

Modeling the Transfer of Sildenafil Across the Placenta with Data of an *Ex-Vivo* Human Cotyledon Perfusion Experiment



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INTRODUCTION

Sildenafil (SIL) is a selective phosphodiesterase 5 (PDE5) inhibitor approved by FDA and EMA for the treatment of erectile dysfunction and pulmonary hypertension (PH) in adults. Over the years, there has been growing interest in the use of SIL during pregnancy for maternal and fetal conditions. It is currently under evaluation as a treatment for several pregnancy complications, such as pre-eclampsia and intrauterine growth restriction and the efficacy of maternally administered SIL in the prevention of PH in neonates with congenital diaphragmatic hernia is explored. To guide selection of the maternal dose required to achieve therapeutic fetal exposure, the trans-placental passage of SIL was quantified in an *ex-vivo* human dually perfused cotyledon experiment¹.

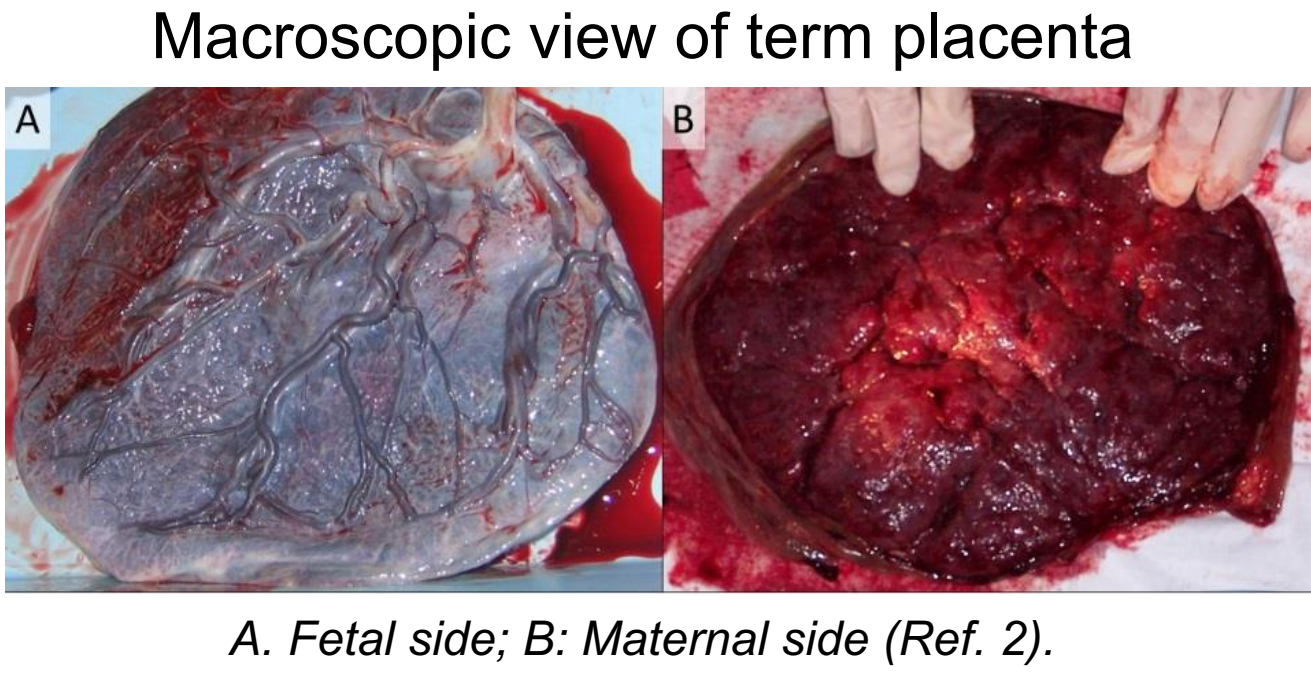
Objective

To estimate the trans-placental transfer parameters of SIL, *i.e.* passive diffusion clearance in the cotyledon (CL_{cot}), placental elimination (k_{PE}) and placental partition coefficient ($K_{p,pl}$) of SIL from this *ex-vivo* human cotyledon perfusion experiment.

METHODS

Ex-Vivo Human Cotyledon Perfusion Experiment

- Six placentas were collected after term delivery from healthy volunteers, cannulated and dually perfused.
- Sildenafil citrate was added to maternal circulation at two concentrations, 50 ng/ml (*i.e.* therapeutic concentration (TC); N=3) and 500 ng/mL (*i.e.* maximum tolerated concentration (MC); N=3).
- Antipyrine (AP), a small non-protein bound molecule that passes the placental barrier by passive diffusion, was used as internal control and added to maternal reservoir at 100 µg/mL (N=6).
- Bovine Serum Albumin (BSA) at a concentration of 30 and 40 mg/mL was added to the maternal and fetal medium, respectively.
- Maternal and fetal circulation was established at a flow rate of 14 mL/min (Q_m) and 6 mL/min (Q_f), respectively.
- Samples were collected from both the fetal and maternal reservoir at 0, 6, 30, 90, 120, 150 and 180 min. Reservoir volumes (V_f and V_m) were 280 mL.
- The cotyledon volumes were assumed to be 23 mL and 35 mL for maternal (V_{mp}) and foetal (V_{fp}), respectively.
- Concentrations of SIL, its desmethyl metabolite (DM)-SIL and AP were determined by validated LC-MSMS or -UV methods.



Data included

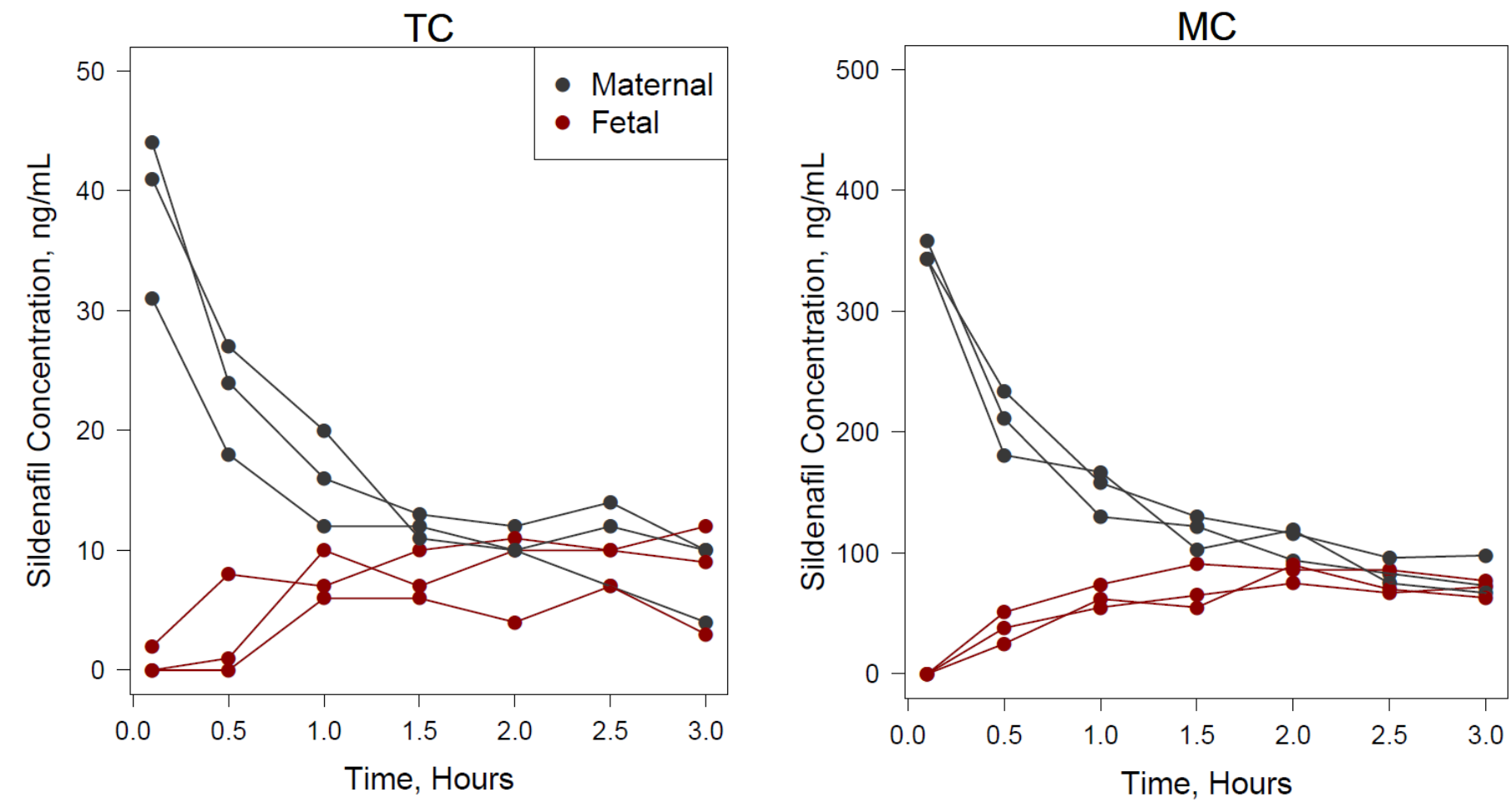
A total of 84 maternal and 63 fetal reservoir samples from 6 placentas were available for the analysis. To stabilize the model, AP data were modeled simultaneously. Only data for SIL are presented.

Model Development

Transfer of SIL across the placenta was modeled as a cotyledon split into maternal (M) and fetal (F) compartments³. Data were analyzed by nonlinear mixed effects modeling using NONMEM[®] 7.3.0⁴. The FOCE method with interaction was used for estimation of the model parameters. Model selection was based on OFV from the NONMEM output and graphical assessment using R for Mac (version 3.4.0). Between-subject variability (IIV) was implemented using an exponential error model and the residual error was described using a proportional model.

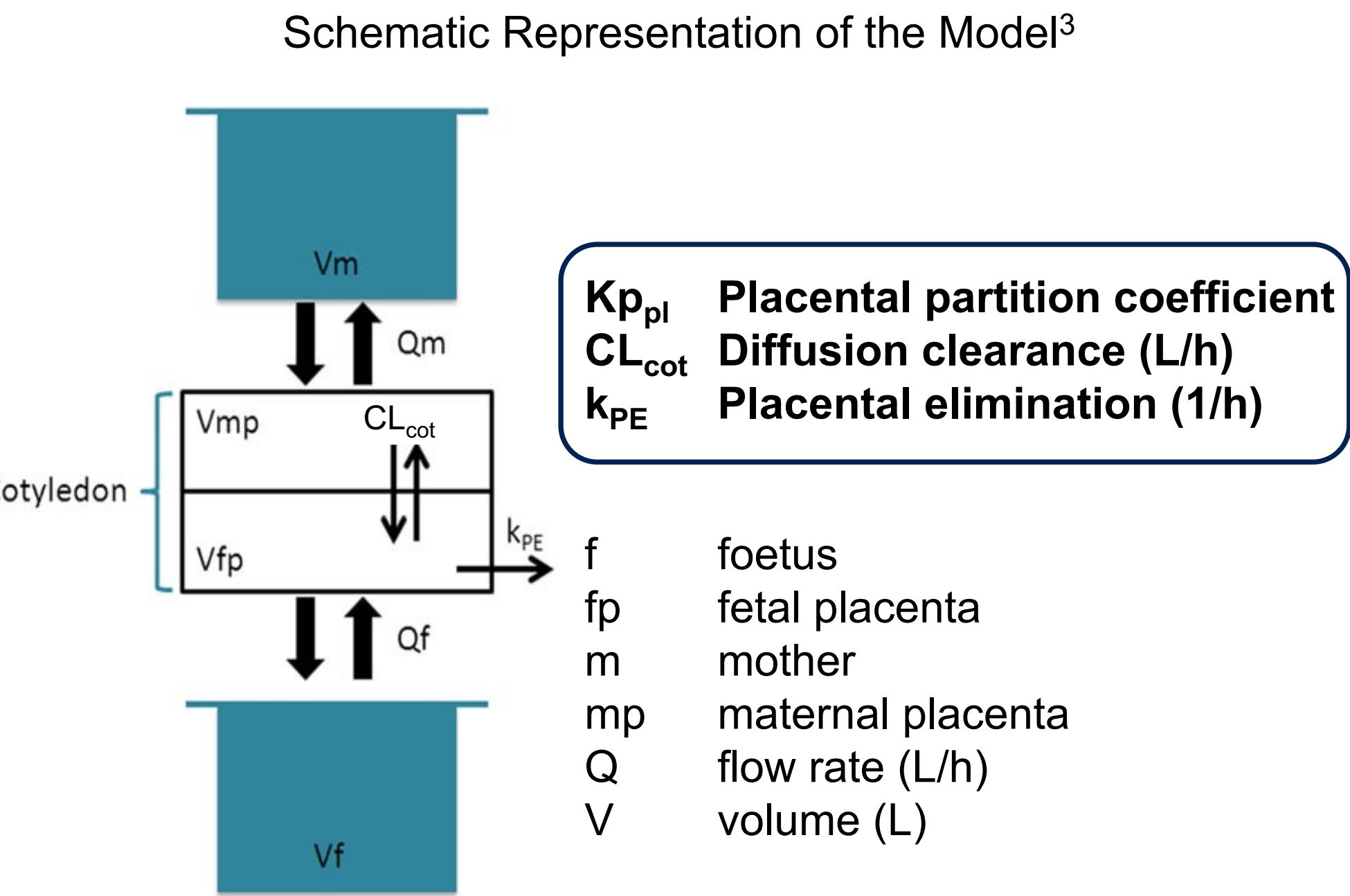
RESULTS

Observed Data of SIL

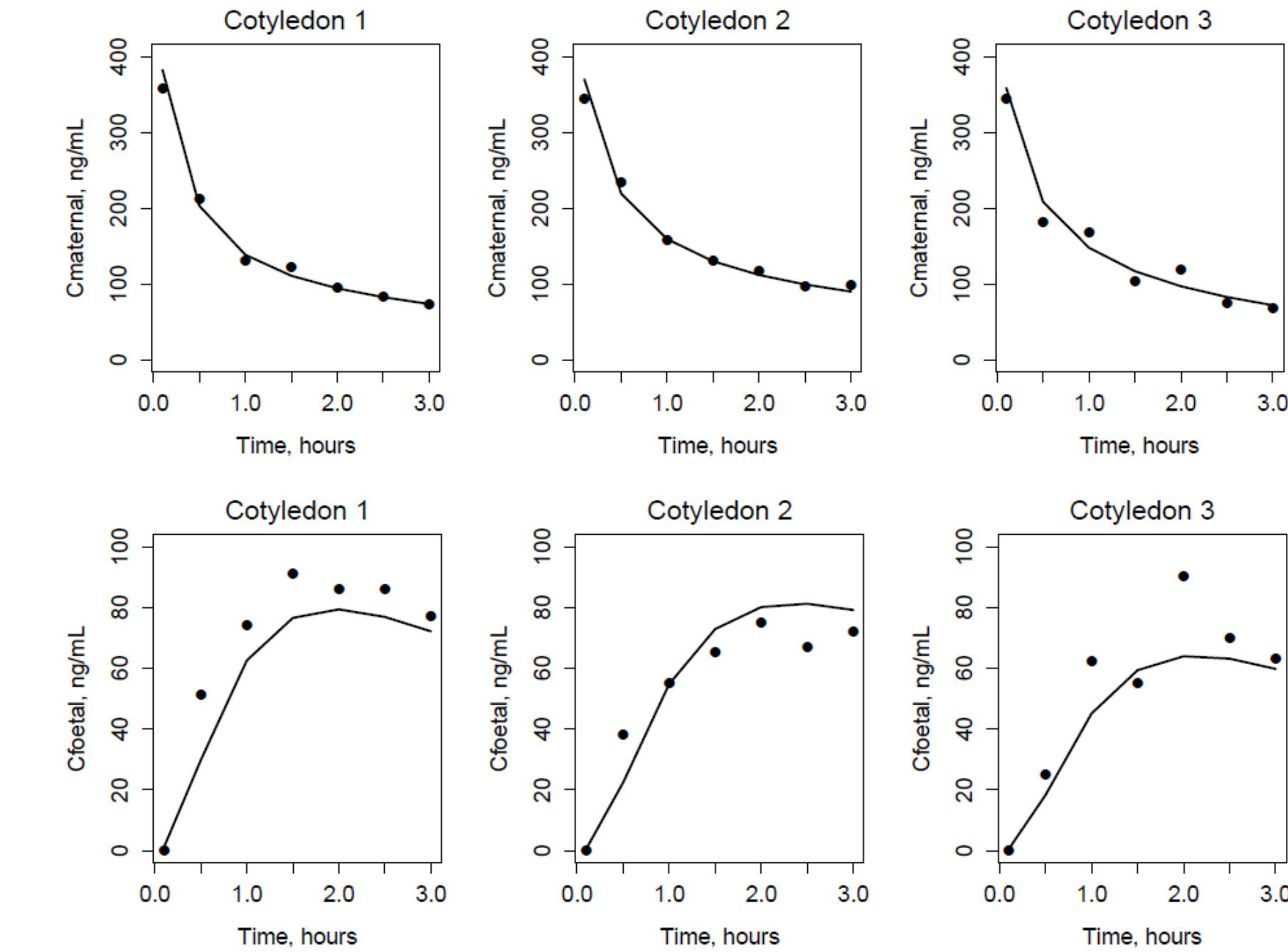


SIL crossed the placenta at both TC and MC. Both maternal and fetal levels reached a plateau at 90-120 min. CL_{cot} was 3.16 ± 0.65 mL/min (MC) and 2.38 ± 1.22 mL/min (TC) at the end of the perfusion, the FM ratio was 0.95 ± 0.22 (TC) and 0.91 ± 0.16 (MC). DM-SIL was not detected in any sample, suggesting negligible placental CYP3A-mediated metabolism.

Transport Model



Model Predicted SIL Concentrations in Maternal and Fetal Reservoirs (MC)



Observed concentrations (closed circles) and model predicted concentrations (solid lines).

A 4-compartment transport model provided an adequate description of the observed *ex-vivo* data of SIL in the fetal and maternal reservoir.

Trans-Placental Transfer Parameters from Final Model

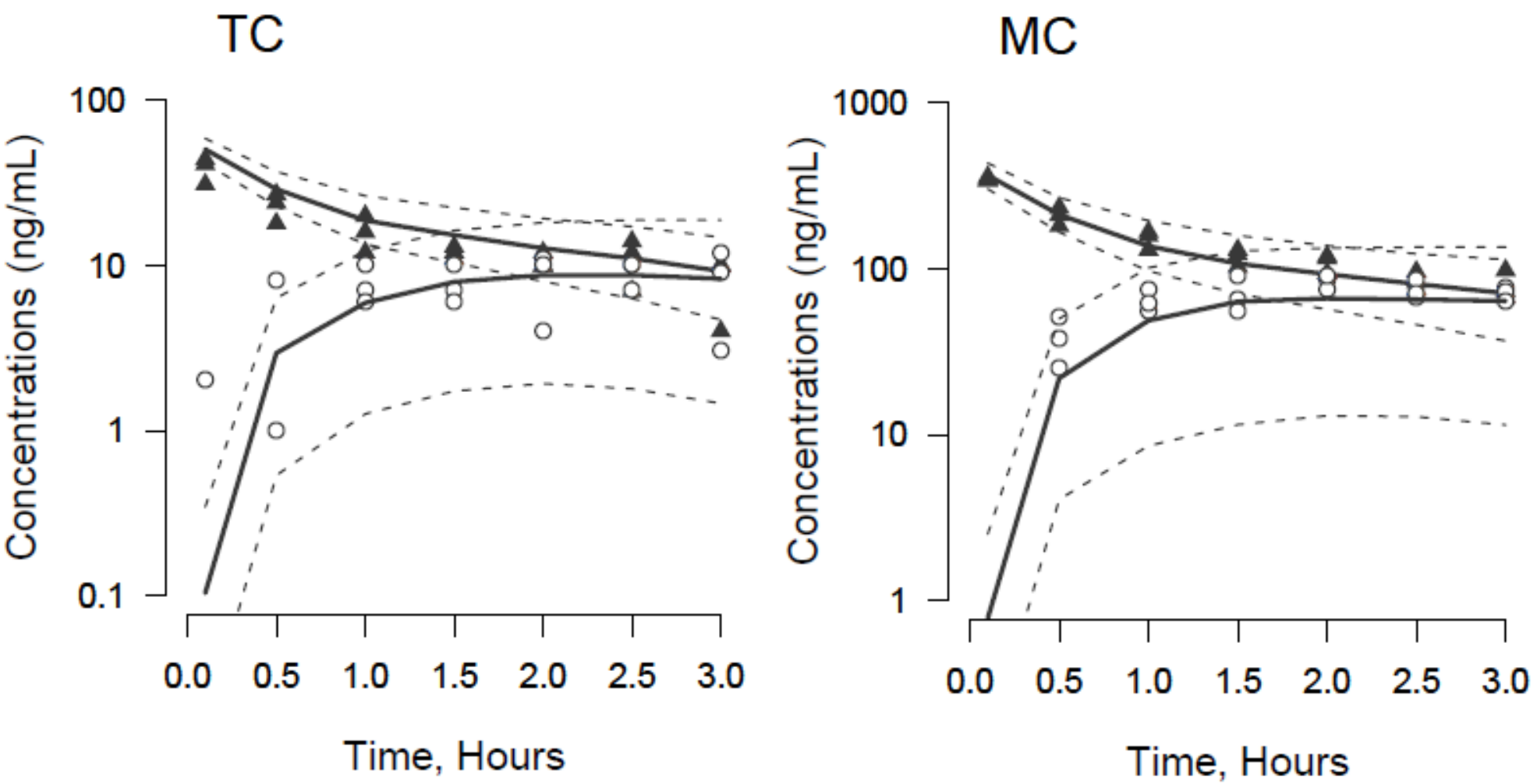
Parameter	Estimate	RSE (%)	IIV (%CV)
$K_{p,pl}$	11.7	11	26
CL_{cot} (L/h)	0.152	60	81
k_{PE} (1/h)	0.584	28	53
$\sigma_{prop} C_m$	10	37	
$\sigma_{prop} C_f$	48	8.9	

RSE relative standard error, σ_{prop} residual variability (proportional)

RESULTS (cont.)

The model parameters of SIL were estimated with acceptable precision. There was considerable IIV on the parameters, especially on CL_{cot} , which might be explained by the possible contribution of other factors like drug transporters, metabolism (other than CYP3A-mediated) and/or protein binding. The estimated k_{PE} was small and may represent leakage and/or metabolism via other pathway(s).

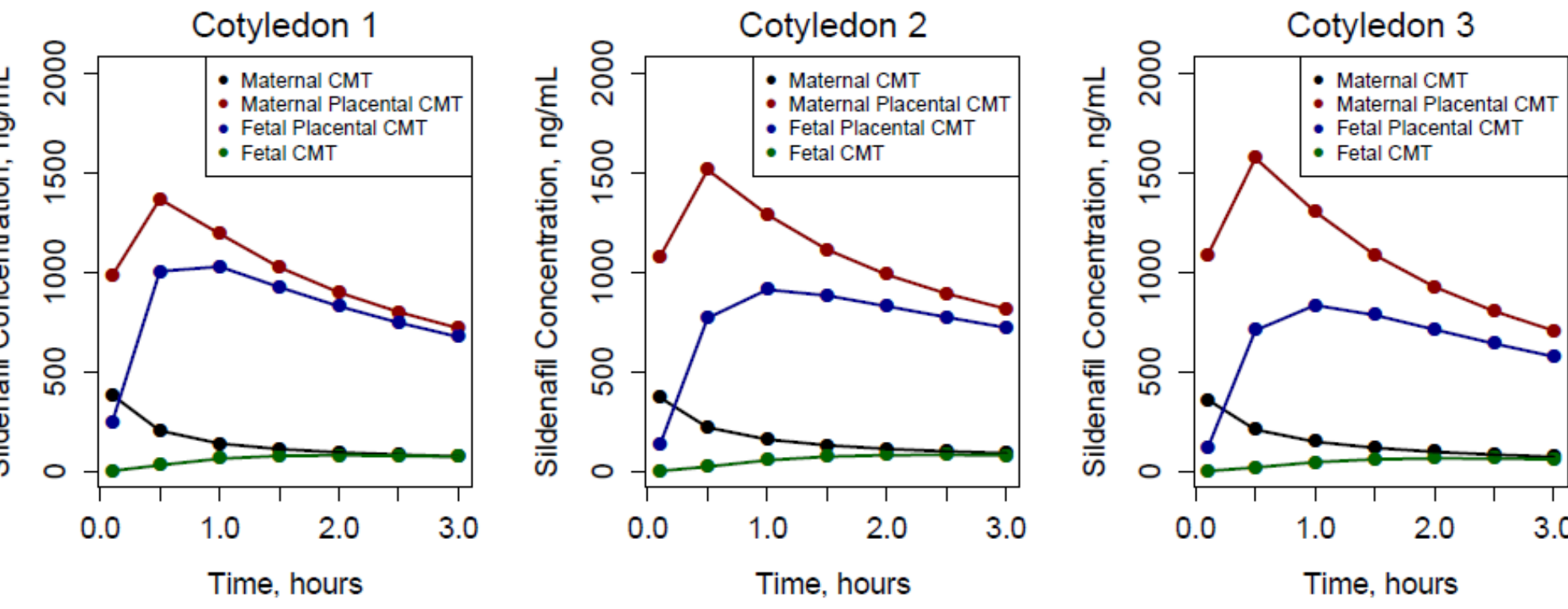
Visual Predictive Check (VPC)



Concentrations observed for fetal side (open circle) and maternal side (closed triangle) compared with estimated median profiles (solid lines) and 95% prediction intervals (black dotted line).

The VPC of the model confirmed that the average prediction matched the observed concentrations. The variability was well estimated for TC, but overpredicted for MC.

Model Predicted Concentrations per Compartment (MC)



CMT=Compartment.

At the end of the infusion, FM ratios were high (> 90%) and SIL seems to accumulate in the placental tissue.

CONCLUSIONS

- SIL crosses the term placenta *ex-vivo*.
- The trans-placental transfer parameters could be estimated from the *ex-vivo* human cotyledon perfusion experiment using the 4-compartment transport model.
- The estimated values of these parameters will be implemented in a pregnant-physiologically based pharmacokinetic (p-PBPK) model in order to be able to predict the PK profiles of SIL in the fetus.

REFERENCES

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ACKNOWLEDGEMENTS

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