A Mechanistic Model to Determine the Uptake and Metabolism of Atorvastatin and Inhibition by Cyclosporine in Rat Hepatocytes Using a High Throughput Method

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# Supplementary Material



Figure S1: Predicted atorvastatin cellular concentration against observed individual concentration for the best fitting macro-rate constant mechanistic model, Model 3 for all data. Points are data, the black solid line represents the line of unity where observed = predicted. **a** where two separate passive rate constants were used (blue circles). **b** where a single passive rate constant and fraction unbound in the cell was used (red triangles)

2-hydroxy atorvastatin and 4-hydroxy atorvastatin were detected within the cell extract at 15 s in the three highest dose groups (25-150 nmol/ml), whilst in the lower dose groups (0.25-5 nmol/ml), detection was variable. The peak area ratio (defined as the ratio of the area of the analyte LCMS peak to that of the internal standard (dextromethorphan) LCMS peak area) of both metabolites was decreased by pre-incubation by CsA, which was similar to the metabolite only at higher atorvastatin incubation concentrations (see Figs. S3 and S4), suggestive of competitive inhibition. The effect of CsA was greatest on 4-hydroxy atorvastatin formation compared to 2-hydroxy atorvastatin formation at lower atorvastatin doses (Figs. S4 and S3 respectively, red dotted lines). Amundsen *et al.* (2012) also showed the inhibition by CsA on midazolam metabolism to be concentration dependent in human liver microsomes.

Table S1. Compartmental mechanistic model ODEs for competitive and non-competitive inhibition of atorvastatin (*A*) uptake by CsA (*C*) in rat hepatocytes.

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|  Micro-rate constant models |
| Compartment | Competitive Inhibition Model 1 (Fig. 2a) | Non-Competitive Inhibition Model 2 (Fig. 2b) |
| Medium | $\frac{dS\_{1}}{dt}=-k\_{aA}S\_{1}T\_{f}-k\_{fA}S\_{1}+k\_{dA}S\_{2}+k\_{bA}S\_{3}$ (1)$\frac{dI\_{1}}{dt}=-k\_{aC}I\_{1}T\_{f}+k\_{dC}I\_{2}$ (2)  | $\frac{dS\_{1}}{dt}= -k\_{aA}S\_{1}T\_{f}-k\_{fA}S\_{1}+k\_{dA}S\_{2}+k\_{bA}S\_{3}-k\_{aA}αS\_{1}I\_{2}+k\_{dA}αI\_{3}$ (10) $\frac{dI\_{1}}{dt}= -k\_{aC}I\_{1}T\_{f}+k\_{dC}I\_{2}-k\_{aC}αS\_{2}I\_{1}+k\_{dC}αI\_{3}$ (11) |
| Transporter | $\frac{dS\_{2}}{dt}= k\_{aA}S\_{1}T\_{f}-\left(k\_{dA}+k\_{tA}\right)S\_{2} $ (3)$\frac{dI\_{2}}{dt}= k\_{aC}I\_{1}T\_{f}-k\_{dC}I\_{2}$ (4)  | $\frac{dS\_{2}}{dt}= k\_{aA}S\_{1}T\_{f}-\left(k\_{dA}+k\_{tA}\right)S\_{2}-k\_{aC}αS\_{2}I\_{1}+ k\_{dC}αI\_{3} $ (12)$\frac{dI\_{2}}{dt}= k\_{aC}I\_{1}T\_{f}-k\_{dC}I\_{2}-k\_{aA}αS\_{1}I\_{2}+(k\_{dA}+k\_{tA})αI\_{3}$ (13)$\frac{dI\_{3}}{dt}= k\_{aC}αS\_{2}I\_{1}+k\_{aA}αS\_{1}I\_{2}-(k\_{dA}+k\_{tA}+k\_{dC})αI\_{3}$ (14) |
| Intracellular | $\frac{dS\_{3}}{dt}= k\_{fA}S\_{1}+ k\_{tA}S\_{2}-k\_{bA}S\_{3}- \frac{V\_{max.met}S\_{3}}{K\_{m.met}\left(1+\frac{I}{K\_{I.met}}\right)+S\_{3}}$ (5) | $\frac{dS\_{3}}{dt}= k\_{fA}S\_{1}+ k\_{tA}S\_{2}-k\_{bA}S\_{3}+ k\_{tA}αI\_{3}- \frac{V\_{max.met}S\_{3}}{K\_{m.met}\left(1+\frac{I}{K\_{I.met}}\right)+S\_{3}}$ (15) |
| Observations (nmol/ml) | $y\_{1}=\frac{1}{V\_{cell}}\left(S\_{2}+S\_{3}\right)$ (6) | $y\_{1}=\frac{1}{V\_{cell}}\left(S\_{2}+S\_{3}+I\_{3}\right)$ (16) |
| Macro-rate constant models |
|  | Competitive Inhibition Model 3 (Fig. 2c) | Non-Competitive Inhibition Model 4 (Fig. 2d) |
| Medium | $\frac{dS\_{1}}{dt}= -k\_{fA}S\_{1}-\frac{V\_{max.up}S\_{1}}{K\_{m.up}(1+\frac{I\_{1}}{K\_{I.up}})+S\_{1}}+k\_{bA}S\_{3}$ (7) | $\frac{dS\_{1}}{dt}= -k\_{fA}S\_{1}-\frac{V\_{max.up}S\_{1}}{(K\_{m.up}+S\_{1})(1+\frac{I\_{1}}{K\_{inact}})}+k\_{bA}S\_{3}$ (17) |
| Intracellular | $\frac{dS\_{3}}{dt}= k\_{fA}S\_{1}+\frac{V\_{max}S\_{1}}{K\_{m}\left(1+\frac{I\_{1}}{K\_{I}}\right)+S\_{1}}-k\_{bA}S\_{3}- \frac{V\_{max.met}S\_{3}}{K\_{m.met}\left(1+\frac{I}{K\_{I.met}}\right)+S\_{3}}$ (8) | $\frac{dS\_{3}}{dt}= k\_{fA}S\_{1}+\frac{V\_{max}S\_{1}}{\left(K\_{m}+S\_{1}\right)\left(1+\frac{I\_{1}}{K\_{inact.up }}\right)}-k\_{bA}S\_{3}- \frac{V\_{max.met}S\_{3}}{K\_{m.met}\left(1+\frac{I}{K\_{I.met}}\right)+S\_{3}}$ (18) |
| Observations (nmol/ml) | $y\_{1}=\frac{1}{V\_{cell}}S\_{3}$ (9) | $y\_{1}=\frac{1}{V\_{cell}}S\_{3}$ (19) |
| States – amount (nmol) | *S*1 and *I*1: atorvastatin and CsA respectively in the medium. *S*2 and *I*2: atorvastatin and CsA respectively bound to transporter. *S*3: intracellular atorvastatin. *I*3: atorvastatin-transporter-CsA complex. |
| Parameters | *kaA* and *kaC* are the transporter association rate constants (/nmol/min), *kdA* and *kdC* are the dissociation rate constants (/min), *ktA* is the transporter to intracellular translocation rate constant for atorvastatin (/min), *Tf* is the amount of free transporter (nmol), α is the degree to which bound CsA affects atorvastatin affinity to transporter (dimensionless): α <1 improved atorvastatin affinity, α >1 decreased atorvastatin affinity. *Vcell* is the cell volume *per* 2x105 cells (0.0011 ml). *Vmax* and *Km* are the maximum transporter uptake velocity (pmol/min) and amount at which 50 % of transporter sites are occupied (nmol). *KI* and *Kinact* are the competitive and non-competitive inhibition constants respectively (nmol). *up* = uptake and *met* = metabolism. |



Figure S2: Atorvastatin concentration against velocity or the inverse of the velocity plots. **a** Yamazaki plot for atorvastatin initial velocity at 15 s against atorvastatin incubation concentration, dashed line = total, dotted line = passive, solid line = saturable uptake, *Vmax.up* = 1253 pmol/min/2x105 cells, *Km.up* = 5-25 nmol/ml. **b** Lineweaver-Burke plot using ‘Active’ from **a,** *Vmax.up* = 158 (RSE = 80 %) pmol/min/2x105 cells, *Km.up* = 0.8 (RSE = 14 %) nmol/ml. **c** Lineweaver-Burke plot for the metabolism of atorvastatin, *Vmax.met* = 51.8 (RSE = 143 %) pmol/min/2x105 cells, *Km.met* = 3.4 (RSE = 5 %) nmol/ml. Data are *n*=3 from separate experiments



Figure S3: Plots of cellular 2-hydroxy atorvastatin peak area ratio against time following the addition of atorvastatin (0.25, 0.5, 2.5, 5, 25, 50 and 150 nmol/ml) in the absence (blue) and presence (red) of 10 nmol/ml CsA. Each time course represents one experiment from one Teflon block trough. Points are data (*n*=1-3) and solid lines are the average peak area ratio



Figure S4: Plots of cellular 4-hydroxy atorvastatin peak area ratio against time following the addition of atorvastatin (0.25, 0.5, 2.5, 5, 25, 50 and 150 nmol/ml) in the absence (blue) and presence (red) of 10 nmol/ml CsA. Each time course represents one experiment from one Teflon block trough. Points are data (*n*=1-3) and solid lines are the average peak area ratio

Table S3: Macro-rate constant model parameter estimates for the inhibition of atorvastatin by CsA in rat hepatocytes (Model 3 – competitive inhibition and Model 4 – non-competitive inhibition). Data are the individual mode of the conditional distribution obtained from Monolix 2018R2, *n*=3. All parameter estimates are scaled to per 1x106 cells. *Vmax* was scaled to pmol/min and *Km,* and *KI* were scaled to nmol/ml

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| Parameter | Model 3 – competitive inhibition | Model 4 – non-competitive inhibition |
| Passive |
| *kfA* (/min) | 1.5 (0.9-2.7) | 1.8 (1-4) |
| *kbA* (/min) | 3 (0.3-19) | 3.3 (0.4-19.6) |
| Transporter |
| *Vmax.up* (pmol/min) | 3220 (1460-13000) | 850 (680-1340) |
| *Km.up* (nmol/ml) | 0.74 (0.18-1.08) | 0.09 (0.04-0.17) |
| *KI.up* (nmol/ml) | 0.1 (0.07-0.22) | 0.06 (0.04-1.11) |
| Metabolism |
| *Vmax.met* (pmol/min) | 296 (158-840) | 364 (211-950) |
| *Km.met* (nmol/ml) | 340 (251-407) | 436 (296-544) |
| *KI.met* (nmol/ml) | 1.1 (1-1.3) | 0.32 (0.27-0.36) |

Table S4: Micro-rate constant model parameter estimates for the non-competitive inhibition of atorvastatin by CsA in rat hepatocytes (Model 2). Data are the individual mode of the conditional distribution obtained from Monolix 2018R2, n=3. All parameter estimates are scaled to per 1x106 cells. *Vmax* was scaled to pmol/min and *Km*, *KI*and *Kinact* were scaled to nmol/ml. Parameters in bold denote those used in main text

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| --- | --- | --- | --- | --- | --- |
| Parameter | Assessment 1 | **Assessment 2** | Assessment 3 | Assessment 4 | Assessment 5 |
| Passive |
| *kfA* (/min) | 1.3 (0.4-20.3) | **1.1 (0.2-23.3)** | 0.9 (0.2-25.8) | 1.1 (0.3-27.4) | 1.1 (0.4-26.3) |
| *kbA* (/min) | 9.7 (1-31.1) | **9.3 (0.6-36.5)** | 7.7 (0.5-39.9) | 9.9 (0.8-42.5) | 8.9 (0.6-41.2) |
| Transporter |
| *kaA* (/nmol/min) | 0.7 (0.2-6.8) | **1.3 (0.6-6.7)** | 1.2 (0.6-4.6) | 1.1 (0.4-5.7) | 1.1 (0.5-5.5) |
| *kaC* (/nmol/min) | 0.26 (0.25-0.29) | **0.18 (0.16-0.2)** | 0.19 (0.17-0.21) | 0.19 (0.18-0.21) | 0.2 (0.2-0.22) |
| *kdA* (/min) | 0.013 (0.012-0.014) | **0.017 (0.015-0.021)** | 0.013 (0.011-0.014) | 0.011 (0.01-0.012) | 0.012 (0.011-0.013) |
| *kdC* (/min) | 0.28 (0.26-0.3) | **0.16 (0.13-0.2)** | 0.3 (0.25-0.36) | 0.18 (0.17-0.19) | 0.29 (0.27-0.3) |
| *To* (nmol) | 3.4 (2.8-3.9) | **2.7 (2-3.6)** | 2.5 (1.4-5.7) | 3.1 (2.1-4.5) | 2.6 (1.7-4.7) |
| *ktA* (/min) | 0.31 (0.3-0.34) | **0.25 (0.23-0.31)** | 0.28 (0.24-0.33) | 0.21 (0.18-0.32) | 0.32 (0.28-0.36) |
| α | 0.012 (0.009-0.021) | **0.013 (0.007-0.023)** | 0.016 (0.013-0.018) | 0.012 (0.01-0.016) | 0.015 (0.012-0.018) |
| Metabolism |
| *Vmax.met* (pmol/min) | 456.5 (324-730) | **393 (284.5-500)** | 344.5 (245-645) | 303 (215.5-550) | 505 (385.5-690) |
| *Km.met* (nmol/ml) | 27.82 (23.64-30.18) | **17.71 (7.98-30.73)** | 28.91 (13.15-45.82) | 9.09 (5.76-12.16) | 55.45 (29.45-83.82) |
| *KI.met* (nmol/ml) | 8.1 (4.2-23.8) | **11 (5.5-29.1)** | 24.4 (9.2-138.2) | 9.5 (1.7-108.7) | 20.9 (13-46) |
| *-2.LL* | 5521 | **5417** | 5491 | 5504 | 5486 |
| *BIC* | 5618 | **5514** | 5587 | 5601 | 5583 |

References

Amundsen, R., Asberg, A., Ohm, I.K. & Christensen, H., 2012. Cyclosporine A- and tacrolimus-mediated inhibition of CYP3A4 and CYP3A5 in vitro. *Drug Metab Dispos,* 40**,** 655-61.