pharmaceutics

# Supplementary Materials: Validation of Pharmacological Protocols for Targeted Inhibition of Canalicular MRP2 Activity in Hepatocytes Using [$^{99m}$Tc]mebrofenin Imaging in Rats

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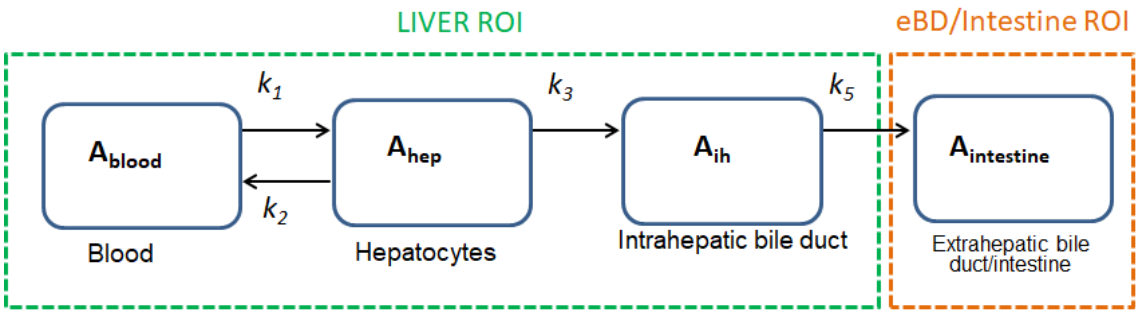


**Figure S1.** Four-compartment pharmacokinetic model. $k_1$ and $k_2$ describe the transfer of [$^{99m}$Tc]mebrofenin between blood and hepatocytes, $k_3$ from hepatocytes into the intrahepatic bile ducts and $k_5$ from the intrahepatic bile ducts to the intestine. ROI means Region of Interest, eBD means Extrahepatic bile duct.

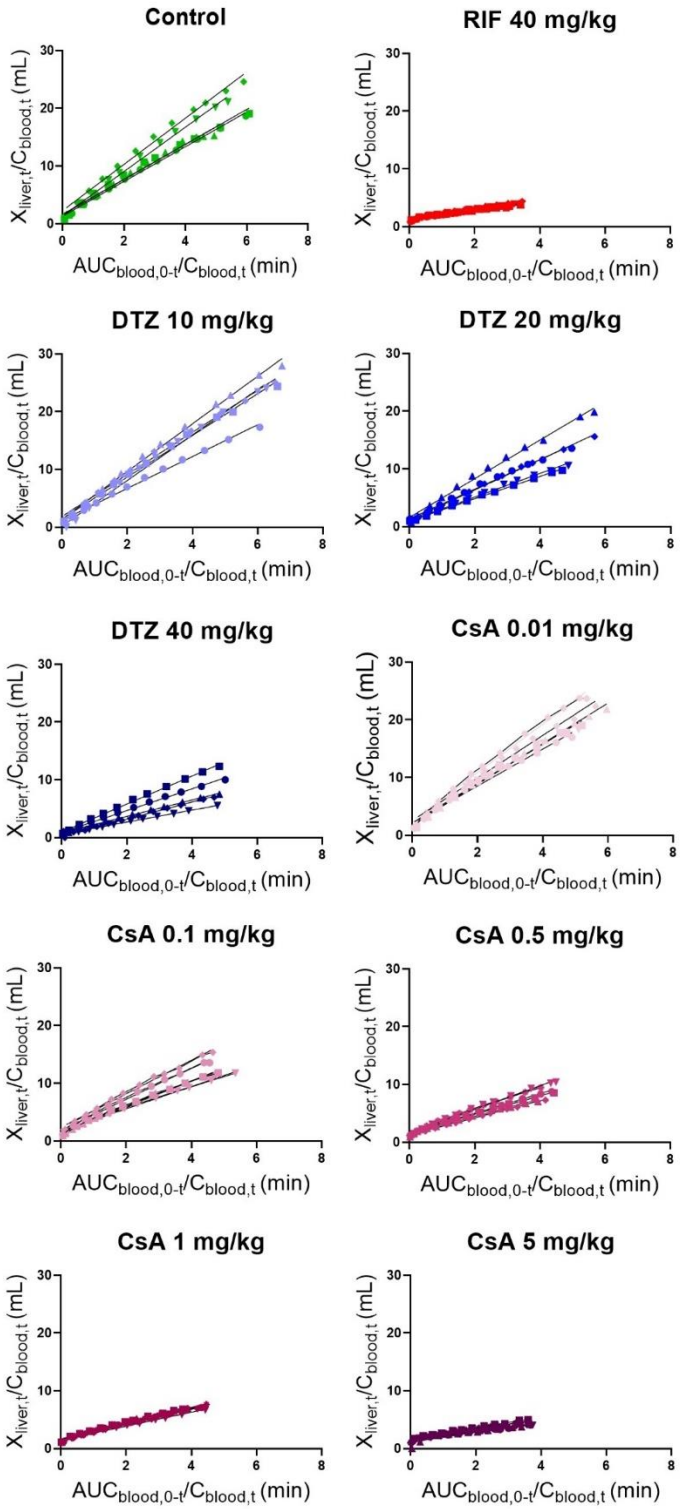


**Figure S2.** Integration plots for all investigated groups to estimate the uptake clearance of [$^{99m}$Tc]mebrofenin from blood into the liver for control animals and animals treated by rifampicin (RIF), diltiazem (DTZ) and cyclosporin A (CsA). $CL_{uptake}$ and $V_E$ correspond to the slope of the linear regression line and the Y-interception respectively.

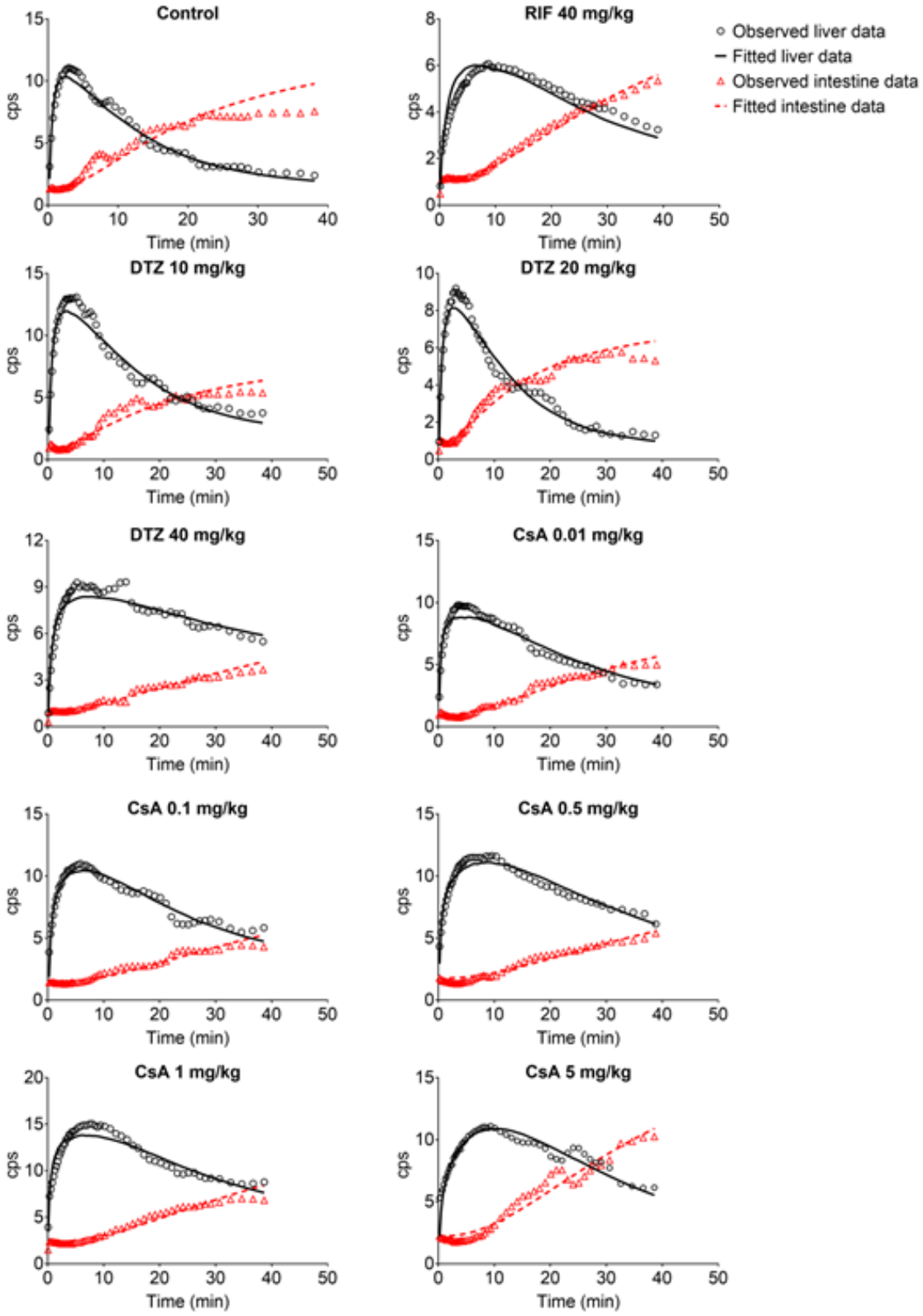


**Figure S3.** Time activity curves (cps: counts per second) of observed and fitted data in one representative subject of each study group for control animals and animals treated by rifampicin (RIF), diltiazem (DTZ) and cyclosporin A (CsA).

**Table S1.** Pharmacokinetic parameters obtained with the four-compartment model for the different study groups.

| Group | $k_1$ (min$^{-1}$) | $k_2$ (min$^{-1}$) | $k_3$ (min$^{-1}$) | $k_5$ (min$^{-1}$) |
|---|---|---|---|---|
| Control | 7.37 ± 1.62 (4.8 - 16.0) | 0.15 ± 0.08 (7.2 - 30.8) | 0.15 ± 0.08 (3.9 - 11.9) | 0.12 ± 0.03 (2.4 - 12.5) |
| RIF (40mg/kg) | 0.89 ± 0.09 * (3.6 - 7.6) | 0.01 ± 0.01 * (28.7 - 125.7) | 0.05 ± 0.02 * (2.4 - 12.7) | 0.64 ± 0.88 (13.6 - 31.7) |
| DTZ (10 mg/kg) | 7.20 ± 0.90 (2.7 - 10.2) | 0.10 ± 0.04 (3.1 - 16.2) | 0.06 ± 0.04 * (1.6 - 6.7) | 0.22 ± 0.16 (4.1 - 15.9) |
| DTZ (20 mg/kg) | 5.21 ± 1.35 * (2.4 - 9.8) | 0.06 ± 0.03 * (3.6 - 25.8) | 0.05 ± 0.01 * (1.3 - 29.1) | 0.31 ± 0.20 (5.2 - 27.8) |
| DTZ (40 mg/kg) | 2.76 ± 0.8 9* (2.6 - 10.2) | 0.02 ± 0.01 * (6.1 - 74.2) | 0.06 ± 0.03 * (1.9 - 10.9) | 0.31 ± 0.24 (6.9 - 27.1) |
| CsA (0.01 mg/kg) | 8.69 ± 1.90 (1.6 - 4.8) | 0.09 ± 0.02 (3.1 - 7.9) | 0.03 ± 0.01 * (1.6 - 7.8) | 0.34 ± 0.31 (6.2 - 18.5) |
| CsA (0.1 mg/kg) | 5.43 ± 1.75 (1.2 - 6.3) | 0.07 ± 0.02 * (3.0 - 11.7) | 0.03 ± 0.01 * (2.9 - 11.4) | 0.10 ± 0.05 (6.7 - 13.6) |
| CsA (0.5 mg/kg) | 3.90 ± 0.67 * (1.4 - 5.7) | 0.05 ± 0.01 * (3.6 - 17.7) | 0.05 ± 0.03 * (6.5 - 9.8) | 0.09 ± 0.04 (5.8 - 19.3) |
| CsA (1 mg/kg) | 3.73 ± 0.39 * (2.2 - 4.6) | 0.04 ± 0.02 * (3.8 - 25.5.) | 0.03 ± 0.02 * (3.2 - 22.4) | 0.13 ± 0.07 (5.7 - 21.9) |
| CsA (5 mg/kg) | 2.81 ± 0.86 * (2.2 - 10.6) | 0.04 ± 0.02 * (10.4 - 37.0) | 0.15 ± 0.08 (6.8 - 37.6) | 0.06 ± 0.02 (2.2 - 42.5) |

Parameter values are given as the mean ± SD (n = 5 for control, RIF, DTZ 10 mg/kg, DTZ 20 mg/kg, DTZ 40 mg/kg, CsA 0.1 mg/kg groups and n = 6 for CsA 0.01 mg/kg, CsA 0.5 mg/kg, CsA 1 mg/kg, CsA 5 mg/kg groups). Values in parentheses express the range in percent coefficient of variation (%CV) of the parameters, which determines parameter precision. $k_1$ and $k_2$ are the rate constants for the transfer of [$^{99m}$Tc]mebrofenin between blood and liver tissue, $k_3$ is the transfer rate constant from liver tissue to the intrahepatic bile ducts, and $k_5$ is the transfer rate constant from the intrahepatic bile ducts to the extrahepatic biliary ducts and intestine. *$p < 0.05$, one-way ANOVA against a reference group (control) followed by a Bonferroni multiple-comparison test.