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Journal

Clinical and Translational Science, 17(12)

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Publication Date

2024-12-01

DOI

10.1111/cts.70103

Peer reviewed

Received: 24 January 2024 | Revised: 9 November 2024 | Accepted: 22 November 2024

DOI: 10.1111/cts.70103

ARTICLE

Effect of *SLC22A1* polymorphism on the pharmacokinetics of proguanil in Korean: A semi-physiologic population pharmacokinetic approach

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Abstract

Proguanil, an antimalarial drug, undergoes hepatic uptake by the polymorphic organic cation transporter 1 (OCT1) and is subsequently metabolized by the cytochrome P-450 2C19 (CYP2C19) enzyme into its active metabolite, cycloguanil. This study aims to evaluate and mechanistically characterize the effect of genetic polymorphism of *SLC22A1*, which encodes OCT1, on the pharmacokinetics (PKs) of proguanil and cycloguanil in Korean. This study was based on a post hoc analysis of the PK results of a CYP2C19 mediated drug–drug interaction study (NCT04568772). Among the 16 CYP2C19 normal metabolizers enrolled in the previous study, 13 were prospectively genotyped for six *SLC22A1* single nucleotide polymorphisms (SNPs) associated with a decreased function of OCT1. Among these, only the SNP *SLC22A1* 1022C>T (rs2282143) was observed, with four subjects being heterozygous (CT) and nine subjects homozygous for the wild-type allele (CC). The CT genotype showed a 1.2-fold higher systemic exposure of proguanil and a 0.6-fold lower exposure of cycloguanil compared to those in subjects with the CC genotype, resulting in a 0.5 to 0.6-fold lower metabolic ratio. Based on the PK and genotype data, a parent-metabolite joint population PK model including a well-stirred liver compartment was developed using a nonlinear mixed-effect modeling approach. The OCT1 activity of the CT genotype was estimated to be 0.42-fold lower compared to the CC genotype. In conclusion, the genetic polymorphism of *SLC22A1* 1022C>T increased the systemic exposure of proguanil, while decreasing the systemic exposure of cycloguanil by reducing the hepatic uptake of proguanil, as mechanistically described by a population PK approach.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Proguanil, an antimalarial drug, undergoes metabolism by its major metabolizing enzyme CYP2C19, leading to the formation of cycloguanil. The

biotransformation occurs within hepatocytes after uptake via the organic cation transporter 1 (OCT1), which is encoded by the highly polymorphic gene *SLC22A1*.

WHAT QUESTION DID THIS STUDY ADDRESS?

To what extent does the genetic polymorphism of *SLC22A1* identified in the Korean population affect the pharmacokinetics of proguanil and cycloguanil?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

Our work suggests the genetic polymorphism of *SLC22A1* 1022C>T, associated with decreased OCT1 activity, reduces the uptake of proguanil into the hepatocyte, affecting its metabolism by CYP2C19. Using a semi-physiologic population pharmacokinetic model, the extent of the decrease in OCT1 activity due to *SLC22A1* 1022C>T polymorphism was mechanistically characterized. Consequently, the *SLC22A1* 1022C>T polymorphism results in the increased systemic exposure of proguanil and decreased the systemic exposure of cycloguanil in the Korean population.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

These findings highlight the importance of genetic polymorphism of transporters, in addition to those in metabolizing enzymes, when interpreting the pharmacokinetics of drugs that primarily undergo hepatic metabolism. Additionally, this study proposes a robust approach to quantitatively assess the transporter activity using a semi-physiologic population PK model.

INTRODUCTION

Proguanil is an antimalarial drug, used in combination with atovaquone for the prophylaxis and treatment of *Plasmodium falciparum* malaria.[1] Through a biotransformation by cytochrome P-450 2C19 isoenzyme (CYP2C19), the active metabolite cycloguanil is formed and inhibits the dihydrofolate reductase.[2,3] In addition to proguanil, drugs such as omeprazole, diazepam, and mephenytoin also undergo metabolism through the CYP2C19 enzyme and are known to show different pharmacokinetics (PKs) associated with CYP2C19 genetic polymorphisms. The relationship between CYP2C19 genetic polymorphisms and PK differences between proguanil and cycloguanil has been thoroughly investigated.[4–6]

Even when considering the CYP2C19 polymorphism, the activity of transporters should also be taken into consideration in order to provide a robust interpretation of the PKs of proguanil and cycloguanil. For hepatic metabolism to take place, the uptake of proguanil by the organic cation transporter 1 (OCT1) should occur in hepatocytes.[7] The significance of OCT1 in this context is crucial due to the highly basic and hydrophilic nature of proguanil,[8] and the likelihood of proguanil moving through passive diffusion is considerably low.

The *SLC22* family comprises genes that encode organic cation transporters, zwitterion/cation transporters, and organic anion transporters.[9] These transporters play a critical role in facilitating the transportation of numerous physiologically charged endogenous and exogenous compounds in both the liver and kidney.[9] OCT1 is encoded by the gene *SLC22A1*, which is highly polymorphic in human.[10] The PKs of substrate drugs of OCT1, including morphine, imatinib, sumatriptan, metformin, and tramadol, have been shown to be significantly influenced by genetic polymorphisms in *SLC22A1*.[11–16] Major single nucleotide polymorphisms (SNPs) that result in alterations to OCT1 activity include 1258–1260delATG (rs202220802), 181C>T (rs12208357), 1201G>A (rs34130495), 1393G>C (rs34059508), 262T>C (rs55918055), and 41C>T (rs34447885).[17]

While the impact of genetic variations of OCT1 on the PKs of proguanil and cycloguanil has been investigated previously,[18] its effect in the Korean population with distinct functional alleles remains unexplored. A global genetic and functional analysis of *SLC22A1* showed that there are ethnic variations in SNP allele frequencies and the extent of decreased OCT1 activities.[17] In the Korean population, genetic variants that are linked to decreased OCT1 function have been identified, including *SLC22A1* 848C>T (rs4646277) with a 2% allele frequency and 1022C>T (rs2282143) with

a 16% allele frequency.[19,20] As different SNP and allele frequencies are identified across ethnicities, the impact of the *SLC22A1* SNP on PKs of proguanil and cycloguanil in Korean requires further research.

Compared to the previous drug–drug interaction (DDI) study of vonoprazan and proguanil conducted in Japanese subjects,[21] slightly different PK characteristics of proguanil were observed in the DDI study of tegoprazan and proguanil conducted in Korean subjects.[22] When proguanil was administered alone, the metabolic ratio of cycloguanil to proguanil was 0.84 in the Japanese study. In contrast, the corresponding ratio was 0.35 in the Korean study.[22] To investigate whether the PK differences observed in Korean originate from the variability in OCT1 activity due to genetic polymorphism, the current retrospective genotyping study was conducted.

A population PK approach is widely used for the analysis of drug PK parameters at a population level and has advantages in quantitatively characterizing the extent of variability.[23] Even though the compartments defined during the population PK analysis are mostly theoretical, a semi-physiologic population PK approach can be utilized when a more comprehensive explanation of data is necessary. Beyond simply comparing the exposure of proguanil and cycloguanil by genotypes, this approach enables further quantification of the effects of intricate changes within physiological compartments, such as alterations in transporter activity, providing a clearer understanding of the impact of genetic polymorphisms.

The aim of this study was to evaluate the effect of genetic polymorphism of *SLC22A1*, the gene encoding OCT1, on the PKs of proguanil and cycloguanil in Korean, and to mechanistically and quantitatively characterize the effect of *SLC22A1* polymorphism on OCT1 activity using a semi-physiologic population PK approach.

METHODS

Study population

Healthy Korean subjects who had completed the previous CYP2C19-mediated DDI study of tegoprazan and proguanil (NCT04568772) were eligible for the *SLC22A1* genotyping. They were aged between 19 and 50 years old, with a body mass index (BMI) ranging from 19.0 to 30.0 kg/m^2, all CYP2C19 normal metabolizers (*1/*1) carrying two wild-type alleles.[22] The institutional review board (IRB) of Seoul National University Hospital approved the study protocol and informed consent form (IRB number: H-2301-010-1391). Participants who gave informed consent for additional blood sampling for genotyping were enrolled.

Genotyping

Peripheral whole blood samples for genotyping were collected in EDTA tubes. Genomic DNA was extracted from the blood samples for the genotyping of *SLC22A1* using a Maxwell® CSC Blood DNA Kit and Maxwell® CSC Instrument (Promega, Madison, WI, USA). TaqMan allelic discrimination assays were conducted using a real-time polymerase chain reaction (PCR) system (Applied Biosystems®, Foster City, CA, USA). The genotyping for the *SLC22A1* was performed with validated TaqMan Genotyping Assays (Table S1). The final reaction volume was 10 microliters each, consisting of 2× TaqMan Universal Master Mix II (or TaqMan Fast Advanced Master Mix), 20×/40× Drug Metabolism Genotyping Assay Mix, DNase-free water, and genomic DNA. The PCRs were performed with the following conditions: an initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 1 min (for TaqMan Fast Advanced Master Mix: initial denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 1 s and annealing/extension at 60°C for 20 s). Allelic discrimination results were assessed using QuantStudio™ 3 Real-Time PCR Instrument and QuantStudio™ Design & Analysis Software (Applied Biosystems).

Data

The PK data of the CYP2C19-mediated DDI study of tegoprazan and proguanil was used for this study.[22] Data consisted of time concentrations and PK parameters of proguanil and cycloguanil after administration of atovaquone/proguanil 250/100 mg alone or co-administration with tegoprazan 50 mg. Data following the co-administration of esomeprazole or vonoprazan were excluded considering the observed DDIs between proguanil and esomeprazole or vonoprazan. PK parameters included maximum concentration (C_{max}) and area under the time-concentration curve from zero to the last measurable point (AUC_{last}) for proguanil and cycloguanil, and the metabolic ratio of cycloguanil. These parameters were calculated as previously described.[22]

Statistical analysis

SAS® software (Version 9.4, SAS Institute, Cary, NC, USA) was used for the statistical analysis. PK parameters (C_{max}, AUC_{last}, metabolic ratio) were summarized and compared across genotypes. Sigmaplot® software (Version 12.5,

Systat Software, San Jose, CA, USA) was used for graphical representation.

Population pharmacokinetic analysis

Software

A joint population PK model of proguanil and cycloguanil was developed using NONMEM software (Version 7.5, ICON Development Solutions, Ellicott City, MD, USA) with Pirana software (Version 2.9.7, Certara, NJ, USA) used as a graphical interface. All parameters were estimated using the first-order conditional estimation with the interaction method. Data processing and visualization were performed using R software (Version 4.1.3). The model evaluation and the stepwise covariate modeling (SCM) were performed using Perl-speaks-NONMEM (Version 5.3.0, Uppsala University, Uppsala, Sweden).

Structural model

To characterize the PK of proguanil and cycloguanil, 1- and 2-compartment disposition with or without transit compartments were tested. Transit compartments for cycloguanil were added one by one and the model appropriateness was evaluated at each step. The absorption of proguanil was evaluated using either a first-order or zero-order absorption process. The inter-individual variability (IIV) of PK parameters was evaluated using the exponential error model on the assumption of being log-normally distributed. For the residual variability, additive, proportional, or combined error model was tested.

For the elimination, a semi-physiologic model including the extracting hepatic compartment was tested. In this model, the metabolism of proguanil was described using the hepatic extraction ratio (E_H) and the hepatic blood clearance (CL_{BH}) (Equations 1 and 2). The fraction unbound in blood (f_{ub}), intrinsic clearance (CL_{int}), and hepatic blood flow (Q_{BH}) were used to define E_H.

$$E_H = \frac{f_{ub} \cdot CL_{int}}{Q_{BH} + f_{ub} \cdot CL_{int}} \quad (1)$$

$$CL_{BH} = E_H \cdot Q_{BH} \quad (2)$$

Fixed values of fraction unbound in plasma (f_u) of 0.25 and a blood-to-plasma concentration ratio (C_B/C_P) of 2.7 was used to calculate f_{ub} (Equation 3).[7,24] Hepatic plasma clearance (CL_H) was also derived from CL_{BH} and C_B/C_P (Equation 4).

$$f_{ub} = f_u / \left(\frac{C_B}{C_P}\right) \quad (3)$$

$$CL_H = CL_{BH} \cdot \left(\frac{C_B}{C_P}\right) \quad (4)$$

The liver volume (V_H) of 1 L and hepatic blood flow (Q_{BH}) of 90 L/h of a typical 70 kg adult was assumed and allometrically scaled based on body weight (Equations 5 and 6). The hepatic plasma flow (Q_H) was calculated from Q_{BH} using hematocrit of 0.45 (Equation 7).

$$V_H = 1\,L \cdot \left(\frac{\text{Body weight (kg)}}{70\,kg}\right) \quad (5)$$

$$Q_{BH} = 90\,L/h \cdot \left(\frac{\text{Body weight (kg)}}{70\,kg}\right)^{0.75} \quad (6)$$

$$Q_H = Q_{BH} \cdot (1 - \text{Hematocrit}) \quad (7)$$

Covariate analysis

Baseline demographic characteristics and laboratory test results were evaluated as potential covariates on PK parameters using SCM. A stepwise forward inclusion and backward elimination process was applied, using significance levels of 0.05 and 0.01, respectively.

Model evaluation

The final model fits were evaluated based on the goodness-of-fit plots. The predictive performance of the model was assessed using visual predictive checks (VPCs). Bootstrap resampling ($N=1000$) was conducted to evaluate the robustness of the final model. The median values and the 95% confidence intervals (CIs) of the parameters from the bootstrap results were compared with the final parameter estimates.

RESULTS

SLC22A1 genotyping

Among 16 subjects who had participated in the previous study,[22] a total of 13 subjects provided written informed consent for genotyping and were included in this study. For the genotyped SNP *SLC22A1* 1022C>T (rs2282143, assay ID: C__15877554_40), nine subjects were homozygous carrying wild-type alleles (CC genotype), and four subjects were heterozygous carrying a single variant allele (CT genotype). For 1258–1260delATG (rs202220802, assay ID: C_191598450_20), 181C>T (rs12208357, assay ID: C_30634096_10), 1393G>C (rs34059508,

assay ID: C_30634080_20), 262T>C (rs55918055, C_30634094_10), and 848C>T (rs4646277, assay ID: C_30634088_10), all 13 subjects carried homozygous wild-type alleles (Table 1).

Demographics

All subjects included in the genotyping analysis were male, and the mean ± standard deviation (SD) values for age, height, body weight, and BMI were 34.31 ± 7.38 years, 172.85 ± 7.37 cm, 76.12 ± 9.82 kg, and 25.52 ± 3.14 kg/m^2, respectively. Demographic characteristics were similar across *SLC22A1* 1022C>T genotypes CC, CT, and those with missing genotypes (Table S2).

Pharmacokinetics

Subjects with the CT genotype showed higher concentration-time profiles of proguanil and lower profiles of cycloguanil compared to those with the CC genotype (Figure 1). The systemic exposure (AUC_{last} and C_{max}) of proguanil in subjects with the CT genotype was higher around 1.2-fold compared to those with the CC genotype (Table 2; Figure S1). In contrast, the systemic exposure of cycloguanil in subjects with the CT genotype was lower around 0.6-fold, and the metabolic ratio calculated by the ratio of AUC_{last} of cycloguanil to proguanil was lower around 0.5- to 0.6-fold compared to those with the CC genotype (Table 2; Figure S2).

Population pharmacokinetic model

Structural model

A total of 160 plasma concentration-time data points for proguanil and 159 data points for cycloguanil from 16 healthy subjects,[22] along with *SLC22A1* genotype data from 13 subjects who subsequently participated in genotyping, was used for the population PK model development.

TABLE 1 Frequency of *SLC22A1* polymorphisms observed in genotyping.

SNPs	Variant	Genotype	*N* (%)
rs2282143	1022C>T	CC	9 (69.23)
		CT	4 (30.77)
rs202220802	1258–1260delATG	ATG	13 (100)
rs12208357	181C>T	CC	13 (100)
rs34059508	1393G>C	GG	13 (100)
rs55918055	262T>C	TT	13 (100)
rs4646277	848C>T	CC	13 (100)

A joint parent-metabolite population PK model of proguanil and cycloguanil was developed (Figure 2). The disposition of proguanil was best described by a 2-compartment model including a permeability considered well-stirred liver compartment. For cycloguanil, a 1-compartment disposition model with 2 transit compartments was applied. The absorption of proguanil from the gut into the liver compartment (K_A) was assumed to follow first-order kinetics. The efflux of cycloguanil from the liver compartment to the central compartment was characterized by its movement through 2 transit compartments. The central volume of distribution of cycloguanil was assumed to be the same as the central volume of distribution of proguanil, due to identifiability issues. The ratio of molecular weight between proguanil hydrochloride (290.19 g/mol) and cycloguanil hydrochloride (288.17 g/mol) was used for molecular weight conversion of the proguainil to cycloguanil.

The IIV of proguanil parameters was incorporated in the apparent clearance of proguanil via non-cycloguanil metabolic pathway (CL/F), central V_d, and CL_{int}. Residual variability for proguanil was modeled using a proportional error model. The IIV of cycloguanil was applied on the transit rate constant (mK_T). Residual variability for cycloguanil was modeled using a combined error model.

Effect of SLC22A1 genetic polymorhism on OCT1 activity

To account for the effect of *SLC22A1* 1022C>T polymorphism on OCT1-mediated hepatocyte uptake in proguanil metabolism, a parameter FUP, representing the relative fraction of OCT1-mediated hepatocyte uptake for the CT genotype compared to the CC genotype, was incorporated into parameter E_H (Equation 8). The *SLC22A1* genotype (CC genotype, GENO = 0; CT genotype, GENO = 1) was applied as an exponent of FUP.

$$E_H = \frac{f_{ub} \cdot CL_{int} \cdot FUP^{GENO}}{Q_{BH} + f_{ub} \cdot CL_{int} \cdot FUP^{GENO}} \quad (8)$$

The hepatocyte uptake of proguanil was estimated to be 0.42-fold lower in the CT genotype, compared to the CC genotype (Table 3).

Mixture model[25] was applied to estimate the genotype of three subjects whose genotype data was missing. Fixed genotype probability derived from our genotyping study was used. Applying the Hardy–Weinberg equation[26,27] to our observation, the expected allele frequency

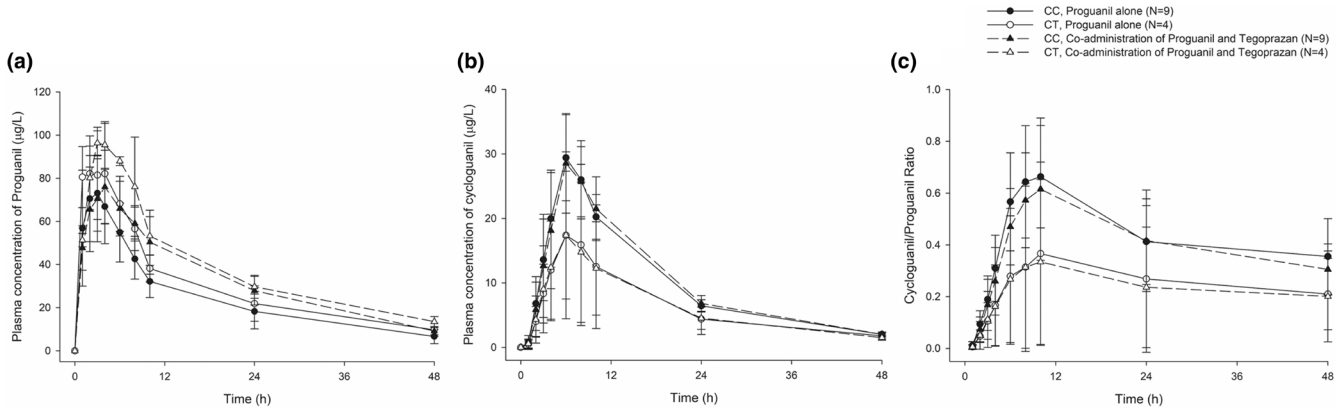

FIGURE 1 Mean plasma concentration-time curves of (a) proguanil, (b) cycloguanil, and (c) cycloguanil/proguanil ratio by *SLC22A1* genotype (CC or CT of 1022C>T polymorphism) following a single oral administration of atovaquone/proguanil 250/100 mg alone or co-administered with tegoprazan 50 mg. Bars represent the standard deviation.

TABLE 2 Summary of pharmacokinetic parameters of proguanil and cycloguanil by *SLC22A1* genotype (CC or CT of 1022C>T polymorphism) following a single oral administration of atovaquone/proguanil 250/100 mg alone or co-administered with tegoprazan 50 mg.

	CC			CT		
Genotype Parameter	**Proguanil alone (*N*=9)**	**Proguanil + Tegoprazan (*N*=9)**	**Total (*N*=9)**	**Proguanil alone (*N*=4)**	**Proguanil + Tegoprazan (*N*=4)**	**Total (*N*=4)**
Proguanil						
AUC_{last} (h*μg/L)	1143.06 ± 345.44	1267.51 ± 382.6	1205.29 ± 359.36	1420.90 ± 356.13	1583.10 ± 477.33	1502.00 ± 399.4
C_{max} (μg/L)	75.8 ± 16.98	82.14 ± 12.79	78.97 ± 14.94	89.08 ± 20.83	97.59 ± 20.82	93.34 ± 19.81
T_{max} (h)	3.00 [1.00–4.00]	3.00 [1.00–4.00]	3.00 [1.00–4.00]	3.50 [2.00–4.00]	1.50 [1.00–4.00]	2.50 [1.00–4.00]
$t_{1/2}$ (h)	16.18 ± 2.64	15.63 ± 1.4	15.90 ± 2.07	18.04 ± 3.73	18.10 ± 4.76	18.07 ± 3.96
Cycloguanil						
AUC_{last} (h*μg/L)	441.21 ± 72.41	448.95 ± 91.63	445.08 ± 80.22	276.08 ± 190.12	282.88 ± 145.45	279.48 ± 156.75
C_{max} (μg/L)	29.41 ± 6.67	28.92 ± 7.22	29.17 ± 6.75	17.49 ± 12.9	17.48 ± 10.03	17.48 ± 10.69
Metabolic ratio[a]	0.4 ± 0.14	0.37 ± 0.13	0.38 ± 0.13	0.21 ± 0.21	0.21 ± 0.2	0.21 ± 0.19
T_{max} (h)	6.00 [6.00–6.00]	6.00 [6.00–8.00]	6.00 [6.00–6.00]	6.00 [6.00–8.00]	6.00 [6.00–8.00]	6.00 [6.00–8.00]
$t_{1/2}$ (h)	11.23 ± 1.27	11.26 ± 1.37	11.24 ± 1.28	12.4 ± 1.66	13.63 ± 2.12	13.02 ± 1.88

Note: Data are presented as arithmetic mean ± standard deviation except for T_{max}, presented as median [minimum–maximum]. AUC_{last}, area under the plasma concentration-time curve (AUC) from time zero to the last observed timepoint; C_{max}, maximum plasma concentration; T_{max}, time to reach maximum plasma concentration; $t_{1/2}$, terminal elimination half-life.

[a]Ratio of AUC_{last} of cycloguanil to AUC_{last} of proguanil.

for the wild type allele was calculated to be 0.832, and the allele frequency of the 1022C>T variant allele was calculated to be 0.168. Based on this allele frequency, the proportions of the CC or CT genotype combinations were calculated and fixed to 0.7 for the CC genotype and 0.3 for the CT genotype. Consequently, the mixture model classified the genotype of three subjects as CC genotype.

Covariate analysis

Potential covariates were screened using SCM analysis: weight and estimated glomerular filtration rate (eGFR) on CL/F; aspartate transaminase (AST) and alanine transaminase (ALT) and weight on CL_{int}; body weight on central volume of distribution (V_d) of proguanil. However, no significant covariates were identified.

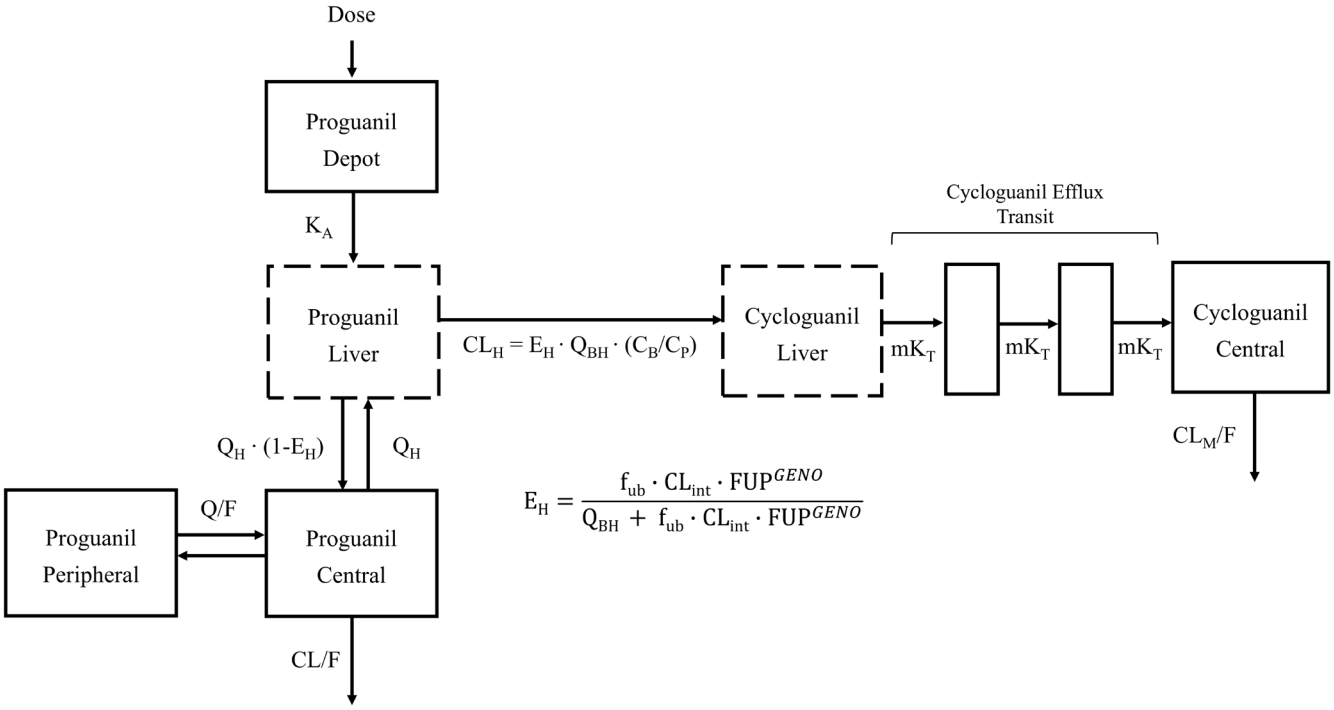

FIGURE 2 Structure of the final parent-metabolite joint population pharmacokinetic model. Central volume of cycloguanil was assumed to be equal to the central volume of proguanil. CL/F, apparent clearance of proguanil via non-cycloguanil metabolic pathway; CL_H, hepatic plasma clearance; CL_{int}, intrinsic clearance; CL_M/F, apparent clearance of cycloguanil; E_H, hepatic extraction ratio; f_{ub}, fraction unbound in blood; GENO, *SLC22A1* CC genotype was assigned to 0, CT genotype was assigned to 1; K_A, absorption rate constant of proguanil; mK_T, transit rate constant of cycloguanil; FUP, relative fraction of OCT1-mediated hepatocyte uptake for the *SLC22A1* 1022C>T CT genotype compared to the CC genotype, *Q*/*F*; apparent inter-compartmental clearance of proguanil; Q_{BH}, Hepatic blood flow; Q_H, Hepatic plasma flow.

Model evaluation

The goodness-of-fit plots and VPC plot demonstrated that the final PK model adequately described the observed data (Figure 3; Figure S3). The median parameters obtained from the bootstrap analysis were similar to the final parameter estimates of the population PK model and were included within the 95% CIs from the bootstrap analysis (Table S3).

DISCUSSION

To our knowledge, this was the first work to evaluate the PKs of proguanil and cycloguanil based on *SLC22A1* genotype in the Korean population. The genetic polymorphism *SLC22A1* 1022C>T, associated with decreased function of OCT1, led to an increase in the systemic exposure of proguanil while decreasing the systemic exposure of cycloguanil. The differences in the metabolic ratio of the CT and CC genotype were around twofold. Moreover, we developed a semi-physiological model of proguanil and cycloguanil to mechanistically explain the effect of the *SLC22A1* 1022C>T polymorphism on OCT1 activity. The result of genotyping and population PK modeling indicates that the decreased OCT1 transporter activity due to genetic polymorphism reduces the transport of proguanil into the hepatocyte, subsequently affecting its metabolism. Significantly, this finding suggests the importance of genetic polymorphisms of transporters in addition to metabolizing enzymes in drugs that primarily undergo hepatic metabolism.

When the Hardy–Weinberg equation was used to estimate the allele frequency, the proportion of the 1022C>T allele was calculated as 16.8%. In a large-scale genotyping study of human organic cation transporters, the allele frequency of the 1022C>T variant was about 16.7% in Koreans.[20] Despite the small sample size, the known genetic frequecy closely aligns with the obserbved frequency in this study, suggesting that these findings could be extrapolated to the entire Korean population. The allele frequencies in Vietnamese, Chinese, and German populations were 5.5%, 11.0%, and 2.0%, respectively,[20] indicating that the impact of this polymorphism may vary among different ethnicities.

When 6 SNPs of *SLC22A1* were genotyped in this study, only the 1022C>T mutation was identified. As the extent in reduction of OCT1 activity varies among different SNPs of *SLC22A1*,[14] it was an advantage to assess the specific

TABLE 3 Parameter estimates of the final population pharmacokinetic model of proguanil and cycloguanil.

Parameters (unit)	Final model		
	Estimate	RSE (%)	Shrinkage (%)
Proguanil			
K_A (1/h)	0.101	6.0	–
CL/F (L/h)	33.5	16.4	–
V_c/F (L)	58.4	24.1	–
Q/F (L/h)	41.4	7.2	–
V_p/F (L)	1400	26.0	–
CL_{int} (L/h)	78.3	18.6	–
FUP	0.416	54.1	–
IIV CL/F (%CV)	31.7	19.6	5.9
IIV V_c/F (%CV)	87.2	20.5	12.2
IIV Cl_{int} (%CV)	53.7	24.7	4.2
Proportional error	0.16	9.1	
Cycloguanil			
mK_T (1/h)	1.03	5.8	
CL_M/F (L/h)	97.0	13.8	
IIV mK_T (%CV)	15.5	18.1	18.8
Proportional error	0.124	12.7	
Additive error (μg/L)	0.372	18.5	

Abbreviations: CL/F, apparent clearance of proguanil via non-cycloguanil metabolic pathway; CL_{int}, intrinsic clearance; CL_M/F, apparent clearance of cycloguanil; CV, coefficient of variation calculated as $\sqrt{e^{\omega^2}-1}\times 100$; FUP, relative fraction of OCT1-mediated hepatocyte uptake for the *SLC22A1* 1022C>T CT genotype compared to the CC genotype; IIV, inter-individual variability; KA, absorption rate constant of proguanil; mK_T, transit rate constant of cycloguanil; Q/F, apparent inter-compartmental clearance of pgoruanil; RSE, relative standard error; V_c/F, apparent central volume of distribution of proguanil; V_p/F, apparent peripheral volume of distribution of proguanil.

effect of the 1022C>T mutation without the presence of other mutations. In addition, because all the subjects were normal metabolizers of CYP2C19, the potential co-effect of the polymorphism in *SLC22A1* and *CYP2C19* was ruled out as a confounding factor. However, further research on the intermediate and poor metabolizers of CYP2C19 in with varying OCT1 activity would also be valuable for the actual clinical setting.

A population PK model of proguanil with a simple 1-compartment structure already exists,[28] but it was limited in its ability to explain the population PKs of proguanil alone without considering its metabolite, cycloguanil. In this study, we developed a joint parent-metabolite model that included a well-stirred liver compartment. The efflux of the metabolite cycloguanil from the liver compartment to the central compartment was modeled using two transit compartments to fit the data. Although the mechanism of cycloguanil efflux after metabolism is unknown, other transporters or passive diffusion could be involved in the transport of cycloguanil. We described this process as occurring through transit compartments with a first-order absorption process, and the data was successfully characterized.

Genotypes of metabolizing enzymes are usually incorporated as covariates for clearance parameters in routine population PK analyses. However, directly applying a transporter genotype as a covariate to systemic clearance is not straightforward, considering that hepatic uptake and clearance do not have a proportional relationship. The concept of hepatic clearance described by a well-stirred model, and the extended clearance theory involving transporter influx, efflux, and diffusion through the membrane is well-established.[29,30] This population PK approach incorporating a well-stirred liver model and the parameter FUP, which represents transporter activity, made it possible to quantify the decrease in OCT1 activity caused by the *SLC22A1* 1022C>T polymorphism. Although there are population PK models that included a well-stirred liver compartment within the base structural model,[31,32] this was the first population PK approach that applied the extended clearance concept accounting for the transporter activity. Our study provides a methodological framework that is potentially applicable to the PK modeling of other drugs influenced by transporter activity.

In addition, during the population PK modeling process, we applied mixture models to estimate the genotype

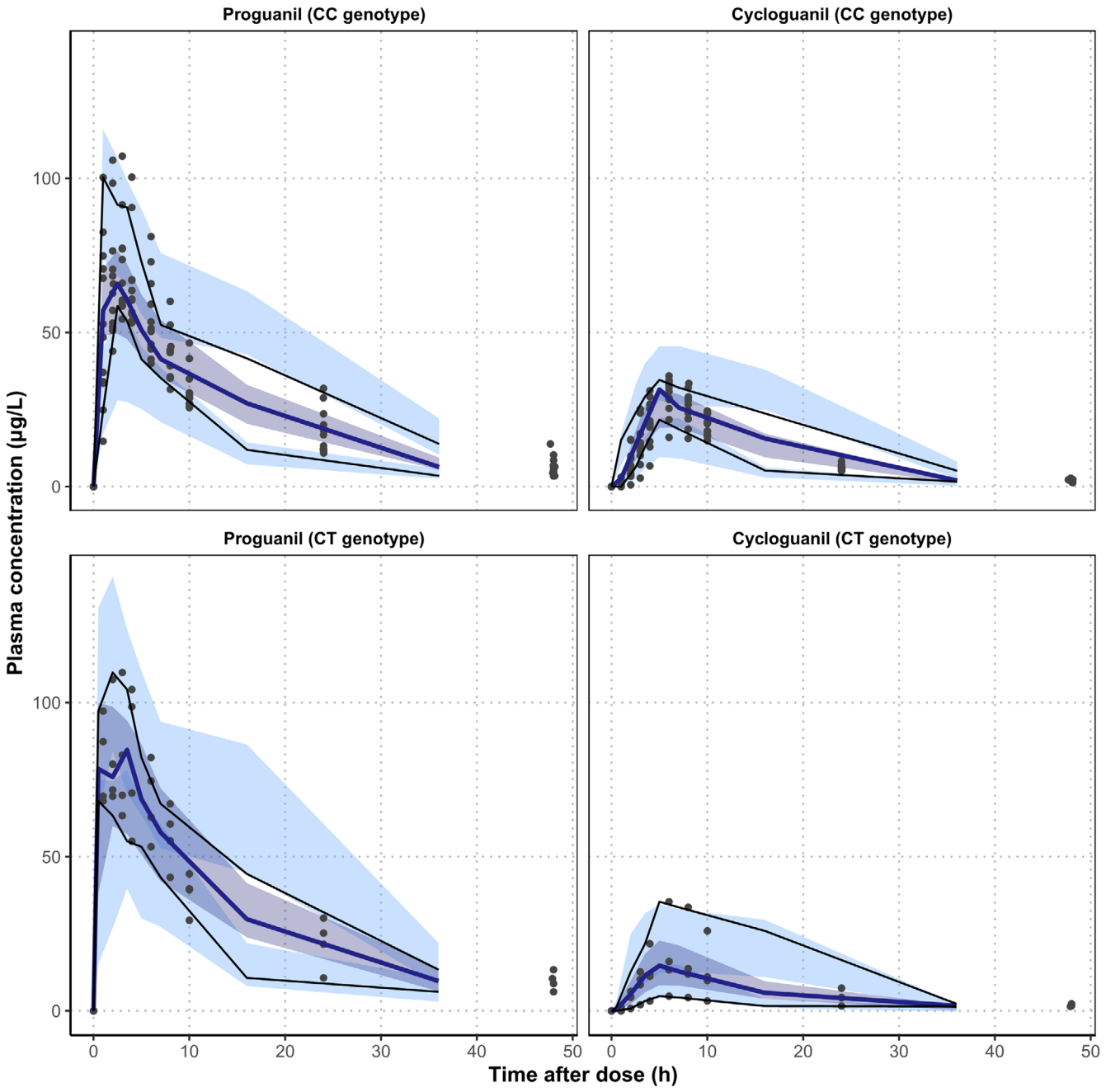

FIGURE 3 Visual predictive checks of the final population model of proguanil and cycloguanil by *SLC22A1* 1022C>T genotype. Top-left panel: Proguanil (CC genotype); Top-right panel: Cycloguanil (CC genotype); Bottom-left panel: Proguanil (CT genotype); Bottom-right panel: Cycloguanil (CT genotype). The closed circles represent the observed data. The blue solid lines represent the medians of the observed data. The black solid lines represent the 5th and 95th percentiles of the observed data. The shaded areas represent the 95% confidence intervals for the medians and for the 5th and 95th percentiles of the simulated data.

of three participants with missing genotype data. Although the genotype subpopulation for *SLC22A1* 1022C>T consists of the three possible genotype combinations (CC, CT, and TT), the probability of the TT genotype was very low. Therefore, the data were assumed to consist of two populations, with the probabilities of the CC and CT genotypes being 0.7 and 0.3, respectively. The mixture model classified the three subjects with missing genotypes of three subjects as CC genotype. This approach was effective in identifying the most likely genotype for the subjects with missing genotype data.

Although the metabolic ratio observed in the DDI study of vonoprazan and proguanil (0.84) remains relatively high in comparison to that of the subjects with the CC genotype in our study (0.4), the effect of *SLC22A1* 1022C>T polymorphism undoubtedly contributes to the

differences between the two study. Renal excretion accounts for approximately 40% to 60% of proguanil elimination.[33] The multidrug and toxin extrusion (MATE) protein 1, MATE2-K, and OCT2 expressed in the proximal tubule cells in the kidney are also known as transporters of proguanil.[7] The urinary excretion fraction was around 18.7% in the vonoprazan and proguanil study,[21] whereas it was around 33.4% in the tegoprazan and proguanil study.[22] Although no PK studies associated with genetic polymorphisms of these transporters have been conducted so far, the activity of transporters playing a major role in the renal elimination of proguanil could potentially be a contributing factor affecting the systemic exposure and disposition.

One of the limitations of this study is that it was a retrospective genotyping study with a relatively small sample size. Even though the differences in the PK profiles of proguanil and cycloguanil between the CC and CT genotypes were shown, a statistical comparison was not conducted due to the small sample size. Another limitation is that the subjects participated in this study were all healthy male volunteers, and the effect of *SLC22A1* polymorphism on the safety and efficacy of proguanil/cycloguanil in patients with malaria or females still need to be investigated.

CONCLUSION

The genetic polymorphism *SLC22A1* 1022C>T led to decreased hepatic uptake of proguanil by OCT1, consequently decreasing the metabolism of proguanil to cycloguanil through CYP2C19. A semi-physiologic population PK model of proguanil and cycloguanil mechanistically quantified the effect of *SLC22A1* 1022C>T on hepatocyte uptake of proguanil. This study suggests the significance of the hepatic transporter influencing the PKs of proguanil and cycloguanil.

AUTHOR CONTRIBUTIONS

All authors wrote the manuscript; J.Y.K., S.H.L., and E.S.Y. designed and performed the research. J.Y.K., S.H.L., C.S.P., and S.C.J. analyzed the data.

ACKNOWLEDGMENTS

J.Y. Khwarg received a scholarship from the BK21FOUR education program.

FUNDING INFORMATION

No funding was received for this work.

CONFLICT OF INTEREST STATEMENT

The authors declared no competing interests for this work.

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How to cite this article: Khwarg J, Yang E, Park CS, Ji SC, Yu K-S, Lee S. Effect of *SLC22A1* polymorphism on the pharmacokinetics of proguanil in Korean: A semi-physiologic population pharmacokinetic approach. *Clin Transl Sci*. 2024;17:e70103. doi:10.1111/cts.70103