemia. This bacterial uricase in plasma retained about 16% of its maximum activity; this percentage of the residual uricase activity at physiological pH was just about one-third of those with common eukaryotic uricases (Zhao et al., 2006; Zhang et al., 2010). Special delivery systems were available to alleviate the unfavorable effect of plasma pH on this bacterial uricase (Tan et al., 2010). Hence, this bacterial uricase shows promise as a drug for handling hyperuricemia.

To model the pharmacodynamics of uricase *in vivo*, the following factors should be considered at least, including (a) the production of uric acid by xanthine oxidase, (b) the excretion of uric acid via the kidneys, (c) the transportation of uric acid from tissue pools into plasma, and (d) the pharmacokinetics of a uricase formation *in vivo*. From the modeled pharmacodynamics, the roles of different properties of uricase formulations in their pharmacological significance to maintain desired levels of plasma uric acid for a required duration can be understood more clearly (Danhof et al., 2007, 2008; Sundy et al., 2007; Yue et al., 2008). Taken together, our practical system, which is based on (1) the ability of uricase formulations to eliminate uric acid from plasma *in vitro*, (2) the pharmacokinetics of uricase formulations *in vivo* in healthy animals, and (3) the modeled pharmacodynamics *in vivo*, is associated with much lower cost and greater efficiency

for preliminary screening of formulations of engineered uricases as potential drugs to handle hyperuricemia.

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