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A Mechanism-Based Pharmacokinetic Enzyme Turnover Model for Dichloroacetic Acid Autoinhibition in Rats

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Abstract

Dichloroacetic acid (DCA), a halogenated organic acid, is a pyruvate dehydrogenase kinase inhibitor that has been used to treat congenital or acquired lactic acidosis and is currently in early-phase clinical trials for cancer treatment. DCA was found to inhibit its own metabolism by irreversibly inactivating glutathione transferase zeta 1 (GSTZ1-1), resulting in nonlinear kinetics and abnormally high accumulation ratio after repeated dosing. In this analysis, a semi-mechanistic pharmacokinetic enzyme turnover model was developed for the first time to capture DCA autoinhibition, gastrointestinal region-dependent absorption, and time-dependent change in bioavailability in rats. The maximum rate constant for DCA-induced GSTZ1-1 inactivation is estimated to be 0.96/h, which is 110 times that of the rate constant for GSTZ1-1 natural degradation (0.00875/h). The model-predicted DCA concentration that corresponds to 50% of maximum enzyme inhibition (EC₅₀) is 4.32 mg/L. The constructed pharmacokinetic enzyme turnover model, when applied to human data, could be used to predict the accumulation of DCA after repeated oral dosing, guide selection of dosing regimens in clinical studies, and facilitate clinical development of DCA.

Keywords

pharmacokinetics; physiological model; inhibition; bioavailability

Introduction

Dichloroacetic acid (DCA), a halogenated organic acid, has been regarded as an antidiabetic, lipid-lowering, and lactate-lowering drug because of its stimulating effect on the activity of the pyruvate dehydrogenase complex in mammalian cells.^{1–3} Clinically, it has been studied as an investigational drug to treat diabetes mellitus⁴ and acquired or congenital forms of

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lactic acidosis.^{5–7} Pharmacological dose of DCA used in human ranges from 10 to 50 mg/kg per day.⁸ Recently, DCA has been proposed as a potential metabolictargeting therapy for cancer because of its pyruvate dehydrogenase complex reactivation effect and substantial evidence for DCA antitumor effect in preclinical *in vitro* and *in vivo* models.⁹ On the other hand, a number of small size, open-label clinical studies showed that DCA has failed to prove the antitumor efficacy or has shown its toxicity.^{10,11} DCA is also an environmental contaminant in chlorine-disinfected water. The hepatocarcinogenicity of DCA in some strains of mice and rats has raised concern with regard to setting a legal upper limit for level of DCA in drinking water.¹² However, no epidemiological evidence is available to implicate an association between chronic consumption of low-dose DCA and human cancer incidence.¹³

As a small-molecule compound with therapeutic potential, DCA has several interesting characteristics in pharmacokinetics. First, after oral dosing, double peaks are observed in DCA concentration-time profiles in rats and humans.^{1,14,15} Saghir and Schultz¹⁵ proposed that the mechanism of the 2 absorption peaks is gastrointestinal (GI) region-dependent absorption, with a rapid absorption from the stomach and upper intestine and a delayed absorption from the colorectal region. Other explanations, such as enterohepatic circulation and variable gastric emptying rate, have also been explored, but seem unlikely to be the mechanism.¹⁵ Second, DCA inhibits its own metabolism. DCA is mainly eliminated by the liver through biotransformation catalyzed by cytosolic and mitochondrial glutathione transferase zeta 1 (GSTZ1-1). It has been reported that DCA can irreversibly inactivate GSTZ1-1 by covalently binding to the enzyme, making itself a mechanism-based self-inhibitor.¹⁶ In line with this autoinhibition mechanism, pharmacokinetic studies conducted in rodents, dogs, and humans all demonstrated clear time-dependent nonlinear kinetics of DCA, with dramatic decrease in clearance as well as abnormally high accumulation ratio after repeated dosing.^{14,17,18} Lastly, DCA demonstrates time-varying concentration-dependent oral bioavailability. It has been reported that the bioavailability of DCA in native rats is 9.68% with 5 mg/kg dose and 80.93% with 100 mg/kg dose, and the bioavailability of DCA in rats with prolonged exposure of DCA is 29.4% with 5 mg/kg dose and nearly 100% with 100 mg/kg dose.¹⁵ The time-dependent and concentration-dependent bioavailability of DCA could be explained by varying hepatic extraction ratios of DCA at different doses and different sampling times. This is not surprising because hepatic extraction ratio of DCA is dependent on its actual hepatic intrinsic clearance, which is both time dependent and concentration dependent due to DCA autoinhibition effect.

Because of the striking nonlinear kinetics of DCA, it is challenging to estimate the concentrations of DCA at different doses/time. To have a better understanding of the underlying processes and assessing the dose regimen adjustment, it is highly valuable to build a mechanism-based mathematic model to quantitatively describe the complicated kinetics of DCA. Currently, there are only a few modeling articles that describe DCA pharmacokinetics,^{16,19,20} and none of them have taken into consideration all those important characteristics in DCA pharmacokinetics as described above. The aim of this analysis was to develop a mechanistic pharmacokinetic model for DCA to characterize the unusual nonlinear pharmacokinetics of DCA observed in rats. Our model accounted for the double-peak phenomena, self-inhibited metabolism, and time- and concentration-dependent

bioavailability. The constructed framework of the semi-mechanistic pharmacokinetic model could be used to facilitate clinical development of DCA as well as assess the dose regimen adjustment that may be needed for DCA in clinical investigations.

Materials and Methods

Data Source

DCA plasma concentration data of rats from a published study¹⁷ was used for development of the mechanistic pharmacokinetic model. In that study, male Sprague-Dawley rats received 50 mg/kg DCA by oral gavage. DCA was administered once daily for 1 day or 2 days. Rat blood samples were collected 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after the last dosing. The rat plasma DCA concentrations were measured by gas chromatography mass spectrometry using previously published methods.^{21,22} A total of 174 observations from 20 rats were used. Sixteen rats (young rats) were 3–4 months of age and 4 rats (adult rats) were 16 months of age. Of the 16 young rats, 6 were dosed for 1 day and 10 were dosed for 2 days; all adult rats were dosed with DCA for 2 consecutive days.

Population Pharmacokinetic Modeling

Population pharmacokinetic modeling was performed using nonlinear mixed effects modeling software NONMEM 7.3.0 (Icon Development Solutions, Ellicott City, MD),²³ with runs and post-run analysis executed by Perl-speaks-NONMEM (PsN) 4.6.0 (Uppsala Pharmacometrics Groups, Uppsala, Sweden)²⁴ and Pirana 2.9.2 (Pirana Software & Consulting, San Francisco, CA). R 3.3.0 (R Core Team, 2016)²⁵ was used for plotting and statistical analysis. The first-order conditional estimation with interaction with a user-defined subroutine (ADVAN 13) was used throughout the model-building process to estimate the typical population parameters, random inter-individual variability (IIV), and residual variability between observed and individually predicted plasma DCA concentrations.

The IIVs of the pharmacokinetic parameters of DCA were estimated by an exponential model as follows:

$$P_i = P_{pop} * e^{\eta_i} \quad (1)$$

where P_i is the estimated parameter value for individual i , P_{pop} is the typical population estimate for the parameter, and η_i is the deviation of P_i from P_{pop} , with η_i assumed to be independent and normally distributed with a mean of 0 and variance ω^2 .

Residual variabilities, denoting the difference between observed and individually predicted DCA concentration, were modeled using additive error model, proportional error model, or combined additive and proportional error model. The combined additive and proportional error model was described as follows:

$$C_{ij} = C_{pred,ij}(1 + \varepsilon_{ij1}) + \varepsilon_{ij2} \quad (2)$$

where C_{ij} and $C_{pred;ij}$ represent the j th observed and predicted DCA concentrations for individual i , ε_{ij1} and ε_{ij2} represent the proportional and additive residual random error for observation j in individual i , with ε_{ij1} assumed to be independent and normally distributed with a mean of 0 and a variance of σ_1^2 , and with ε_{ij2} assumed to be independent and normally distributed with a mean of 0 and a variance of σ_2^2 . By setting σ_1 or σ_2 to 0, the resulting residual error model would be in the form of additive model or proportional model.

Absorption Model Development

The double peaks observed in the absorption phase of DCA was modeled by 2 parallel first-order absorption: one represents the immediate first-order absorption from stomach and upper intestine and the other represents the delayed first-order absorption process from the lower intestine. The parallel first-order absorption model was compared with the single first-order absorption model to see whether the additional absorption pathway would improve model fit.

Disposition Model Development

Several base disposition models, including 1/2-compartment models with linear/nonlinear (Michaelis-Menten)/mixed-order elimination from central compartment, were tested during the model-building phase. As noted earlier, DCA autoinhibition is caused by covalent binding of DCA with its major metabolizing enzyme GSTZ1-1, forming an inactive intermediate that is highly susceptible to further degradation. Therefore, the autoinhibition phenomenon of DCA is modeled using an enzyme turnover model with increasing DCA concentration leading to increment of GSTZ1-1 enzyme degradation rate constant. In the absence of DCA, the physiological process of production and elimination of GSTZ1-1 can be described by the following differential equation:

$$\frac{dA_{GSTZ1-1}}{dt} = k_{in} - k_{out} * A_{GSTZ1-1} \quad (3)$$

where $A_{GSTZ1-1}$ denotes the amount of GSTZ1-1 enzyme, k_{in} denotes the zero-order production rate of GSTZ1-1, and k_{out} denotes the first-order elimination rate constant of GSTZ1-1. At steady state, the production rate of GSTZ1-1 equals its elimination rate. Normalizing $A_{GSTZ1-1}$ to 1, we get $k_{in} = k_{out}$. The value assigned to $A_{GSTZ1-1}$ is a symbolic value chosen to simplify the calculation. The actual $A_{GSTZ1-1}$ at steady state is trivial to DCA model parameter estimates as it can be cancelled out in further steps. It should be noted that k_{in} is not a valid enzyme production rate in this case, but getting an accurate estimate of k_{in} is not the focus of current analysis.

In the presence of DCA, the degradation of GSTZ1-1 is stimulated, with the effect of DCA concentration on GSTZ1-1 enzyme degradation rate constant being modeled as an E_{max} model. The new degradation rate constant with the presence of DCA, which is denoted by k'_{out} , is described as follows:

$$k'_{out} = k_{out} + \frac{E_{max}}{EC_{50} + C} * C \quad (4)$$

where C represents DCA concentration at the inhibition site, E_{max} represents the maximum change in enzyme degradation rate, and EC_{50} represents the DCA concentration that results in 50% of the E_{max} effect. The inhibition site was assumed to be located in either the central compartment or a separate inhibition compartment. In the first case, C is the plasma concentration; in the second case, C is the concentration in an additional hypothetical inhibition compartment with a transfer rate constant between the central compartment and the inhibition compartment. k_{out} in model was fixed to 0.00875 L/h based on literature-reported GSTZ1-1 degradation rate constant in rats.²⁶ With regard to the inhibitable clearance of DCA, 2 different models were tested, one with 100% of DCA clearance being influenced by DCA-mediated GSTZ1-1 enzyme inhibition effect and the other with a portion of clearance being free from DCA autoinhibition.

Attempts were made to model the time-varying concentration-dependent bioavailability of DCA. It has been reported that DCA is nearly completely absorbed from the GI tract of rodents, as indicated by limited fecal recovery and good urine and exhaled air recovery of C^{14} after DCA oral dosage in rodents.^{27,28} Therefore, in our model, DCA is assumed to be completely absorbed from the GI tract and the presystemic loss of DCA is assumed to be caused solely by hepatic first-pass effect. Absorption rate-dependent extraction model^{29,30} was implemented to account for the change in hepatic extraction ratio along with the change in hepatic intrinsic clearance. The bioavailability of DCA can be calculated by

$$F = 1 - ER \quad (5)$$

where ER stands for the hepatic extraction ratio of DCA and can be calculated by

$$ER = \frac{Cl_i}{Q + Cl_i} \quad (6)$$

where Cl_i represents hepatic intrinsic clearance and Q represents rat hepatic blood flow. Q is calculated based on rat weight and literature-reported rat cardiac output³¹ and liver fraction of cardiac output,³² which is 2.58 L/h/kg^{3/4}. Based on “Well-Stirred” model,³³

$$Cl_h = \frac{Q * Cl_i}{Q + Cl_i} \quad (7)$$

where Cl_h denotes hepatic clearance. Because DCA is reported to be minimally bound to plasma proteins,¹ the free fraction of drug term in the original “Well-Stirred” model is

regarded as one in this case and not displayed in formula in this case. Both linear and nonlinear hepatic intrinsic clearance were tested in absorption rate–dependent model. The mathematical implementation of linear hepatic intrinsic clearance is

$$Cl_i = Cl_0 * A_{GSTZ1-1} + Cl_1 \quad (8)$$

in which Cl_0 represents the fraction of DCA hepatic intrinsic clearance that can be inhibited by DCA exposure, Cl_1 represents the portion of DCA hepatic intrinsic clearance that is free from DCA autoinhibition, and $A_{GSTZ1-1}$ represents the relative amount of available GSTZ1-1 enzyme. The details about incorporation of nonlinear hepatic intrinsic clearance in absorption rate–dependent extraction model were documented in another publication.³⁰ Another method developed by Gordi et al.,³⁴ previously used to incorporate autoinduction-induced change in artemisinin bioavailability, was also tried as an alternative method to describe the time course of DCA bioavailability.

As both young rats and adult rats with different body weights were included in the animal study, allometric scaling theory was used to relate DCA pharmacokinetic disposition parameters as well as rat hepatic blood flow to weight, with the allometric exponent fixed to 0.75 for clearance and hepatic blood flow, and 1 for volume of distribution.³⁵ Age was used as a binary variable (young/adult) and explored as a potential covariate on Cl_1 , Cl_0 , E_{max} , and DCA central volume of distribution. The effects of age on parameters were modeled as:

$$P = P_{young} * \theta_{adult}^{IND_{adult}} \quad (9)$$

where P is the typical pharmacokinetic parameter in that population, P_{young} is the pharmacokinetic parameter in young rats, θ_{adult} is the multiplicative factor describing the increase or decrease in P associated with the old rats, and IND_{adult} is an indicator variable equal to 1 for adult rats and 0 for young rats.

Model Selection and Evaluation

Model selection was based on biological and physiological plausibility, goodness-of-fit plots, individual fitted plots, stability of parameter estimates, prediction-corrected visual predictive checks (pcVPCs), normalized prediction distribution errors (NPDE),³⁶ and objective function value. The likelihood ratio test was used for comparing nested models where a decrease in the NONMEM objective function ($-2 \log$ likelihood) of 3.84 points was necessary to consider the improvement in model performance statistically significant at $\alpha = 0.05$. Bootstrap analysis of 1000 resamples with replacement was used to obtain the mean, standard error, and confidence interval of the final model parameter values as well as to evaluate the stability of the parameter estimates.

Model Simulation

Simulation was performed on the basis of the final parameter estimates of the selected model to predict the concentration-time profile for multiples doses of 50 mg/kg DCA (once daily

for 5 days) in a rat with a weight of 0.25 kg. In addition, DCA hepatic intrinsic clearance and extraction ratio were simulated over time throughout the dosing period. In addition, percentage of remaining DCA hepatic intrinsic clearance compared with pretreated DCA hepatic intrinsic clearance value was calculated.

Results

The final structure model was a 1-compartment disposition model incorporating a parallel first-order absorption model, an enzyme turnover component, and a rate-dependent extraction component with linear intrinsic hepatic clearance and a component of intrinsic hepatic clearance being free from DCA autoinhibition. The framework of the final DCA semi-mechanistic enzyme turnover model is shown in Figure 1.

A parallel first-order absorption model was selected over a single first-order absorption model because of its physiological plausibility and good model fitting in the absorption phase. A 1-compartment enzyme turnover model with concentration in central compartment linked to enzyme inhibition effect was selected to describe DCA disposition in rats with current data because of its physiological plausibility, successful convergence, reasonably good goodness-of-fit plots, and good model stability. The absorption rate-dependent extraction with linear or nonlinear hepatic intrinsic clearance and Gordi's method were all tried to account for the time-varying bioavailability of DCA but the latter 2 failed to converge. Only absorption rate-dependent extraction with linear hepatic intrinsic clearance succeeded in convergence, leading to a decrement of 28.8 in objective function value compared with the model without the time-varying bioavailability component.

Figure 2 depicts the goodness-of-fit plots, including scatter plots of the observed versus population-predicted concentration data (Fig. 2a), observed versus individual-predicted concentrations (Fig. 2b), conditional weighted residuals versus time (Fig. 2c), and population-predicted concentrations (Fig. 2d). In the population observed versus predicted concentration plot (Fig. 2a), there is a trend of overprediction at lower range of concentrations and underprediction at higher range of observations. The individual-predicted concentrations versus observed concentrations (Fig. 2b) were symmetrically distributed around the line of identity without bias, indicating that the final model describes DCA pharmacokinetics adequately at individual levels. In addition, conditional weighted residuals are distributed uniformly around the zero line when plotted either by time (Fig. 2c) or by population-predicted concentrations (Fig. 2d); these observations further suggest absence of significant systematic bias in the model fit. The individual fitting plots are given in Figure 3. Figure 4 shows the unstratified pcVPC of the final semi-mechanistic model, and Figure 5 shows the pcVPCs of the final model stratified by dosing days. The 5th, 50th, and 95th percentiles of the observations were within the 95% confidence interval of the corresponding prediction percentiles for the final model in pcVPC plots. The final model demonstrated a mean NPDE of -0.001 with a variance of 1.125. And the NPDE distribution agrees well with the theoretical $N(0,1)$ distribution (Figs. 6a and 6b). Additionally, no obvious trends were observed in the NPDE versus time and NPDE versus population predicted concentration plots (Figs. 6c and 6d). The goodness-of-fit plots, individual fitting plots, pcVPCs, and NPDE diagnostic plots all indicated that the semi-mechanistic model

adequately described the trend in rat DCA concentration-time profiles at both preinhibited and inhibited states.

In our final model, IIV terms were placed on absorption rate constant of the delayed absorption site (K_{a2}), absorption lag time for the delayed absorption site (T_{lag}), relative fraction of the agent absorbed in immediate rapid absorption site (F_1), and the portion of intrinsic clearance not inhibitable by DCA dosing (Cl_1). Off-diagonal IIV variance-covariance matrices were considered sufficient in describing the random effects and was selected over the full variance-covariance matrices. A combined proportional and additive residual error model best described the unexplained residual variability. Results from covariate test showed that age does not have significant impact on Cl_1 , Cl_0 , E_{max} , or volume of distribution.

The pharmacokinetic parameter estimates and bootstrap results for the final model are summarized in Table 1. All fixed effects parameter estimates are precisely estimated with reasonable standard errors (<40%). The absorption rate constant that corresponds to the immediate absorption compartment (2.88/h) is about 15 times that of the delayed absorption compartment (0.195/h). The estimated lag time for DCA entering the delayed absorption compartment is 1.4 h. The overall estimated intrinsic DCA hepatic clearance without the impact of DCA exposure is the addition of the estimates of Cl_0 and Cl_1 , which is $4\text{ L/h/kg}^{3/4}$. $Cl_1/(Cl_0 + Cl_1) \times 100\%$ gives the percentage of the DCA hepatic intrinsic clearance susceptible to DCA autoinhibition, which is 99%. The maximum rate constant for DCA-induced GSTZ1-1 inactivation is estimated to be 0.96/h, which is 110 times that of the rate constant for GSTZ1-1 natural degradation (0.00875/h). The model-predicted DCA concentration that corresponds to 50% of maximum enzyme inhibition (EC_{50}) is 4.32 mg/L.

Figure 7a shows the simulated DCA concentration-time profile for a 250-g rat after repeated oral daily administration of 50 mg/kg DCA for 5 days. From visual inspection of the simulated profile, steady state was reached approximately on the fourth day of dosing. Predicted DCA hepatic intrinsic clearance (Fig. 7b) decreases sharply after the first dose with a rebound before the second dose, resulting in an intrinsic clearance (Fig. 7c) being approximately 20% of its pretreated level at the time of second dosing. The change in DCA hepatic intrinsic clearance (Fig. 7c) becomes less dramatic shortly after the second dose and remains stable with small fluctuations along with the change in DCA plasma concentration. The hepatic extraction ratio for DCA was about 60% before DCA dosing and decreases to less than 20% after the second dosing along with the drop in DCA intrinsic clearance.

Discussion

A population pharmacokinetic model was successfully developed in this study to describe the complicated nonlinear pharmacokinetic profiles of DCA in rats. The proposed mechanistic pharmacokinetic enzyme turnover model was able to capture DCA autoinhibition, GI region-dependent absorption, and time-dependent change in bioavailability in rats. The model structure reflects current knowledge about DCA absorption and disposition and is in line with the results of *in vitro* and *in vivo* experiments.

The double peaks observed in the absorption phase after DCA oral dosing were well captured using a parallel first-order absorption model, which mechanistically mimics GI region-dependent absorption. Apart from GI region-dependent absorption, other common mechanisms for the second-peak phenomena include enterohepatic circulation and varying gastric-emptying rate. Based on the fact that GSTZ1-1, the primary enzyme responsible for DCA biotransformation, is a phase II enzyme and substrates of phase II enzymes are prone to undergoing enterohepatic recirculation, the intuitive explanation of the double-peak phenomenon of DCA would be GSTZ1-1-mediated enterohepatic recirculation. However, although GSTZ1-1 is a member of GST, it shows little activity with conventional GST substrates. Instead, GSTZ1-1 catalyzes DCA through a different mechanism—oxygenating DCA to glyoxylic acid.³⁷ As a result, DCA is not eliminated through biliary secretion and is unlikely to go through extensive enterohepatic recirculation.³⁸ Another evidence for DCA not undergoing enterohepatic recirculation is that the second peak is absent when DCA was administered intravenously,¹⁵ which should persistently exist if the underlying mechanism responsible for the second peak phenomena is enterohepatic recirculation. Variable gastric emptying is also not likely the cause because the fasting or fed condition, which would influence gastric-emptying rate, does not affect the appearance of the second peak.¹⁵ Therefore, although the exact underlying mechanism responsible for the double-peak phenomenon observed in DCA absorption phase remains unclear, GI region-dependent absorption represents the most plausible mechanism among all proposed explanations. Further investigations are warranted to validate the proposed mechanism.

The enzyme turnover model, with DCA central compartment concentration increasing the degradation rate of GSTZ1-1, was successfully used to model the autoinhibition metabolism of DCA. The typical preinhibited DCA intrinsic hepatic clearance in rat is estimated to be 4L/h/kg^{3/4}. Based on the simulation result (Fig. 7), with 50 mg/kg once-daily dose, DCA has a fast and dramatic effect in inhibiting its own metabolism, as shown by the sharp decrease in model-predicted DCA intrinsic clearance (Fig. 7b) shortly after the first dosing. A close-to-maximum inhibition was achieved after the second dosing, then the DCA intrinsic hepatic clearance remained relatively constant with repeated daily oral dosing. The hepatic extraction ratio (Fig. 7c) was about 60% before DCA treatment and quickly dropped to less than 20% after the second dosing. This changes DCA from a drug with intermediate extraction to a drug with low extraction. This indicates that hepatic clearance of DCA can be dependent (when it has intermediate extraction ratio)/relatively independent (when it has low extraction ratio) on hepatic blood flow, depending on the real-time DCA hepatic intrinsic clearance. The existence of a portion of DCA intrinsic hepatic clearance free from DCA self-inhibition is in accordance with a previously published study.¹⁶ There are 2 possible explanations for the existence of a portion of DCA clearance not influenced by the presence of DCA: (1) alternative DCA metabolic pathway exists apart from GSTZ1-1-mediated metabolism; (2) DCA is unable to fully inhibit GSTZ1-1 activity in rats with the current dosing scheme. To date, GSTZ1-1 is the only enzyme identified in the direct biotransformation of DCA. Alternative pathways of DCA metabolism involving free-radical formation and reductive dechlorination to monochloroacetic acid have been proposed,¹ but experimental study failed to validate the existence of this secondary DCA metabolic pathway in liver.^{39,40} Further studies are needed to unravel the mechanism.

There are limitations with our current analysis. As the pharmacokinetic model only included observations from one dose level, concentration-time profiles from other dose levels cannot be reliably simulated. Also, the concentrations were only measured for 2 days, and it is predicted that 50 mg/kg dosing in rats can result in nearly maximum inhibition in DCA metabolism after 2 days as seen in the simulation plot. However, there is no direct evidence to show whether the DCA metabolic capacity can decrease in a different pattern with additional dosing. Another limitation is model scale-up. Because of the interspecies difference in preinhibited liver GSTZ1-1 activity, GSTZ1-1 turnover rate, and the response of GSTZ1-1 inhibition in presence of DCA, the semi-mechanistic pharmacokinetic model cannot be directly scaled to other species, including human, by allometric scaling. In an *in vitro* study, in which mouse, rat, or human liver cytosol was incubated with various concentrations of DCA, the calculated rate constants for DCA-dependent inactivation of GSTZ1-1 were different between species, with the order being rat > mouse > human.⁴¹ In another *in vitro* study, there is a 5-fold difference in intrinsic metabolic clearance of DCA calculated from the metabolism experiment using liver cytosol of naive rats and human.¹⁵ In a physiologically based pharmacokinetic model that used observed plasma DCA concentrations in naive and 2 g/L DCA pretreated rats and mice, the estimated reduction in metabolism between naive and 2 g/L pretreated rodents was 99% in rats and 76% in mice, indicating species difference in DCA inhibition constant or maximum inhibition effect.¹⁶ With the large interspecies difference in enzyme activities and responses to DCA inhibition, the scale-up of DCA pharmacokinetics should be done only after carefully evaluating the enzyme kinetic differences between species. Although the enzyme kinetics differ dramatically between rats and human, the double-peak phenomena, the autoinhibition metabolism and dose- and time-dependent bioavailability are observed for both species. Therefore, the structure of the semi-mechanistic pharmacokinetic model developed based on rat plasma DCA concentrations should be easily adapted to model human data.

In summary, a mechanistic pharmacokinetic turnover model was successfully built to describe the complicated nonlinear pharmacokinetics of DCA in rats. It is the first mechanistic model that incorporates the processes of DCA autoinhibition, GI region-dependent absorption, and time-dependent change in bioavailability at the same time. The established model framework, when applied to human data, could be used to predict the accumulation of DCA after repeated oral dosing, guide selection of dosing regimens in clinical studies, and facilitate clinical development of DCA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations used

DCA	dichloroacetic acid
GSTZ1-1	glutathione transferase zeta 1
GI	gastrointestinal

IIV	inter-individual variability
pcVPC	prediction-corrected visual predictive check

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Figure 1.

Schematic diagram of the DCA semi-mechanistic enzyme turnover model. F1, fraction of drug that gets into absorption site I; T_{lag} , lag time in absorption for absorption site II; K_{a1} , absorption rate constant for absorption site I; K_{a2} , absorption rate constant for absorption site II; ER, hepatic extraction ratio; Q, hepatic blood flow; Cl_i , hepatic intrinsic clearance; Cp, plasma concentration; Cl_h , hepatic clearance; Cl_0 , the portion of intrinsic hepatic clearance that can be inhibited by DCA dosing; Cl_1 , the portion of intrinsic hepatic clearance that is not inhibited by DCA dosing; $AGSTzeta$, amount of GSTZ1-1 enzyme; k_{in} , zero-order input rate for enzyme synthesis; k_{out} , baseline first-order rate constant for enzyme degradation before DCA inhibition; k'_{out} , actual enzyme degradation rate constant with the presence of DCA; E_{max} , maximum change in enzyme degradation rate; EC_{50} , concentration that results in 50% of the E_{max} effect.

Figure 2. Goodness-of-fit plots. (a) Observed versus population-predicted DCA concentrations. (b) Observed versus individual-predicted DCA concentrations. (c) Conditional weighted residuals versus time. (d) Conditional weighted residuals versus population-predicted DCA concentrations.

Figure 3.

Individual model-predicted concentrations. Empty circles, observed DCA concentrations; solid line, model-predicted DCA concentrations.

Figure 4.

Prediction-corrected visual predictive check. The open circles represent the observed concentrations, the solid lines represent the median of the observed data, the dashed line represents the 5th and 95th percentiles for the observed data, and the shaded areas represent the 95% confidence intervals surrounding the 5th, 50th, and 95th of the prediction percentiles.

Figure 5. pcVPC stratified by sampling time (1 day after dosing and 2 days after dosing). The left panel is the pcVPC for observations after the first dose. The right panel is the pcVPC for observations after the second dose. The denotation of the plots is the same as in Figure 4.

Figure 6. NPDE plots: (a) Q-Q plot of the NPDE; (b) histogram of the NPDE; (c) NPDE versus time; (d) NPDE versus predicted concentrations.

Figure 7. Simulated time profiles of DCA concentration (a), DCA hepatic intrinsic clearance (b), and DCA hepatic extraction ratio (c) for a 250-g rat orally dosed with 50 mg/kg DCA for 5 days.

Table 1

Final Model Parameter Estimates and Bootstrap Results

Parameter	Bootstrap Mean	Bootstrap RSE%	Bootstrap 95% CI
K_{a1} (/h)	2.88	10	2.24, 3.57
K_{a2} (/h)	0.195	19	0.14, 0.29
Cl_0 (L/h/kg ^{3/4})	3.95	15	3.23, 5.77
Cl_1 (L/h/kg ^{3/4})	0.048	26	0.0188, 0.0736
V (L/kg)	0.350	12	0.250, 0.435
K_{out} (/h)	0.00875 FIXED ¹⁶		
E_{max} (/h)	0.96	22	0.79, 1.57
EC_{50} (mg/L)	4.32	36	3.34, 9.09
T_{lag} (/h)	1.4	12	1.01, 1.70
$F1$	0.511	9	0.41, 0.63
Q (L/h/kg ^{3/4})	2.58 FIXED ^{19,20}		
$\omega^2(K_{a2})$	0.607	33	0.176, 1.03
$\omega^2(T_{lag})$	0.288	52	0.041, 0.717
$\omega^2(F1)$	0.233	31	0.075, 0.333
$\omega^2(Cl_1)$	1.24	49	0.412, 2.801
σ^2 (Proportional error)	0.0466	21	0.028, 0.068
σ^2 (Additive error)	0.256	48	0.066, 0.515

RSE, relative standard error.