

# Pharmacokinetics and Pharmacodynamics of Glucarpidase Rescue Treatment After High-dose Methotrexate Therapy Based on Modeling and Simulation

TOSHIMI KIMURA<sup>1</sup>, YUTAKA FUKAYA<sup>2</sup>, YUKIHIRO HAMADA<sup>2</sup>,  
KENICHI YOSHIMURA<sup>3</sup> and HIROSHI KAWAMOTO<sup>4</sup>

<sup>1</sup>Department of Pharmacy, Juntendo University Hospital, Tokyo, Japan;

<sup>2</sup>Department of Pharmacy, Tokyo Women's Medical University Hospital, Tokyo, Japan;

<sup>3</sup>Center for Integrated Medical Research, Hiroshima University Hospital, Hiroshima, Japan;

<sup>4</sup>Department of Pediatric Oncology, National Cancer Center Hospital, Tokyo, Japan

**Abstract.** *Background:* Model-informed approaches are important in drug development, including for dose optimization and the collection of evidence in support of efficacy. *Materials and Methods:* We developed a modified Michaelis–Menten pharmacokinetics/pharmacodynamics model and used it to conduct simulations of glucarpidase at doses between 10 and 80 U/kg rescue treatment after high-dose methotrexate therapy. We carried out a dose-finding modeling and simulation study before a phase II study of glucarpidase. Monte-Carlo simulations were conducted using the deSolve package of R software (version 4.1.2). The proportion of samples in which the plasma methotrexate concentration was less than 0.1 and 1.0  $\mu\text{mol/l}$  at 70 and 120 h after methotrexate treatment was evaluated for each dosage of glucarpidase. *Results:* The proportion of samples in which the plasma methotrexate concentration was less than 0.1  $\mu\text{mol/l}$  at 70 h after methotrexate treatment was 71.8% and 89.6% at 20 and 50 U/kg of glucarpidase, respectively. The proportion of samples in which the plasma methotrexate concentration was less than 0.1  $\mu\text{mol/l}$  at 120 h after methotrexate treatment was 46.4% and 59.0% at 20 and 50 U/kg of glucarpidase, respectively. *Conclusion:* We determined a recommended glucarpidase dose of 50 U/kg to be ethically acceptable. A rebound in the serum concentration of methotrexate may be observed in many

patients after the administration of glucarpidase, and long-term monitoring (over 144 h) of the serum methotrexate concentration may be needed after the administration of glucarpidase. Its validity was confirmed in the phase II study and glucarpidase was approved for manufacturing in Japan.

Methotrexate is an antineoplastic agent for which the primary route of excretion is urine (1-3). Nonsteroidal anti-inflammatory drugs inhibit organic anion transporter-3, resulting in delayed elimination of methotrexate, which is excreted in the urine as an intact drug via this transporter (4). Other metabolites of methotrexate include 7-hydroxymethotrexate (less than 10% methotrexate) and 2,4-diamino-*N*-10-methylpteroic acid (less than 5% methotrexate) (1, 2, 5, 6). Severe side-effects have been reported in association with high doses of methotrexate (such as pancytopenia, nephrotoxicity, hepatotoxicity, and mucositis); moreover, treatment of these side-effects (for instance, through the use of leucovorin, dialysis, and plasmapheresis) has shown limited efficacy (7, 8).

Glucarpidase (CPG2) is an enzymatic drug (a homodimeric protein of 390 amino acids with a molecular weight of 83 kDa, isolated from *Pseudomonas* spp.) that directly hydrolyzes methotrexate into glutamic acid and 2,4-diamino-*N*-10-methylpteroic acid (9). Plasma methotrexate concentrations have been reported to decrease to less than 95% within 1 h of the administration of CPG2 (10-14).

CPG2 has been approved in Europe and the United States of America for use at a clinical dose of 50 U/kg. Although CPG2 has been reported to be safe and effective at a dose of 50 U/kg, dose-finding studies of CPG2 have not been conducted in humans. We previously conducted a phase I study in healthy Japanese volunteers to evaluate the safety and pharmacology of doses of 20 or 50 U/kg of CPG2 (identifier: JMA-IIA00078) (15). No dose-limiting toxicities or remarkable clinical findings were observed in an earlier phase I study of CPG2 administered at two dose levels (10, 16).

*Correspondence to:* Yutaka Fukaya, Department of Pharmacy, Tokyo Women's Medical University Hospital, 8-1 Kawada-cho, Shinjyuku-ku, Tokyo 162-8666, Japan. Tel.: +81 333538111, Fax: +81 352697385, e-mail: fukaya.yutaka@twmu.ac.jp

**Key Words:** Glucarpidase, methotrexate, modeling and simulation, pharmacokinetics, pharmacodynamics.



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We carried out a modeling and simulation study aimed to provide dosing recommendations before a phase II study of CPG2 is conducted in Japanese patients receiving methotrexate at high doses. Model-informed approaches are important for drug development, including for dose optimization and the collection of evidence in support of efficacy (17). Modeling and simulation are methods used to explore optimal drug doses using efficacy and safety information from different treatment arms. We developed a modified Michaelis–Menten pharmacokinetics (PK)/pharmacodynamics (PD) model and used the model to conduct simulations of CPG2 at doses of between 10 and 80 U/kg as a rescue treatment after high-dose methotrexate therapy in order to clarify the recommended dose (20 or 50 U/kg) of CPG2 for a future phase II study (including pediatric patients).

**Materials and Methods**

*Development of a modified Michaelis–Menten PK/PD model for the decomposition of methotrexate by CPG2.* CPG2–methotrexate PK/PD analysis was performed based on the known population PK properties of methotrexate. The default structural model was a two-compartment model (Figure 1) and the following differential equations were used:

$$\begin{aligned} dX_c/dt &= -(K_r + K_d + K_{12}) \times X_c + K_{21} \times X_p & \text{(Equation 1)} \\ dX_p/dt &= K_{12} \times X_c - K_{21} \times X_p \end{aligned}$$

In Equation 1,  $X_c$  and  $X_p$  are the quantities of methotrexate in the central and peripheral compartments, respectively.  $K_r$  and  $K_d$  are the elimination constants from the central compartment via renal excretion and the metabolic pathway, respectively.  $K_{12}$  and  $K_{21}$  are the intercompartmental constants between the central and peripheral regions, respectively. This differential equation represents the decomposition of methotrexate by CPG2 as a catalyst:  $dX_c/dt = -(K_d \times X_c)$ . CPG2 degrades methotrexate into its inactive metabolites. Using the Michaelis–Menten equation, the enzymatic properties of CPG2 were characterized as follows:

$$dX_c/dt = -V_{max} \times [X_c] / (K_m + [X_c]) \quad \text{(Equation 2)}$$

In Equation 2, the maximum degradation rate,  $K_m$ , is the Michaelis–Menten constant, and  $[X_c]$  is the substrate concentration.

Because  $V_{max}$  has units of mass/time, and the enzyme is in constant interaction with its substance, it can be exchanged based on the mass balance equation as follows:

$$V_{max} \text{ (mass/time)} = [\text{catalyst}] \times \alpha \text{ (mass/volume} \times \text{volume/time)} \quad \text{(Equation 3)}$$

In Equation 3, alpha converts the constant between  $V_{max}$  and the catalyst concentration into a patient-specific parameter similar to individual clearance.

Using a constant, alpha, and catalyst concentration, Equation 1 was rearranged with the modified Michaelis–Menten equation as follows:

$$dX_c/dt = -(K_r + K_{12}) \times X_c + K_{21} \times X_p - \alpha \times [\text{catalyst}] \times [X_c] / (K_m + [X_c]) \quad \text{(Equation 4)}$$

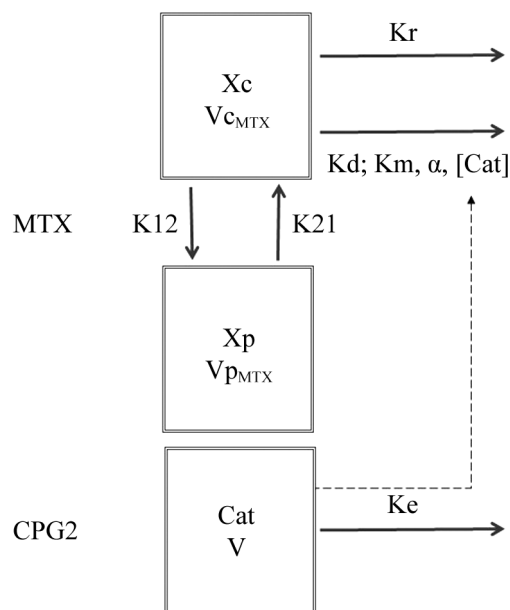


Figure 1. Structure of the modified Michaelis–Menten pharmacokinetics/pharmacodynamics model of methotrexate (MTX) decomposition by glucarpidase. Cat: Catalyst;  $K_{12}$ : distribution rate constant for transfer from central to peripheral;  $K_{21}$ : distribution rate constant for transfer from peripheral to central;  $K_d$ : Degradation rate constant;  $K_e$ : elimination rate constant of glucarpidase;  $K_m$ : Michaelis–Menten kinetics of methotrexate hydrolysis by glucarpidase;  $K_r$ : Renal elimination rate constant of methotrexate;  $V$ : volume of distribution of glucarpidase;  $V_{cMTX}$ : central compartment volume of distribution of methotrexate;  $V_{pMTX}$ : peripheral compartment volume of distribution of methotrexate;  $X_c$ : amount of central compartment of methotrexate;  $X_p$ : amount of peripheral compartment of methotrexate;  $\alpha$ : conversion constant.

*Simulation of PK/PD modeling of CPG2 rescue treatment after high-dose MTX therapy.* We carried out a dose-finding modeling and simulation study before a phase II study of CPG2. Monte Carlo simulations were conducted using the deSolve package of R software (version 4.1.2), based on the modified Michaelis–Menten PK/PD model for methotrexate decomposition by CPG2. The reported PK parameters of methotrexate and CPG2 were used in the simulations (Table I). The value of  $\alpha$  was fixed at  $V_{max}$  of 800 mol/s at 1 mol/l of CPG2 (18). After methotrexate treatment at a dose of 1 g/m<sup>2</sup> for 4 h, CPG2 was intravenously administered at a dose range of 10–80 U/kg. The PK profiles of 500 virtual patients were simulated using eight CPG2 doses of 10, 20, 30, 40, 50, 60, 70 and 80 U/kg. In this simulation, virtual patients were assumed to have a weight of 60 kg, body surface area of 1.73 m<sup>2</sup>, creatinine clearance of 80 ml/min, and methotrexate clearance of 10%.

**Results**

*Monte-Carlo simulations using the modified Michaelis–Menten PK/PD model of CPG2 rescue treatment after high-dose methotrexate therapy.* The PK parameters used in the Monte-Carlo simulations are shown in Table II. Monte-Carlo

Table I. Pharmacokinetic parameters of methotrexate (MTX) and glucarpidase (CPG2).

Drug (Reference)	Pharmacokinetic parameters	Value	Units
MTX (3)	Clearance	$5.57 \times (\text{CL}_{\text{Cr}}/80.0)^{0.112}$	l/h
	Central compartment volume of distribution ( $V_c$ )	26.9	l
	Peripheral compartment volume of distribution ( $V_p$ )	2.27	l
	$V_c$ and $V_p$ intercompartment clearance ( $Q$ )	0.0778	l/h
CPG2 (16)	Clearance	0.086	ml/min/kg
	Volume of distribution at steady state ( $V_{\text{dss}}$ )	67.9	ml/kg
	Maximum drug concentration ( $C_{\text{max}}$ )	2.86	mg/ml
	Elimination half-life ( $t_{1/2}$ )	9.97	min
(18)	Michaelis-Menten constant with methotrexate hydrolysis by CPG2 ( $K_m$ )	86	$\mu\text{mol/l}$

CL<sub>Cr</sub>: Creatinine clearance.

Table II. Pharmacokinetic parameters of methotrexate (MTX) and glucarpidase (CPG2) in Monte-Carlo simulation.

Drug	Pharmacokinetic parameters	Units	Mean	Standard deviation	95% CI
MTX	Clearance	l/h	0.615	0.215	0.596-0.634
	Central compartment volume of distribution ( $V_c$ )	l	26.683	9.749	25.828-27.537
	Peripheral compartment volume of distribution ( $V_p$ )	l	2.253	1.038	2.162-2.344
	$V_c$ and $V_p$ intercompartment clearance ( $Q$ )	l/h	0.078	0.036	0.075-0.081
CPG2	Clearance	l/h	0.310	0.062	0.304-0.315
	Volume of distribution at steady state ( $V_{\text{dss}}$ )	l	4.114	0.802	4.043-4.184

CI: Confidence interval.

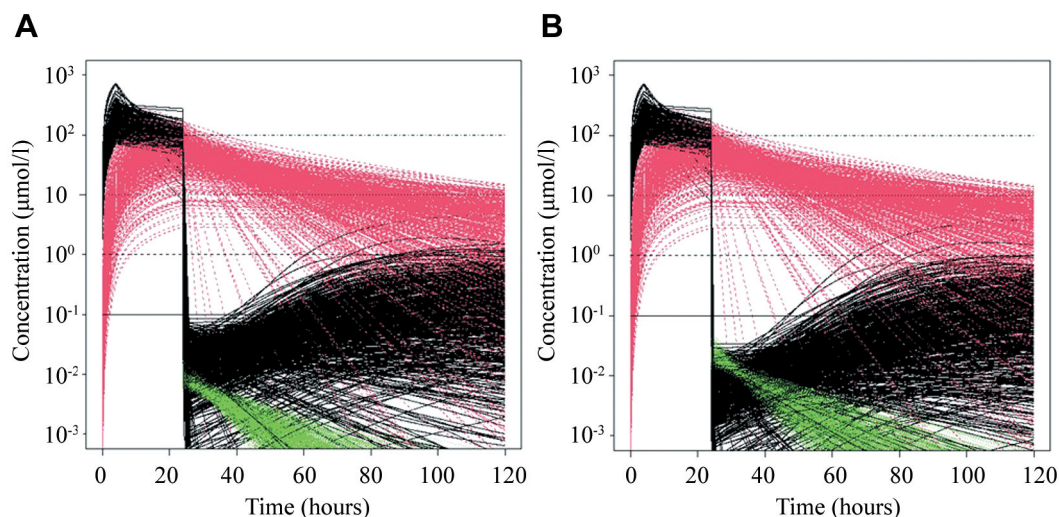


Figure 2. Plasma (black lines) and peripheral (red lines) methotrexate and plasma glucarpidase (green lines) log-concentrations versus time profile for therapy with 20 U/kg (A) and 50 U/kg (B) of glucarpidase using Monte-Carlo simulation.

simulations were performed to evaluate the plasma and peripheral methotrexate concentrations, and plasma CPG2 concentration (Figure 2). The mean plasma methotrexate concentration tended to decrease as the dose of CPG2 increased (Figure 3). The mean plasma methotrexate concentration at 70

h after methotrexate treatment was less than 0.1  $\mu\text{mol/l}$  at all doses of CPG2 apart from 10 U/kg, but at 120 h it was above 0.1  $\mu\text{mol/l}$  at all doses of CPG2.

The proportion of samples in which the plasma methotrexate concentration was less than 0.1  $\mu\text{mol/l}$  at 70 h after

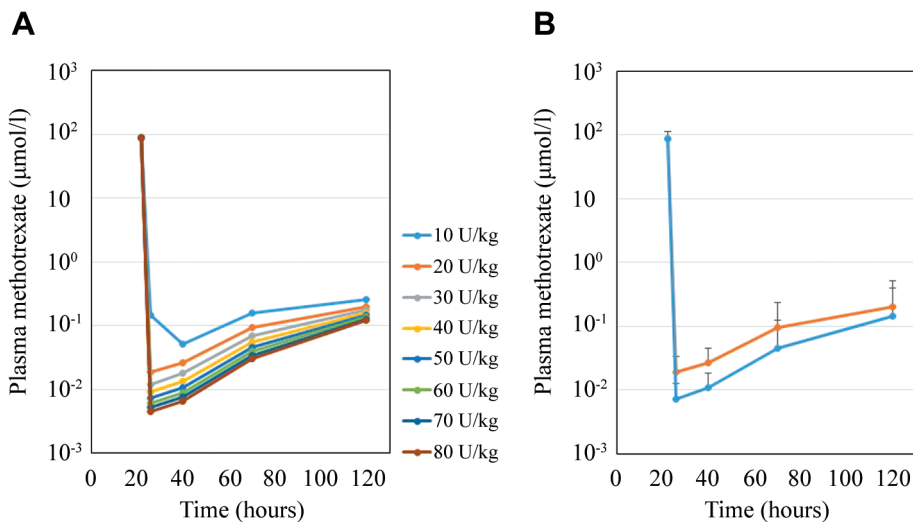


Figure 3. Mean plasma methotrexate concentration (log) versus time profile at all doses of glucarpidase (A) and highlighting those at 20 and 50 U/kg (B) (mean + standard deviation).

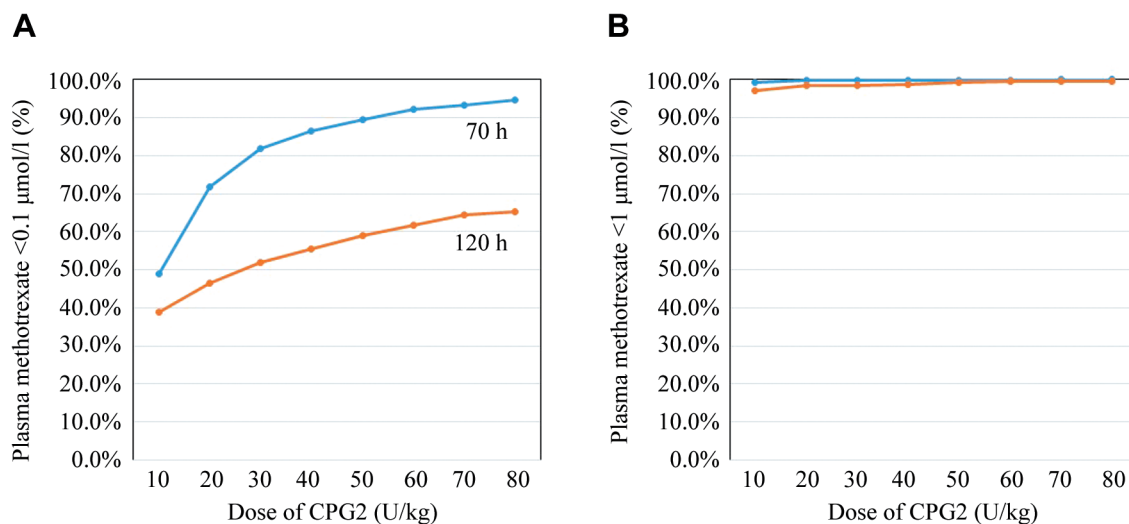


Figure 4. The proportion of samples in which the plasma methotrexate concentration was less than 0.1  $\mu\text{mol/l}$  (A) and less than 1.0  $\mu\text{mol/l}$  (B) at 70 and 120 h after methotrexate treatment according to the dose of glucarpidase (CPG2).

methotrexate treatment was 71.8% and 89.6% at 20 and 50 U/kg of CPG2, respectively (Figure 4). The proportion of samples in which the plasma methotrexate concentrations was less than 0.1  $\mu\text{mol/l}$  at 120 h after methotrexate treatment was 46.4% and 59.0% at 20 and 50 U/kg of CPG2, respectively.

### Discussion

Using the modified Michaelis–Menten PK/PD model of methotrexate decomposition by CPG2, Monte-Carlo simulation revealed the efficacy of CPG2 at a dose range of

10-80 U/kg. Simulations applying the Michaelis–Menten model have been reported in the oncology field, but to our knowledge, this is the first report covering CPG2 (19, 20). This model was considered appropriate because the trend of the blood concentration of methotrexate with CPG2 administration approximated previously reported clinical results (10-14). According to the assessment of CPG2 by the Food and Drug Administration and European Medicines Agency, the primary efficacy endpoint of CPG2 is a clinically important reduction, which is a methotrexate concentration  $\leq 1 \mu\text{mol/l}$  in all samples collected after the

first dose of CPG2 (18). In this study, we evaluated the proportion of samples in which the plasma methotrexate concentration was less than 0.1  $\mu\text{mol/l}$  at 70 h after methotrexate administration in order to consider clinical methotrexate toxicity (21). To reduce its toxicity, the methotrexate concentration should ultimately be less than 0.1  $\mu\text{mol/l}$ . A rebound of the serum concentration of methotrexate was observed in many virtual patients after the administration of CPG2, and in many exceeded the toxic range of methotrexate at 70 h based on the simulation using the reported parameters. Long-term monitoring (over 144 h) of serum methotrexate concentration might be needed after the administration of CPG2, even when nontoxic serum levels of methotrexate are observed at 70 h.

CPG2 is an enzymatic drug, and because the enzymatic reaction involves competition for substances in the body, individual differences occur depending on various conditions, such as body temperature. Therefore, it is necessary to establish a sufficiently safe dose that considers intra- and interindividual variations. However, it is possible that low doses of CPG2 may not reduce the concentration of methotrexate to 0.1  $\mu\text{mol/l}$ , which is its safe concentration threshold. This suggests that a low dose of CPG2 may not be sufficient to reduce methotrexate concentration, depending on certain factors, such as patient condition and methotrexate concentration before CPG2 administration. At CPG2 doses of 50 U/kg or higher, the percentage of virtual patients in which the methotrexate concentration was reduced to 0.1  $\mu\text{mol/l}$  was about 90%, with the effect leveling off at higher doses (Figure 3 and Figure 4). A PK study of healthy Japanese volunteers revealed a positive proportional relationship between the CPG2 dose, the plasma drug concentration, and the area under the blood concentration–time curve (15), which was similar to that observed in volunteers of Caucasian and African descent (16). Therefore, we decided to recommend a CPG2 dose of 50 U/kg for a phase II study.

From previous reports, it is known that CPG2 does not infiltrate intracellularly because the distribution volume of CPG2 is as small as 51 ml/kg, from which it can be inferred that most of the CPG2 is distributed into the blood (16). The blood volume in children ( $9.8 \pm 4.6$  years) is reported to be linearly related to body weight (boys:  $52.3 \pm 8.3$  ml/kg,  $R=0.944$ ; girls:  $47.9 \pm 7.7$  ml/kg,  $R=0.943$ ), indicating that the maximum drug concentration at a 50 U/kg dose would be similar to that in adults (22). In addition, CPG2 is an enzyme that is rapidly metabolized independent of organ function; therefore, the PK of CPG2 in pediatric patients is considered to be similar to that in adults. Consequently, to reduce the concentration of methotrexate in pediatric patients, the same CPG2 concentration as in adults is required, and a CPG2 dosage of 50 U/kg is also recommended for pediatric patients.

This model and simulation dose-finding phase II study has a limitation. Although the analysis was based on PK

parameters in adults, the phase II study included pediatric patients (identifier: JMA-IIA00097).

The methotrexate concentration changes under treatment with CPG2 were clarified by this PK/PD model and simulation study. Based on this simulation study, the phase II study was conducted with CPG2 dose of 50 U/kg, and CPG2 was approved for manufacturing in Japan (identifier: JMA-IIA00097).

## Conclusion

We decided to recommend a CPG2 dose of 50 U/kg as being ethical for the planning of a phase II study. As a rebound in the serum methotrexate concentration may be observed in many patients after the administration of CPG2, long-term (over 144 h) monitoring of serum methotrexate concentration may be needed after the administration of CPG2, even when nontoxic serum levels of methotrexate are observed at 70 h.

## Conflicts of Interest

The Authors declare no conflicts of interest.

## Authors' Contributions

Conceptualization, H.K., K.Y., and T.K.; data analysis, interpretation, and drafting, Y.F., T.K., and Y.H. All the Authors critically reviewed and revised the article draft and approved the final version for submission.

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