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Population pharmacokinetics of mizoribine in pediatric patients with kidney disease

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Abstract

Background   The present study aimed to obtain information enabling optimisation of the clinical effect of mizoribine (MZR) in pediatric patients with kidney disease.

Methods   A total of 105 pediatric patients with kidney disease treated at our institutions were enrolled. Kidney transplant patients were excluded. Population pharmacokinetic analysis of MZR was performed based on serum concentration data. Area under the curve from time zero to infinity (AUC_∞) and maximal concentration (C_{max}) were calculated by Bayesian analysis.

Results   In children, the appearance of MZR in the blood tended to be slower and the subsequent rise in blood concentration tended to be more sluggish, compared to healthy adults. Apparent volume of distribution and oral clearance were also higher in children compared to adults. A significant positive correlation was observed between patient age and AUC_∞. There were significant differences of AUC_∞ and C_{max} by age group. No relationship was observed between the administration method of MZR and serum concentration.

Conclusion   The pharmacokinetics of MZR was different in children compared to adults. To obtain the expected clinical efficacy, the regular MZR dosage schedule (2–3 mg/kg/day) might be insufficient for pediatric patients. In particular, younger patients might require a higher dosage of MZR per unit body weight.

Keywords   Mizoribine · Pediatrics · Drug monitoring · Therapeutics

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Introduction

Mizoribine (MZR) is a selective inhibitor of inosine monophosphate dehydrogenase in the pathway responsible for de novo synthesis of guanine nucleotides, resulting in the suppression of T- and B-lymphocyte proliferation [1, 2]. MZR has been used successfully in the treatment of immune-mediated diseases, including transplantation [3], kidney diseases [4–7], and rheumatic diseases [8, 9] in both children and adults. MZR is highly safe compared to other immunosuppressive drugs, but has mild immunosuppressive effects with low doses up to 3 mg/kg/day. Recent clinical reports have indicated that the efficacy of MZR may depend on the peak serum level of the drug [10–15]. Accordingly, a peak serum MZR level of at least 2.5–3.0 µg/ml is now thought to be needed to sustain long-term efficacy in the treatment of patients with glomerular diseases [11, 13]. However, when using the conventional daily low-dose MZR protocol (2–3 mg/kg) in patients with renal disease, the peak blood level of the drug usually remains at around 1.0 µg/ml [10], which may explain the previously reported relatively mild immunosuppressive efficacy of MZR in clinical practice [4, 12]. Furthermore, no correlation exists between the dosage of MZR and the serum concentration, and individual differences are large [3]. This has led to differences in the evaluated clinical efficacy of MZR.

Evaluation of drug efficacy requires pharmacokinetic parameters estimated using several serum samples. However, Bayesian analysis can be used to predict individual pharmacokinetic parameters from a small number of concentration data points [16], allowing pharmacokinetic parameters to be determined from a limited number of samples. Accordingly, an optimal MZR administration schedule for children has been sought. To date, however, population pharmacokinetic (PPK) data have only been available for healthy adults [17].

In the present study, to establish the optimal dosage schedule for obtaining the expected clinical effect of MZR in pediatric patients with kidney disease, PPK analysis of MZR was performed at our institutions, and we evaluated the pharmacokinetic characteristics of MZR.

Patients and methods

A total of 105 pediatric patients with kidney disease being treated at our institutions were targeted, and testing was performed 213 times. Kidney transplant patients were excluded. In all patients, kidney function was maintained (creatinine clearance, $\geq$90 ml/min/1.73 m^2 based on the new Schwartz equation [18]). The study was conducted in remission phase of the patients' diseases. The patients had no edema and their serum albumin levels were normal.

Some subjects had been administered steroids or other immunosuppressants concomitantly with MZR. Background data for the studied patients and details of their diseases are shown in Table 1. In conducting the study, we provided a full explanation of the details in writing to all the patients and their guardians, and asked them to provide written consent, as approved by the ethics committee of each participating institution. The study conformed to the tenets of the 2000 Declaration of Helsinki. The study was approved by the local ethics board, and written informed consent was obtained from the parents of each subject. The ethics committee approval number in Aichi Children's Health and Medical Center is 200605.

Pharmacokinetic data

MZR [Bredinin®; (AsahiKASEI, Tokyo)] was generally administered once daily or twice daily before meals (before 30 min to just before the start of a meal). In some patients who were given multiple administrations, postprandial administration (from just after to 2 h after the end of a meal), and administration between meals (on empty stomach from 2 h after the end of a meal to 30 min before the start of the next meal) were also tested in this study. The MZR dosage for target patients was 1.0–13.5 mg/kg (40–650 mg) per administration. For serum collection, a blood vessel was secured by inserting an indwelling needle, and serum was sampled just before oral administration and from 1 to 24 h after oral administration, at a total of 1–13 time points. Testing was performed 1–7 times. Collected serum samples were centrifuged (3000 rpm, 15 min), and separated serum samples were stored frozen at −20 °C or below until concentration analysis.

Serum MZR concentrations were determined by Asahi Kasei Pharma by high-performance liquid chromatography (HPLC) according to the method of Hosotsubo et al. [19]. The quantification limit of MZR was 0.05 µg/ml in serum.

Population pharmacokinetic analysis

Using serum MZR concentration data for a total of 984 points in 213 pharmacokinetic trials as a base, PPK parameters and inter-individual variations were estimated using the NON-MEM program (Version 5 Level 1.1 [20]). Estimation of PPK parameters was performed using the first-order method based on the assumption that the apparent volume of distribution correlated with oral clearance. Good absorption of MZR from the digestive tract of animals and adult humans has been demonstrated, and in each case, MZR was excreted by the body without being metabolized [21]. A one-compartment model was, therefore, adopted for the present pharmacokinetic analysis. Using a one-compartment model in which first-order absorption was assumed to be the

Table 1 Demographics of pediatric kidney disease patients

Total patients	105		
Male/female	53/52		
Age, mean ± SD (minimum–maximum) (years)	10.36 ± 4.11 (1–18.1)		
Height, mean ± SD (cm)	132.86 ± 20.76		
Body weight, mean ± SD (kg)	34.86 ± 14.04		
Serum creatinine, mean ± SD (mg/dl)	0.434 ± 0.142		
eGFR, mean ± SD (ml/min/1.73 m^2)	120.71 ± 27.26		
Serum albumin, mean ± SD (mg/dl)	3.7 ± 0.7		
Oral dose of MZR, mean ± SD (minimum–maximum) (mg/kg)	5.90 ± 2.38 (1.0–13.5)		
Total trials	213		
Total blood samples	984		
Total urinal collections	68		

Disease	Number (male/female)	Age, mean ± SD (years)	Serum albumin, mean ± SD (g/dl)	Oral dose of MZR, mean ± SD (mg/kg)
INS	74 (41/33)	9.95 ± 4.1	3.7 ± 0.8	6.02 ± 2.48
IgAN	10 (7/3)	10.3 ± 3.6	4.1 ± 0.7	4.94 ± 1.28
SLE	10 (0/10)	13.7 ± 2.3	3.7 ± 0.5	6.14 ± 2.61
HSPN	8 (5/3)	7.8 ± 2.6	3.6 ± 0.5	4.74 ± 1.11
JIA	1 (0/1)	14	3.8	6.39 ± 0.86
ATIN	1 (0/1)	15.2	4.7	
TINU	1 (0/1)	17.3	4.6	7.18 ± 3.50

INS idiopathic nephrotic syndrome, *IgAN* IgA nephropathy, *SLE* systemic lupus erythematosus, *HSPN* Henoch–Schönlein purpura nephritis, *JIA* juvenile idiopathic arthritis, *ATIN* acute tubulo-interstitial nephritis, *TINU* tubulo-interstitial nephritis and uveitis syndrome

analytical model, ADVAN2 and TRANS2 were selected from NONMEM-PREDPP library subroutines, and the absorption lag time (ALAG), absorption rate constant (KA), apparent volume of distribution (V/F), and oral clearance (CL/F) were calculated (where F denotes bioavailability). We estimated PPK parameters based on the assumption that the apparent volume of distribution correlated with oral clearance. Furthermore, the obtained PPK parameters were assumed to show a normal logarithmic distribution. The equations used for the calculation of the various parameters were as follows.

Absorption lag time in the ith pharmacokinetic trial (ALAG$_i$):

$$\text{ALAG}_i = \theta_1 \cdot \exp(\eta_{\text{ALAG}_i})$$

where θ_1 is the predicted population mean of the absorption lag time (in h), and η_{ALAG_i} is a random variable distributed normally with a mean of zero and variance of ω^2_{ALAG}.

Absorption rate constant in the ith pharmacokinetic trial (KA$_i$):

$$\text{KA}_i = \theta_2 \cdot \exp(\eta_{\text{KA}_i})$$

where θ_2 is the predicted population mean of the absorption rate constant (in h^{-1}), and η_{KA_i} is a random variable distributed normally with a mean of zero and a variance of ω^2_{KA}.

Apparent volume of distribution (V/F_i) and oral clearance (CL/F_i) in the ith pharmacokinetic trial:

$$V/F_i = \theta_3 \cdot \text{WT} \cdot \exp(\eta_{V/F_i})$$

$$\text{CL}/F_i = \theta_4 \cdot \text{CL}_{\text{cr}} \cdot 60/1000 \cdot \exp(\eta_{\text{CL}/F_i})$$

where WT is the body weight (in kg), and $\theta_3 \cdot \text{WT}$ is the predicted population mean of the apparent volume of distribution (in l). CL_{cr} is the creatinine clearance (in ml/min), and $\theta_4 \cdot \text{CL}_{\text{cr}} \cdot 60/1000$ is the predicted population mean of oral clearance (in l/h). Random variables, η_{V/F_i} and η_{CL/F_i}, are assumed to be distributed normally with means of zero and co-variance of $\omega^2_{V/F}$, $\omega_{V/F,CL/F}$, and $\omega^2_{\text{CL}/F}$.

Creatinine clearance (CL_{cr}) (in ml/min) was calculated as follows:

$$\text{CL}_{\text{cr}} = (0.413 \cdot \text{HT}/S_{\text{cr}})/(\text{BSA}/1.73)$$

$$\text{BSA} = 0.007184 \cdot \text{WT}^{0.425} \cdot \text{HT}^{0.725}$$

where HT is height (in cm), WT is body weight (in kg), BSA is body surface area (in m^2), and S_{cr} is the serum creatinine concentration (in mg/dl).

Finally, the jth observed serum concentration in the ith pharmacokinetic trial (C_{ij}) was assumed to be randomly and normally distributed from the predicted value(C^*_{ij}):

$$C_{ij} = C^*_{ij} + \varepsilon_{ij}$$

where ε_{ij} is a random variable that describes intra-individual variability with a mean of zero and a variance of σ^2.

Calculation of parameters in each case was performed using ALAG, KA, V/F and CL/F obtained by Bayesian analysis. In addition, calculation of pharmacokinetic parameters [area under the curve from time zero to infinity (AUC_∞), maximal concentration (C_{max}), and maximal serum concentration time (T_{max})] in each case was performed using the pharmacokinetic analysis software package WinNonlin^R Ver 5.2 (Pharsight Corp., Mountain View, CA, USA).

Effects of aging on pharmacokinetic parameters

Patients overall were divided into three age groups: infants and preschool-age children (<6 years old); school-age children (6–11 years old); and adolescents (>11 years old). Classification into age groups was performed with reference to "Guidance for clinical studies of drugs in child population (2000)" (Ministry of Health, Labour and Welfare, Japan). Patients who underwent the test twice or more belonging to more than one age group were counted separately. Differences in AUC_∞, C_{max} and T_{max} among age groups were examined on the basis of changes in serum concentration in each patient calculated by Bayesian analysis. Units for MZR dosage were unified to milligrams per kilogram body weight, and all AUC_∞ and C_{max} values were corrected based on the dosage received in each case. All T_{max} values were evaluated without any correction.

Effects of MZR administration method on pharmacokinetic parameters

For examination of the effects of meals on pharmacokinetics, patients were divided into three groups according to differences in administration method: preprandial administration; postprandial administration; and inter-meal administration. Effects on AUC_∞ and C_{max} were then examined. In the present examination, none of the patients who underwent multiple tests were reassigned to different administration groups.

Statistical analysis

Data obtained are expressed as mean ± standard deviation (SD), and are shown with the coefficient of variation (%), as an index of scattering. To determine correlations, the regression equation and Spearman's correlation coefficient (rs) were obtained using the statistical analysis software package SPSS Statistics (IBM SPSS, Tokyo, Japan), and the significance of rs was tested by Spearman's correlation coefficient test. To examine the effects of meals and age differences, significance was tested using Scheffe's F test. In each test, the significance level was set at 5 %.

Results

Pediatric population pharmacokinetic parameters of MZR

Means and 95 % confidence intervals for each PPK parameter when MZR was administered to pediatric patients, as calculated by NONMEM analysis, are shown in Table 2. Each 95 % confidence interval was obtained as the mean ± 1.96 · standard errors of the mean (SE). Mean values of ALAG and KA were calculated as 0.513 h and 0.793 h^{-1}, respectively. In comparison with the known average values for healthy adults (ALAG, 0.349 h; KA, 0.869 h^{-1}) [17], the appearance of MZR in the blood tended to be slower, and the subsequent rise in blood concentration tended to be more sluggish. Mean values of V/F and CL/F were also calculated to be 1.29 · WT L and 2.79 · CL$_{cr}$ L/h, respectively, and were higher than those of known average values in healthy adults (V/F, 0.834 · WT L; CL/F, 1.93 · CL$_{cr}$ L/h) [17]. Furthermore, based on the ω value of each parameter, significant inter-individual variations of MZR pharmacokinetics were observed in children. Although examples of serum MZR concentration–time curves in individual patients prepared from PPK parameters are not shown, the predictability determined by Bayesian analysis was mostly good.

Linearity between patient age and AUC_∞

Results are shown in Fig. 1. A significant positive correlation was observed between age and AUC_∞ (rs = 0.422, $p < 0.0001$). Although the data are not shown in the figure, we also examined data by converting MZR dosage units to milligram per square meter of body surface area and

Table 2 Population pharmacokinetic parameters of mizoribine in pediatric kidney disease patients

Parameter	Estimate	95 % CI
θ_1 (h)	0.513	0.426–0.600
θ_2 (h^{-1})	0.793	0.678–0.908
θ_3 (l/kg)	1.29	1.13–1.45
θ_4	2.79	2.60–2.99
ω^2_{ALAG}	0.480	0.221–0.739
ω^2_{KA}	0.513	0.355–0.671
$\omega^2_{V/F}$	0.306	0.234–0.378
$\omega_{V/F,CL/F}$	0.192	0.144–0.240
$\omega^2_{CL/F}$	0.225	0.164–0.286
σ (µg/ml)	0.103	0.0806–0.121

ALAG absorption lag time, *KA* absorption rate constant, *V/F* apparent volume of distribution, *CL/F* oral clearance, *F* extent of bioavailability
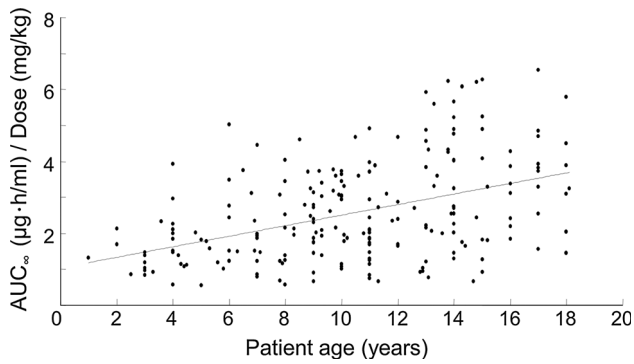
Fig. 1 Plot of area under the serum MZR concentration–time curve from time zero to infinity (AUC_∞) against patient age for all patients. *Solid line* is the correlation line ($rs = 0.422$, $p < 0.0001$). *MZR* mizoribine

milligrams per milliliter total volume of body fluid in patients, and the results were seen to be similar to those obtained with milligrams per kilogram body weight.

Relationship between patient age and AUC_∞, C_{max} and T_{max}

Statistical characteristics for each age group are shown in Table 3. Results for AUC_∞ and C_{max} are presented in Fig. 2a, b. Upon comparison of the mean ± SD, each parameter showed significant differences among age groups, and a tendency for an increase with age was observed [AUC_∞/dose (µg h/ml)/(mg/kg); 1.52 (<6 years), 2.36 (6–11 years), 3.37 (>11 years), C_{max}/dose (µg/ml)/(mg/kg); 0.30 (<6 years), 0.43 (6–11 years), 0.51 (>11 years)]. On the other hand, mean T_{max} was calculated as 2.510 ± 0.695 h for all groups, almost the same as the

known T_{max} for healthy adults (median T_{max} ranges from 2 to 2.5 h for 3, 6, 9 and 12 mg/kg MZR) [21].

Relationship between administration method and AUC_∞, C_{max}

Results are shown in Fig. 3a, b. In the present examination, the number of patients with postprandial and inter-meal administration was lower than that of patients with preprandial administration. However, no significant differences were detected among these groups.

Discussion

For accurate evaluation of the efficacy of MZR in pediatric patients, we considered it necessary to clarify the pharmacokinetic parameters specific to children, and conducted a large-scale multicenter PPK analysis. We established details of MZR pharmacokinetics for pediatric groups separated by different parameters, and slower absorption of the drug from the digestive tract in comparison with adult males was demonstrated [17]; higher levels of both V/F and CL/F were clarified. For V/F, a large difference was identified between children and adults. There are two possible reasons for this: lower bioavailability of the drug in children; and/or a larger actual distribution volume (Vd) in children, in comparison to adults. We are planning to clarify whether bioavailability and Vd change with age or remain constant.

Marked individual differences in MZR pharmacokinetics in children are frequently experienced in routine clinical settings. We used AUC_∞ as an index reflecting the actual proportion of dose absorbed in the body, and examined the

| **Table 3** Demographic variables in age strata	Infants and preschool children 1–5 years	Children 6–11 years	Adolescents 12–18 years	Total
Total number[a]	20	50	35	105
Male/female	12/8	26/24	15/20	53/52
Trial times				
1	13	22	21	56
2	4	17	4	25
≥3	3	12	13	28
Sampling time points				
1[b]	2	8	12	22
2–4	1	1	1	3
5–7	16	38	23	77
8–13	1	4	2	7

[a] Patients belonging to multiple strata throughout the study period were counted in each stratum

[b] Samples were taken at 2 h post-dose

Fig. 2 Relationship between patient age and AUC_∞ (**a**) and maximal serum MZR concentration (C_{max}) (**b**). *Each column represents mean ± SD. *$p < 0.01$. *n.s* not significant, AUC_∞ area under the curve from time zero to infinity, *MZR* mizoribine
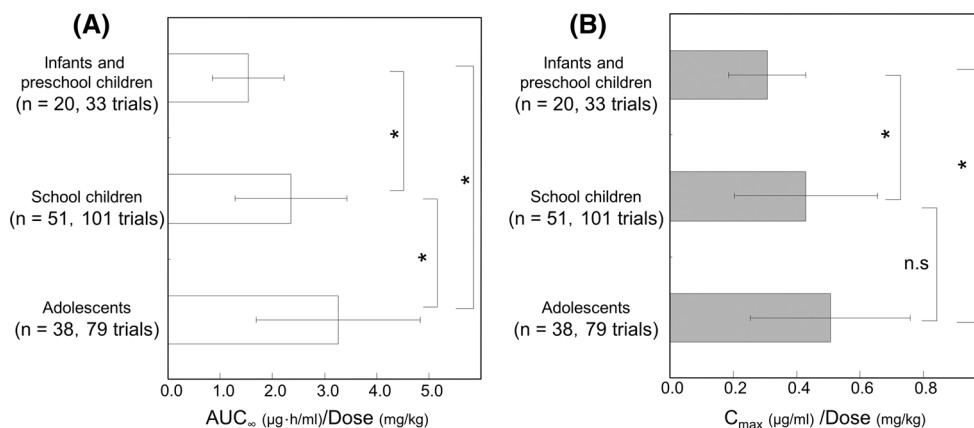
Fig. 3 Effects of MZR administration method on AUC_∞ (**a**) and C_{max} (**b**). *Each column represents mean ± SD. AUC_∞ area under the curve from time zero to infinity, *MZR* mizoribine
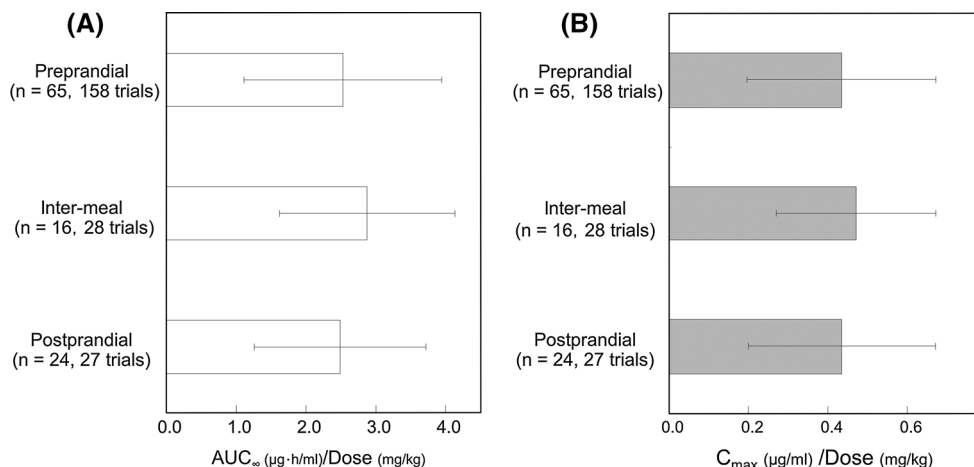
relationship between patient age and AUC_∞. Furthermore, to consider cases of similar AUC_∞ values even when different amounts of the drug were administered, all AUC_∞ values were divided by the oral dose for comparative examination. This correction allowed the bioavailability in each individual to be reflected.

We first examined the correlation between patient age and AUC_∞, revealing a significant positive correlation (Fig. 1). This suggests that AUC_∞ decreases due to reductions in the oral bioavailability of MZR in younger patients. Furthermore, a tendency for up- and down-scattering of AUC_∞ values was seen among individuals in the same age groups. We will examine the possibility that factors other than patient age have an effect on the pharmacokinetics of MZR, particularly its bioavailability.

In a comparison of age-related MZR efficacy (Fig. 2), both AUC_∞ and C_{max} per unit dosage of MZR increased with patient age, and the inter-group differences were significant. On the other hand, T_{max} is almost the same as in healthy adults. Measurement of blood concentrations at 2 h (C2) or 3 h (C3) after MZR administration was considered important in the calculation of pharmacokinetic parameters

in individual patients. When MZR is used for the treatment of pediatric kidney disease, a strategy for increasing the dosage for single administration becomes necessary.

An experiment using a rat small intestine loop model has demonstrated competitive inhibition of MZR absorption with concomitant use of nucleic acids [22], and possible effects of the time after a meal until administration or the contents of a meal on MZR absorption have been indicated for postprandial administration. Although the comparison was performed in a small number of cases (Fig. 3), no significant differences were detected among the preprandial, postprandial and inter-meal administration groups. We have previously demonstrated that equilibrative nucleoside transporters (ENTs) are largely responsible for absorption of MZR using the rat small intestine [23], and the lack of an effective administration method may be reflected in the fact that ENT plays a major role in MZR absorption in humans.

Recent studies have shown that serum MZR concentrations >2.5–3.0 μg/ml are sufficient for an expectation of clinical efficacy [11, 13]. From the findings in this study, high-dose treatment of MZR should be applied for

pediatric patients to obtain the expected clinical effect. The estimated optimal dose to obtain 3.0 µg/ml in each age is 10.00 mg/kg (<6 years), 6.97 mg/kg (6–11 years) and 5.88 mg/kg (>11 years). Whether a significant relationship exists between C_{max}/AUC of MZR and clinical efficacy remains unclear. Further studies with high-dose MZR treatment based on the findings in this study are necessary to clarify the optimal dosage schedule for obtaining expected clinical efficacy.

In this study, some patients had been administered steroids or other immunosuppressants concomitantly with MZR. These drugs might influence the pharmacokinetics of MZR. This is an important limitation of this study. Further studies to clarify the effects of concomitant drugs on the pharmacokinetics of MZR are necessary.

In conclusion, despite the limitations of this study, our findings suggest that high-dose treatment with MZR should be applied for pediatric patients to obtain the expected clinical effect of MZR. The optimal dose of MZR should be considered in each age for pediatric patients.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare.

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