Supporting Information

Comparative hepatic and intestinal metabolism and pharmacodynamics of statins

Anne M. Filppula, Päivi Hirvensalo, Heli Parviainen, Vilma E. Ivaska, K. Ivar Lönnberg, Feng Deng, Jenni Viinamäki, Mika Kurkela, Mikko Neuvonen, Mikko Niemi

Department of Clinical Pharmacology, University of Helsinki, Helsinki, Finland (A.M.F., P.H., H.P., V.E.I., K.I.L., F.D., J.V., M.K., M.Ne., M.Ni.)

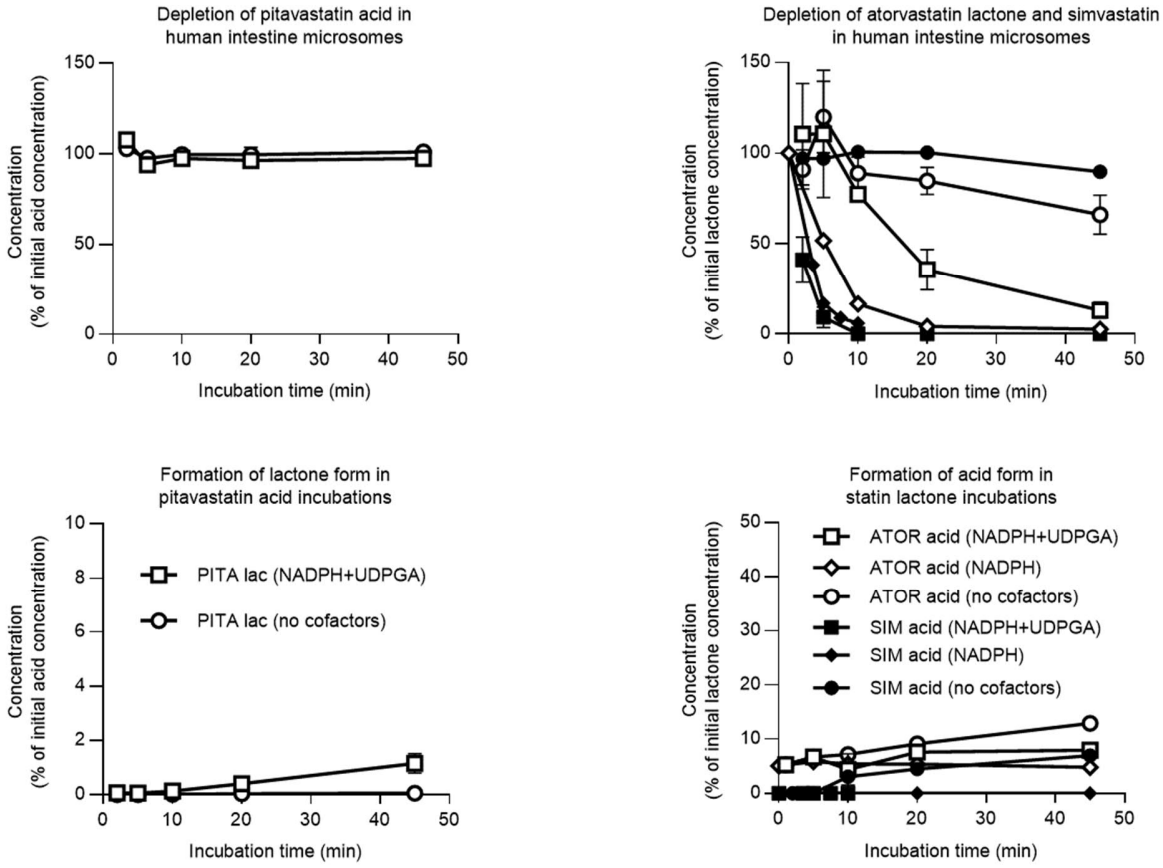
Individualized Drug Therapy Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland (A.M.F., P.H., H.P., V.E.I., K.I.L., F.D., J.V., M.K., M.Ne., M.Ni.)

Department of Clinical Pharmacology, HUS Diagnostic Center, Helsinki University Hospital, Helsinki, Finland (M.Ni.)

Corresponding author: Prof. Mikko Niemi, e-mail: mikko.niemi@helsinki.fi

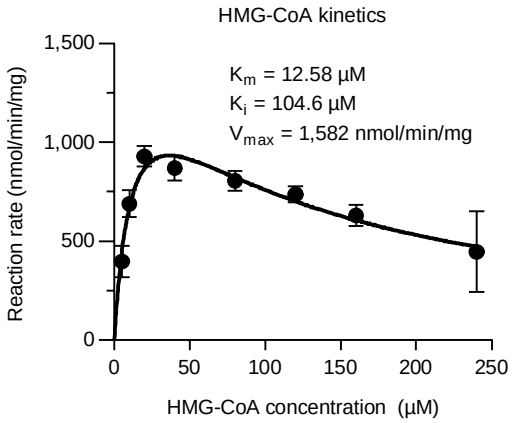
Manuscript number: DMD-AR-2021-000406

Journal title: Drug Metabolism & Disposition



Supplementary Fig. 1.

Acid-lactone conversion in human intestine microsomal incubations. In human intestine microsomal depletion experiments, marginal lactone formation (below the quantification limit) was observed for pitavastatin in incubations fortified with NADPH + UDPGA. For atorvastatin lactone, acid concentrations increased up to 8% and 13% of the initial lactone concentration in NADPH+UDPGA and no cofactor incubations. In NADPH incubations, no increase in acid concentrations was observed. For simvastatin (lactone), there seemed to be an increase in acid concentrations in incubations lacking cofactors but not in those containing cofactors.



Supplementary Fig. 2.

The kinetics of mevalonate formation by HMG-CoA reductase was best described by a substrate inhibition model. In pharmacodynamic experiments, HMG-CoA was incubated with HMG-CoA reductase (0.9 µg/ml) and NADPH in buffer for 3 min. The results shown describe mean and standard deviation values of triplicate incubations. HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; K_i, inhibition constant; K_m, Michaelis-Menten constant; V_{max}, maximal reaction rate.

Supplementary Tables

Supplementary Table 1.

The characteristic multiple reaction monitoring (MRM) transitions of the analytes and internal standards.

Analyte	Analyte mass-to-charge ratios (*m/z*)	Internal standard (concentration in stop solution)	Internal standard mass-to-charge ratios (*m/z*)	Ionization (+/-)
Atorvastatin	559.1-440.1	Atorvastatin-d5 (100 nM)	564.1-445.1	+
Atorvastatin lactone	541.1-448.1	Atorvastatin lactone-d5 (100 nM)	546.1-453.1	+
2-hydroxyatorvastatin	575.1-440.1	2-hydroxyatorvastatin-d5 (40 ng/ml)	580.1-445.1	+
2-hydroxyatorvastatin lactone	557.1-448.1	2-hydroxyatorvastatin lactone-d5 (40 ng/ml)	562.1-453.1	+
4-hydroxyatorvastatin	575.1-440.1	4-hydroxyatorvastatin-d5 (30 ng/ml)	580.1-445.1	+
4-hydroxyatorvastatin lactone	557.1-448.1	4-hydroxyatorvastatin lactone-d5 (30 ng/ml)	562.1-453.1	+
3R,5S-Fluvastatin	410.1-348.1	Fluvastatin-d8 (100 nM)	418.1-356.1	-
3S,5R-Fluvastatin	410.1-348.1	Fluvastatin-d8 (100 nM)	418.1-356.1	-
Pitavastatin	422.1-290.1	Pitavastatin-d5 (100 nM)	427.1-295.1	+
Pitavastatin lactone	404.1-290.1	Pitavastatin-d5 (100 nM)	427.1-295.1	+
Pravastatin	442.1-269.1	Pravastatin-d9 (100 nM)	451.1-269.1	+
Rosuvastatin	482.1-258.1	Rosuvastatin-d6 (100 nM)	488.1-264.1	+
Simvastatin (lactone)	436.1-285.1	Simvastatin (lactone)-d6 (50 nM)	442.1-285.1	+
Simvastatin acid	437.1-303.1	Simvastatin acid-d6 (50 nM)	443.1-303.1	+

Supplementary Table 2.

Incubation concentrations (C_0) of the statin compounds tested and literature K_m values from HLM and recombinant CYP and UGT incubations, collected from the University of Washington Drug Interaction Database (September 16, 2020). K_m values from disease HLMs (e.g. diabetic HLM) and variant rCYP values were excluded.

Compound	C_0 in HLMs, HLC, rCYPs (µM)	C_0 in HIMs (µM)	Literature median K_m values in HLMs (µM)	Literature median K_m values in rCYPs and rUGTs (µM)
Atorvastatin	0.05	1	31 (range 1.2-49) (n=15)	30 (range 4-55) (n=13)
Atorvastatin lactone	0.05	1	6.3 (range 0.62-14) (n=7)	37 (range 1.4-64) (n=6)
2-hydroxyatorvastatin	0.05	n/d	n/a	n/a
2-hydroxyatorvastatin lactone	0.05	n/d	n/a	n/a
4-hydroxyatorvastatin	0.05	n/d	n/a	n/a
4-hydroxyatorvastatin lactone	0.05	n/d	n/a	n/a
3R,5S-Fluvastatin	0.05	1	Racemic: 0.5 (0.2-0.7) (n=3)	Racemic: 2.8 (1.1-7.1) (n=5)
3S,5R-Fluvastatin	0.05	1	Racemic: 0.5 (0.2-0.7) (n=3)	Racemic: 2.8 (1.1-7.1) (n=5)
Pitavastatin	0.04	1	51 (49-78) (n=3)	115 (10-220) (n=2)
Pitavastatin lactone	0.03	1	n/a	n/a
Pravastatin	0.1	1	HLMs: 8,889 (3,480-20,987) (n=4) HIMs: 4,925 (4,560-5,290) (n=2)	n/a
Rosuvastatin	0.01	0.2	n/a	220 (range 16-259) (n=3)
Simvastatin (lactone)	0.05	0.05	14 (range 2.1-36) (n=8)	35 (n=1)
Simvastatin acid	0.05	0.05	62 (range 47-416) (n=4)	28 (range 16-88) (n=6)

C_0, initial incubation concentration; HIMs, human intestine microsomes; HLMs, human liver microsomes; K_m, Michaelis-Menten constant; n/a, not available; rCYP, recombinant CYP; rUGT, recombinant uridine 5'-diphospho-glucuronosyltransferase.

Supplementary Table 3.

Comparison of CL_{int} values obtained in the depletion experiment in NADPH-supplemented HLMs with those found in the literature. The CL_{int} values (mean of triplicates) of the present experiments have been corrected for potential depletion in negative controls. In addition, previous CL_{int} data from depletion studies were collected from the literature (Fujino *et al.*, 2003; Fujino *et al.*, 2004; Gertz *et al.*, 2010; Gertz *et al.*, 2011; Nishimuta *et al.*, 2013; Varma *et al.*, 2014; Hirvensalo *et al.*, 2019) and metabolite formation CL_{int} data from the University of Washington Drug Interaction Database (September 16, 2020).

Compound	$f_{u,mic}$ at 0.2 mg/ml	HLM CL_{int} (µl/min/mg)	HLM $CL_{int,u}$ (µl/min/mg) [a]	Literature median HLM CL_{int} (µl/min/mg) [b] (n = number of studies)
Atorvastatin	0.683 ± 0.049	37.5 ± 4.2	54.9 ± 6.2	Depletion: 16 (range 3.0-59) (n=4) Metabolite formation: 13 (range 4.1-46) (n=8)
Atorvastatin lactone	0.238 ± 0.025	3,740 ± 502	15,700 ± 2110	Depletion: 1,892 (n=1) Metabolite formation: 923 (range 4.2-2,949) (n=3)
2-hydroxyatorvastatin	0.530 ± 0.036	6.27 ± 0.64	11.8 ± 1.2	n/a
2-hydroxyatorvastatin lactone	0.216 ± 0.015	843 ± 82	3,900 ± 379	n/a
4-hydroxyatorvastatin	0.510 ± 0.040	79.9 ± 13	157 ± 25	n/a
4-hydroxyatorvastatin lactone	0.482 ± 0.093	122 ± 13	252 ± 28	n/a
3R,5S-Fluvastatin	0.747 ± 0.019	19.3 ± 2.93	25.8 ± 3.9	Depletion: 39 (n=1) Depletion (racemic): 33 (range 6-76) (n=3) Metabolite formation (racemic): 67 (range 23-68) (n=3)
3S,5R-Fluvastatin	0.735 ± 0.015	14.8 ± 1.8	20.2 ± 2.4	Depletion: 33 (n=1) Depletion (racemic): 33 (range 6-76) (n=3) Metabolite formation (racemic): 67 (range 23-68) (n=3)

Compound	$f_{u,mic}$ at 0.2 mg/ml	HLM CL_{int} (µl/min/mg)	HLM $CL_{int,u}$ (µl/min/mg) [a]	Literature median HLM CL_{int} (µl/min/mg) [b] (n = number of studies)
Pitavastatin	0.948 ± 0.051	<0.1	<0.1	Depletion: 1.4 (range 0.25-2.5) (n=2) Metabolite formation: 2.52 (range 1.9-3.1) (n=3)
Pitavastatin lactone	0.186 ± 0.066	23.6 ± 6.3	124 ± 34	Depletion: 5 (range 4.6-5.4) (n=2)
Pravastatin	0.739 ± 0.087	<0.1	<0.1	Depletion: 0 (n=1) Metabolite formation: 0.017 (range 0.0055-0.026) (n=4)
Rosuvastatin	0.768 ± 0.092	<0.1	<0.1	Depletion: 0.55 (range: 0-1.1) (n=2) Glucuronide formation: 0.5 (n=1)
Simvastatin (lactone)	0.147 ± 0.008 (at 0.1 mg/ml)	7,420 ± 599	50,490 ± 4070	Depletion: 4,000 (range 1,959-7,100) (n=3) Metabolite formation: 86 (range 37-287) (n=8)
Simvastatin acid	0.660 ± 0.039	44.3 ± 3.9	67.2 ± 5.9	Depletion: 28 (n=1) Metabolite formation: 18 (range 0.4-20) (n=4)

CL_{int}, intrinsic clearance; $CL_{int,u}$, unbound intrinsic clearance; $f_{u,mic}$, fraction unbound in microsomes; HLM, human liver microsome.

[a] Corrected for nonspecific binding in HLMs, using the measured $f_{u,mic}$ values.

[b] Correction for nonspecific binding unknown.

Supplementary Table 4.

Comparison of CL_{int} values obtained in HIM incubations with those found in the literature. The CL_{int} are from Gertz *et al.* (2011); Nishimuta *et al.* (2013) and the University of Washington Drug Interaction Database (September 16, 2020). The CL_{int} values (mean of triplicates) of the present experiments have been corrected for potential depletion in negative controls.

Compound	$f_{u,mic}$ at 0.2 mg/ml	HIM CL_{int} (µl/min/mg) [a]	HIM $CL_{int,u}$ (µl/min/mg) [b]	Literature median HIM CL_{int} (µl/min/mg) [c] (n = number of studies)
Atorvastatin	n/d	<0.1	<0.1	Depletion: 14 (n=1)
Atorvastatin lactone	0.637 ± 0.027	724 ± 20	1,140 ± 30	n/a
Pitavastatin	0.871 ± 0.053	7.0 ± 1.0	8.0 ± 1.2	n/a
Pravastatin	n/d	<0.1	<0.1	Metabolite formation: 0.011 (range: 0.006-0.016) (n=2)
Simvastatin (lactone)	0.068 ± 0.029	1,540 ± 20	22,670 ± 320	Depletion: 1,900 (range 1,859-3,480) (n=2)

CL_{int}, intrinsic clearance; $CL_{int,u}$, unbound intrinsic clearance; $f_{u,mic}$, fraction unbound in microsomes; HIM, human intestine microsome; n/d, not determined.

[a] The data of atorvastatin, pitavastatin and pravastatin are from the depletion experiment with NADPH+UDPGA, those of atorvastatin lactone and simvastatin from the inhibition experiment with NADPH.

[b] Corrected for nonspecific binding in HIMs, using the measured $f_{u,mic}$ values.

[c] Correction for nonspecific binding unknown.

Supplementary Table 5

Comparison of the IC_{50} values (nM) obtained in the present pharmacodynamic experiment with those of some previous studies.

Compound	Present study	Aoki *et al.* (1997)	Holdgate *et al.* (2003) [a]	Kathawala (1991)	McTaggart *et al.* (2001)	Perchellet *et al.* (2009)
Atorvastatin	13.1 ± 3.2	n/d	8.0	n/d	8.2	n/d
2-hydroxyatorvastatin (*ortho*-hydroxyatorvastatin)	12.1 ± 4.2	n/d	n/d	n/d	n/d	n/d
4-hydroxyatorvastatin (*para*-hydroxyatorvastatin)	~100 [b]	n/d	n/d	n/d	n/d	n/d
3R,5S-fluvastatin	8.58 ± 2.61	n/d	28 [c]	2.4	27.6 [c]	172.8 [c]
3S,5R-fluvastatin	~100 [b]	n/d	28 [c]	80	27.6 [c]	172.8 [c]
Pitavastatin	12.4 ± 1.8	6.8	n/d	n/d	n/d	n/d
Pravastatin	12.6 ± 3.7	48	44	n/d	44.1	66.1
Rosuvastatin	4.37 ± 1.13	n/d	3.5	n/d	5.4	n/d
Simvastatin acid	19.7 ± 2.0	16	11	n/d	11.2	134
Enzyme source	Recombinant enzyme	Rat liver microsomal enzyme	Recombinant enzyme	Rat liver microsomal enzyme	Recombinant enzyme	Recombinant enzyme

IC_{50}, inhibitor concentration producing 50% inhibition; n/d, no data.

[a] Inhibition constant (K_i) values.

[b] The highest concentrations tested were 100 and 500 nM.

[c] The fluvastatin compound tested was likely the racemic mix (not clearly stated in the paper).

References

Aoki T, Nishimura H, Nakagawa S, Kojima J, Suzuki H, Tamaki T, Wada Y, Yokoo N, Sato F, Kimata H, Kitahara M, Toyoda K, Sakashita M, and Saito Y (1997) Pharmacological profile of a novel synthetic inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. *Arzneimittelforschung* 47**:**904-909.

Fujino H, Saito T, Tsunenari Y, Kojima J, and Sakaeda T (2004) Metabolic properties of the acid and lactone forms of HMG-CoA reductase inhibitors. *Xenobiotica* 34**:**961-971.

Fujino H, Yamada I, Shimada S, Yoneda M, and Kojima J (2003) Metabolic fate of pitavastatin, a new inhibitor of HMG-CoA reductase: human UDP-glucuronosyltransferase enzymes involved in lactonization. *Xenobiotica* 33**:**27-41.

Gertz M, Harrison A, Houston JB, and Galetin A (2010) Prediction of human intestinal first-pass metabolism of 25 CYP3A substrates from in vitro clearance and permeability data. *Drug Metab Dispos* 38**:**1147-1158.

Gertz M, Houston JB, and Galetin A (2011) Physiologically based pharmacokinetic modeling of intestinal first-pass metabolism of CYP3A substrates with high intestinal extraction. *Drug Metab Dispos* 39**:**1633-1642.

Hirvensalo P, Tornio A, Neuvonen M, Kiander W, Kidron H, Paile-Hyvarinen M, Tapaninen T, Backman JT, and Niemi M (2019) Enantiospecific Pharmacogenomics of Fluvastatin. *Clin Pharmacol Ther* 106**:**668-680.

Holdgate GA, Ward WH, and McTaggart F (2003) Molecular mechanism for inhibition of 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase by rosuvastatin. *Biochem Soc Trans* 31**:**528-531.

Kathawala FG (1991) HMG-CoA reductase inhibitors: an exciting development in the treatment of hyperlipoproteinemia. *Med Res Rev* 11**:**121-146.

McTaggart F, Buckett L, Davidson R, Holdgate G, McCormick A, Schneck D, Smith G, and Warwick M (2001) Preclinical and clinical pharmacology of Rosuvastatin, a new 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor. *Am J Cardiol* 87**:**28B-32B.

Nishimuta H, Nakagawa T, Nomura N, and Yabuki M (2013) Species differences in hepatic and intestinal metabolic activities for 43 human cytochrome P450 substrates between humans and rats or dogs. *Xenobiotica* 43**:**948-955.

Perchellet JP, Perchellet EM, Crow KR, Buszek KR, Brown N, Ellappan S, Gao G, Luo D, Minatoya M, and Lushington GH (2009) Novel synthetic inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity that inhibit tumor cell proliferation and are structurally unrelated to existing statins. *Int J Mol Med* 24**:**633-643.

Varma MV, Bi YA, Kimoto E, and Lin J (2014) Quantitative prediction of transporter- and enzyme-mediated clinical drug-drug interactions of organic anion-transporting polypeptide 1B1 substrates using a mechanistic net-effect model. *J Pharmacol Exp Ther* 351**:**214-223.