# Interactions of Human P-glycoprotein with Simvastatin, Simvastatin Acid, and Atorvastatin

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***Purpose.*** In this study, P-glycoprotein (P-gp) mediated efflux of simvastatin (SV), simvastatin acid (SVA), and atorvastatin (AVA) and inhibition of P-gp by SV, SVA, and AVA were evaluated to assess the role of P-gp in drug interactions.

***Methods.*** P-gp mediated efflux of SV, SVA, and AVA was determined by directional transport across monolayers of LLC-PK1 cells and LLC-PK1 cells transfected with human MDR1. Inhibition of P-gp was evaluated by studying the vinblastine efflux in Caco-2 cells and in P-gp overexpressing KBV1 cells at concentrations of SV, SVA, and AVA up to 50 μM.

***Results.*** Directional transport studies showed insignificant P-gp mediated efflux of SV, and moderate P-gp transport [2.4–3.8 and 3.0–6.4 higher Basolateral (B) to Apical (A) than A to B transport] for SVA and AVA, respectively. Inhibition studies did not show the same trend as the transport studies with SV and AVA inhibiting P-gp ($IC_{50}$ ~25–50 μM) but SVA not showing any inhibition of P-gp.

***Conclusions.*** The moderate level of P-gp mediated transport and low affinity of SV, SVA, and AVA for P-gp inhibition compared to systemic drug levels suggest that drug interactions due to competition for P-gp transport is unlikely to be a significant factor in adverse drug interactions. Moreover, the inconsistencies between P-gp inhibition studies and P-gp transport of SV, SVA, and AVA indicate that the inhibition studies are not a valid means to identify statins as Pgp substrates.

**KEY WORDS:** P-glycoprotein; simvastatin; atorvastatin; HMG-CoA; reductase inhibitor; drug interactions.

## INTRODUCTION

Statin cholesterol lowering drugs have dramatically reduced cardiovascular disease associated with elevated cholesterol. In general statins are considered to be safe, and the cholesterol lowering benefits significantly outweigh any risks associated with the drugs (1). However, at higher doses drug-drug interactions have resulted in elevated systemic drug levels resulting in development of rhabdomyolysis, a rare, but potentially life threatening condition (2–4). These drug interactions have primarily been ascribed to elevated systemic statin levels resulting from decreased clearance associated with inhibition of Phase I or Phase II metabolism by co-administered drugs (5–7). Recently, the potential for drug-drug interactions resulting from inhibition of P-glycoprotein (P-gp) has received considerable attention as a contributing factor in statin drug interactions (7–10).

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**ABBREVIATIONS:** SV, simvastatin; SVA, simvastatin acid; AVA atorvastatin; VBL, vinblastine; CsA, cyclosporin A; P-gp, P-glycoprotein; L-MDR1, human MDR1 transfected LLC-PK1 cells.

P-gp is an ATP driven efflux pump capable of transporting a wide variety of structurally diverse compounds from the cell interior into the extracellular space (for review see references 11 and 12). P-gp is constitutively expressed in the intestine, kidney, liver, brain microvascular endothelia, and placenta (13–14) in a manner consistent with protection from xenobiotics. In the intestine, liver and kidney the expression of P-gp is polarized such that its activity could restrict absorption and aid elimination of P-gp substrates, thus minimizing systemic exposure of some drugs. The putative role of P-gp in drug disposition has lead to speculations regarding the potential for drug interactions at the level of P-gp. In the case of simvastatin (SV), atorvastatin (AVA), and other HMG-CoA reductase inhibitors, several publications have speculated that elevation of systemic statin levels may be due to inhibition of P-gp mediated statin transport by co-administered drugs (7–10). Such drug interactions require that P-gp mediated transport is sufficient to significantly limit absorption or facilitate elimination of the drugs. However with the exception of AVA (11), direct evidence that statins are subject to transport by P-glycoprotein has been lacking. Instead, SV, AVA, and other statins have been judged to be P-gp substrates primarily based on their ability to inhibit P-gp (7–10). Although P-gp inhibition could account for alterations in the PK of other P-gp substrates, it is not sufficient to identify if a drug itself is transported by P-gp. In this study we evaluated P-gp transport of SV and its active metabolite, simvastatin acid (SVA), and AVA as well as inhibition of P-gp by these statins. The results clearly show a disparity between inhibition results and P-gp transport of the drugs. Moreover the extent of transport by P-gp and the potency for inhibition of P-gp suggest that inhibition of P-gp is unlikely to account for SV associated drug interactions.

## MATERIALS AND METHODS

### Chemicals

Simvastatin, simvatatin acid, pravastatin, and atorvastatin were prepared at Merck Research Laboratories (Rahway, NJ). $^3$H-Vinblastine sulfate (VBL; 17.0 mCi/mg) was purchased from Amersham Bioscience, (Piscataway, NJ). Unlabeled and $^3$H-ritonavir (RIT; 1Ci/mmole) were purchased from Moravek Biochemicals (Brea, CA). Cyclosporin A (CsA) and unlabeled VBL were purchased from Sigma Chemical Company (St. Louis, MO). All other reagents were analytical grade.

### Cell Culture

Fetal calf serum (FCS), Opti-MEM, glutamine, non-essential amino acids (NEAA), Dulbecco's modified Eagle's medium (DMEM), Medium 199, and penicillin-streptomycin solution were obtained from from GibcoBRL (Carlsbad, CA). LLC-PK1 cells and LLC-PK1 cells expressing human (MDR1) P-glycoprotein were obtained from The Netherlands Cancer Institute and used under a license agreement. The cells were maintained in Medium 199 supplemented with 10% fetal bovine serum and 2 mM L-glutamine, and nonessential amino acids (1%, vol/vol) in 95% air/5% CO2 humidified incubator at 37°C. Caco-2 cells (passage 25-29) were obtained from American Type Culture Collection (Rockville, MD) and were maintained in Opti-MEM supplemented with 5% FCS,

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2 mM L-glutamine, and nonessential amino acids (1%, vol/vol) at 37°C in a humidified atmosphere of 5% $CO_2$/95% air. The drug-sensitive human epidermoid carcinoma cell line KB3-1 and its VBL-selected MDR variant KBV1 (17) were provided by Dr. Michael M.Gottesman (National Cancer Institute) and used under a license agreement. Both cell lines were maintained in DMEM high glucose containing 10% FCS, 50 U/ml penicillin, 50 μg/ml streptomycin, and 2 mM L-glutamine at 37°C in a humidified atmosphere of 5% $CO_2$/95% air. KB-V1 cells were grown in the continuous presence of VBL (1 μg/ml).

### P-glycoprotein Inhibition Studies

Inhibition studies were performed by evaluating inhibition of directional transport of Ritonavir in L-MDR1 cells and VBL in Caco-2 cells, and inhibition of P-gp restricted accumulation of VBL in L-MDR1 cells and KBV1 cells. Caco-2 cells were grown on filters using the Biocoat HTS Caco-2 assay system (Becton Dickinson) in accordance with the manufacturer's instructions with the exception that the cells were maintained in basal seeding media for 3–5 days prior to inducing differentiation with Entero-STIM media. After 2 days in Entero-STIM media, filters were rinsed one time with HBSS buffered with 10 mM Hepes (pH 7.4). Transport of [$^3$H]-VBL in the apical to basolateral direction (A-B) and in the basolateral to apical direction (B-A) was measured in the presence of the indicated concentrations of SV, SVA, and AVA. The drugs were present in both the receiver and donor (apical and basolateral) solutions. VBL transport was allowed to proceed for 2 h at 37°C after which the receiver, donor and dosing solutions were collected and the total radioactivity in the samples was measured. IC50 values were calculated based on the difference in the Papp in the A to B and B to A direction.

Inhibition of directional transport of ritonavir was performed on L-MDR1 cell grown on 96-well multiscreen Caco-2 filter plates (Millipore, Corp, Bedford, MA). The cells were plated at $6 \times 10^4$ cells per well and maintained on the filters for five days prior to the transport study. Inhibition of directional transport of ritonavir by the SV, SVA, and AVA was studied using the protocol described above for the Caco-2 studies.

Measurements of the accumulation of [$^3$H]-VBL in KB3.1 and KBV1 cell monolayers were performed at 37°C in HBSS with 10 mM Hepes (pH 7.4) in accordance with Yamazaki *et al.* (16). Cell monolayers were prepared by plating $1 \times 10^6$ cells per well in six-well tissue culture dishes the day before use. Blank wells were prepared simultaneously using the same media to assess background. After overnight incubation at 37°C, cell monolayers were washed once with serum-free medium, and then 3 ml of HBSS was added containing 100 nM [$^3$H]-VBL with no additional drug or the indicated concentration of SV, SVA, AVA, or CsA. Incubation in the drug-containing medium was carried out for one hour, after which the reaction was terminated by removing the medium and washing the monolayers two times with phosphate buffered saline. The plates were then turned upside down to drain briefly before the addition of 1.5 ml of 0.25% trypsin, 1 mM EDTA in HBSS without $Ca^{2+}$ and $Mg^{2+}$. The cell-associated total radioactivity was measured in trypsin-solubilized cell suspension by liquid scintillation counting. Similar experiments were performed to measure the accumulation of [$^3$H]-VBL in LLC-PK1 and L-MDR1 cell monolayers with the exception that the studies were performed in 24-well plates in which the cells were plated at 2 X $10^5$ cells per well. Accumulation of vinblastine was determined from the radioactivity in the total cell lysate released with 100mM NaOH after neutralization with an equal volume of 100 mM HCL.

### *In Vitro* P-glycoprotein Transport Studies

Directional transport studies were performed in LLC-PK1 cells (parental control cell line) and LLC-PK1 cells expressing genes for human (MDR1) P-glycoprotein (LMDR1 cells) (18). Cells were plated on HTS filter plates (Becton Dickinson) at 1.5 E5 cells/well five days prior to the transport studies. Prior to initiating the transport studies, the culture media on each side of the filters was replaced with 10 mM Hepes buffered Hank's balanced salt solution pH 7.4 (HBSS). Twenty-four-well tissue culture plates were used for the basolateral compartment. Transport studies were initiated by adding 0.5 ml of HBSS to the receiver side and 0.5 ml HBSS containing 5μM SV, SVA, or AVA to the donor side. The donor side is the apical compartment for A to B transport and the basolateral side for B to A transport. Transport studies on 1 μM verapamil were run in parallel as positive controls to verify activity of P-gp. The plates and filters were then placed in a 37°c incubator for 2 h after which the filter units were separated from the receiver plates, and samples were collected for analysis. The statins were quantified in the receiver and donor compartments by LC/MS/MS using pravastatin as an internal standard. SV, SVA, and AVA were separated on a betasil C-18 column (2.1 × 50mm) over 3.5 min using a gradient from 10% acetonitrile in 0.2% ammonium acetate pH 4.5 to 90% acetonitrile over the first minute. The SV and AVA were detected in positive ion mode using parent ion/fragment transitions of 419/285 and 557/453 respectively. Pravastatin and SVA were detected in negative ion mode using 423/321 and 435/319 transitions respectively. Verapamil transport was determined by LC/MS in positive ion mode monitoring parent ion was monitored at m/z = 455, using a linear gradient from 15% to 90% acetonitrile in 0.05% formic acid on an inertsil ODS column (Varian, Inc., Walnut Creek, CA).

## RESULTS

### Inhibition of P-gp by SV, SVA, and AVA

Inhibition of P-gp by the SV, SVA, and AVA was evaluated using two complementary P-gp assays: i) studying the impact of the statins on P-gp restricted drug accumulation in cells, and ii) evaluating inhibition of P-gp mediated directional transport. The results of these studies are summarized in Table I. Directional transport studies were performed in Caco-2 cells, which express an uptake transport system for AVA, and in L-MDR1 cells, human MDR1transfected LLC-PK1 cells used for the statin transport studies. In Caco-2 cells, vinblastine showed 5- to 18-fold higher B to A transport than A to B transport with the difference in permeability coefficients ($\Delta Papp = Papp_{B\ to\ A} - Papp_{A\ to\ B}$) of 0.86-1.6 X $10^{-5}$ cm/s. In the presence of the P-gp inhibitor CsA (5μM) ΔPapp was reduced to 0.4 to 4.2 × $10^{-6}$ cm/s reflecting 73–94% inhi-

**Table I.** Inhibition of P-gp Mediates Transport of Vinblastine by SV, SVA, and AVA: Effect on Directional Transport and KBV1 Cellular Accumulation

| Inhibitor \ Cell line (substrate) | % Inhibition directional transport | | Cellular accumulation | |
|---|---|---|---|---|
| | L-MDR1 cells (ritonavir) | Caco-2 cells (vinblastine) | L-MDR1/LLC PK-1 (vinblastine) | KBV1/KB-3-1 (vinblastine) |
| Control | 0% | 0% | 8% | 12% |
| SV 1 μM | ND | 26% | 5% | 14% |
| SV 10 μM | ND | 44% | 9% | 18% |
| SV 25 μM | 30% | 60% | 33% | 47% |
| SV 50 μM | 100% | 71% | 34% | 128% |
| SVA 1 μM | ND | 14% | ND | 12% |
| SVA 10 μM | ND | 13% | ND | 14% |
| SVA 50 μM | 15% | 9% | 6% | 17% |
| AVA 50 μM | 9% | 43% | 5% | 15% |
| CsA 10 μM | ND | 94% | 68% | 114% |

ND, not determined.
* No directional transport of ritonavir detected in control LLC-PK1 cells.

bition of P-gp. SV and AVA both inhibited directional transport of VBL at relatively high concentrations (10–50 μM). In contrast to SV, the active open acid form SVA did not significantly inhibit P-gp mediated VBL transport at concentrations as high as 50 μM. Since intrinsic directional transport of VBL was observed for in the control LLC-PK1 cells, results from inhibition of directional transport using VBL as a substrate would lead to ambiguous interpretation in the L-MDR1 cells. Consequently inhibition of P-gp mediated directional transport in the L-MDR1 cells were performed using 5 μM ritonavir as a P-gp substrate. Five micromolar ritonavir showed no directional transport in control LLC-PK1 cells but had 30-fold higher B to A transport than A to B transport in L-MDR1 cells with the difference in permeability coefficients (ΔPapp) of $4.1 \times 10^{-5}$ cm/s. Results from inhibition studies with SV and SVA in the L-MDR1 cells were similar to those in Caco-2 cells with the exception that 25 μM SV was slightly less potent at inhibiting P-gp efflux of ritonavir. However, in contrast to results in the Caco-2 cells, 50 μM AVA did not significantly inhibit P-gp efflux in the L-MDR1 cells. This most likely reflects the presence of an uptake transporter in the Caco-2 cells (15) which is absent in the L-MDR1 cells, and is consistent with the very high concentrations required to observe inhibition of P-gp by AVA in other cell types (8).

Results in the transport models were complemented by evaluating inhibition of P-gp mediated [$^3$H]-VBL efflux by human P-gp in L-MDR1 cells and in the human P-gp over expressing cell line KBV1. Accumulation of [$^3$H]-VBL was determined in monolayers of L-MDR1 cells and of KB-V1 cells and in their low P-gp expressing counterparts LLC-PK1 and KB3.1 cells in the presence of 0–50 μM SV, SVA, AVA, or in the presence of 10 μM CsA. After 60 min incubation VBL accumulation in KB-V1 cells was 15% of that in drug-sensitive parent cell line KB-3-1. CsA (10 μM) completely reversed the attenuated accumulation in KB-V1 cells. Similarly, vinblastine accumulation in the L-MDR1 cells was only 8% of that in the LLC-PK1 cells and increased to 68% in the presence of 10 μM CsA. In contrast to the directional transport studies in the L-MDR1 cells, intrinsic transporters had little influence on the cell accumulation of vinblastine in as much as P-gp inhibition only resulted in a 2- to 2.5-fold increase in vinblastine accumulation in LLC-PK1 cells, while yielding a 20-fold increase in cell accumulation in the L-MDR1 cells. The results of the uptake studies are consistent with L-MDR transport results showing SV inhibited P-gp efflux of VBL, while SVA and AVA had no significant effect on VBL accumulation (Table I).

### P-gp Transport of SV, SVA, AVA

Figure 1 shows the time course for transport of 5 μM SV, SVA and AVA across LLC-PK1 and L-MDR1. No significant directional transport was observed for SV, SVA, or AVA in the parental cell line LLC-PK1. In the human P-gp expressing L-MDR1 cell monolayers AVA and SVA showed 6.4- and 3.4-fold higher B to A transport than A to B, while no directional transport was observed for the lactone prodrug SV. Under the conditions of the assay less than 10% of the SV was converted to SVA, and no conversion was observed of the free acids SVA and AVA to the lactone forms. Additional transport experiments were performed at SV concentrations ranging from 2 to 25 μM, to ensure that saturation of P-gp transport was not resulting in underestimates of SV transport. SV did not show significant directional transport in the MDR1 transfected cells with B to A/ A to B transport ratios ranging from 1.2 to 1.9. Under similar conditions L-MDR1 transport ratios for SVA and AVA ranged from 2.4 to 3.8 and 3.0 to 6.4, respectively. No directional transport was observed for SV, SVA, or AVA in control LLC-PK1 cell lines at 2–25 μM and the passive permeability observed was 17 to $19 \times 10^{-6}$ cm/s, 19 to $20 \times 10^{-6}$ cm/s, and $7 \times 10^{-6}$ cm/s for SV, SVA, and AVA, respectively. Under the conditions of the assay, recovery of SVA and AVA were 80–100% but recovery of SV ranged from 50% at 2 and 5 μM to 80% at 25 μM. To improve mass balance at the lower concentrations of SV, 0.1% BSA was added to the transport buffers. Addition of BSA to the transport buffers increased mass balance at all the concentrations to 80–90% of the added drug, but did not influence the transport ratios SV, SVA, or AVA at any of the concentrations, indicating that the ratios observed are not a consequence of non-specific binding.

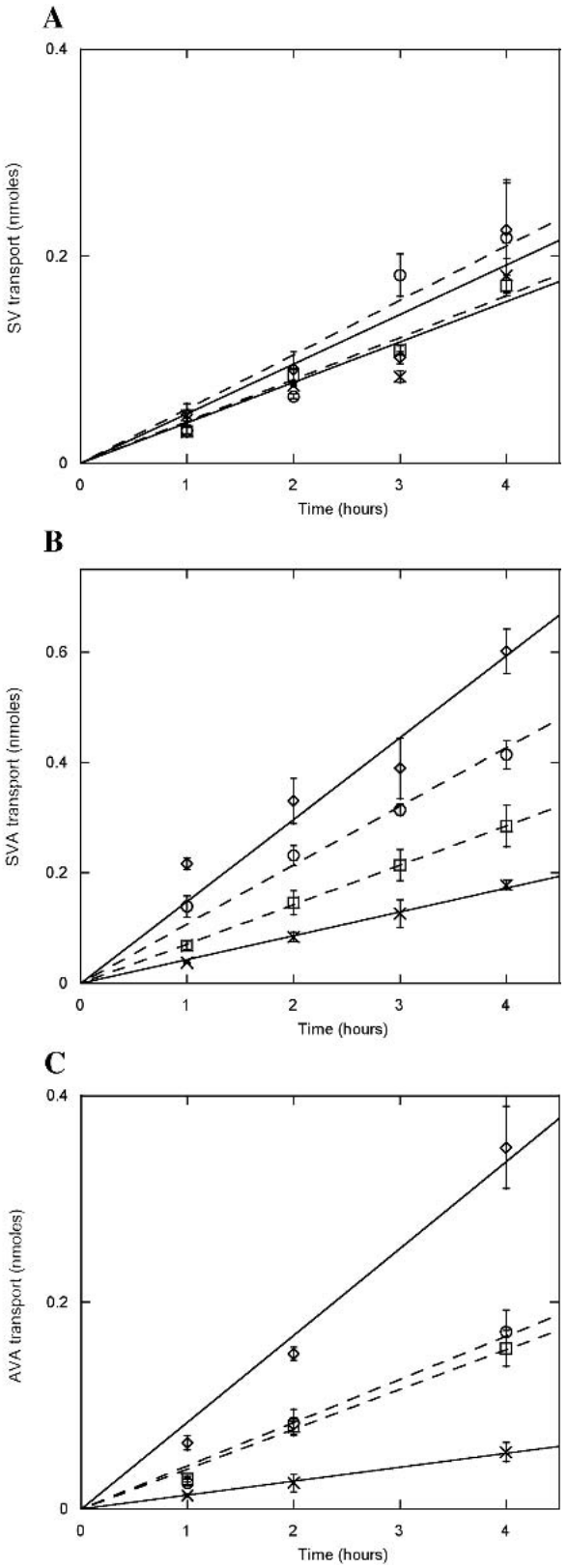


**Fig. 1.** Transport of (A) SV, (B) SVA, and (C) AVA across LLC-PK1 cells (broken line, ○, □) and L-MDR1 cells (solid line, ◊, X) in the basolateral to apical (○,◊) and apical to basolateral (□, X) direction. Five μM SV, SVA, or AVA was dosed to the basolateral or apical compartment, and drug transport from the basolateral to apical and apical to basolateral compartment was quantified by LC/MS/MS at 1, 2, 3, and 4 h after dosing.

## DISCUSSION

In this study, we evaluated P-gp mediated transport of SV, SVA and AVA, and inhibition of P-gp by these drugs. Results from inhibition of vinblastine and ritonavir efflux by SV, SVA and AVA were consistent with previously reported P-gp inhibition studies (8–10). SV inhibits P-gp with an $IC_{50}$ of 20–50 μM while SVA did not show significant P-gp inhibition at concentrations as high as 50 μM. AVA inhibited P-gp mediated vinblastine efflux in Caco-2 cells, but did not show significant P-gp inhibition in KBV1 or L-MDR1 cells. The discrepancy in inhibitory properties of AVA in the different cell lines most likely reflects different uptake mechanism of AVA. For inhibition of P-gp to be detected, AVA must be present at sufficient intracellular concentrations to significantly impact P-gp activity. In transport experiments AVA showed low passive permeability in LLC-PK1 cells. Therefore failure to observe inhibition of P-gp in the KBV1 and L-MDR1 cells may reflect the poor passive membrane permeability restricting accumulation of sufficient intracellular AVA levels to cause significant inhibition of P-gp activity. In Caco-2 cells intestinal anion transporters facilitate uptake of by AVA (15,19). Results from transport studies did not show a correlation with the inhibition studies. SV which inhibits human P-gp is not subject to significant transport by human P-gp even at concentrations an order of magnitude lower than its $IC_{50}$ for P-gp efflux. Based on the B to A/ A to B transport ratios of 2.4–3.8 and 3.0–6.4 for SVA and AVA, respectively, both SVA and AVA are considered moderate P-pg substrates, but only AVA inhibited P-gp. Collectively these results indicate that inhibition of P-gp does not predict P-gp mediated transport of these statins.

When considering potential for pharmacokinetic drug-drug interactions with P-gp a drug should be evaluated to determine if it is a P-gp inhibitor thus potentially influencing the disposition of other drugs, and if it is a substrate for P-gp in which case disposition of the drug itself could be impacted by other P-gp inhibitors. Previous reports evaluating interactions of various statins with P-glycoprotein have (8–10) extrapolated results from P-gp inhibition assays to identify compounds as substrates for P-gp. However the lack of correlation between directional transport and inhibition studies seen for SV, SVA and AVA indicate that the assumption that P-gp substrates will inhibit P-gp at reasonable concentrations is incorrect. Inhibition studies would incorrectly identify SV as a P-gp substrate, and would not identify SVA as a substrate yielding both false positive and false negative results. A second surrogate assay was applied in one study in which modulation of P-gp-ATPase activity was evaluated (8). In those studies AVA was observed to inhibit P-gp-ATPase activity while simvastatin enhanced ATPase activity. Based on these diametrically apposed results it was concluded that both AVA and SV are substrates for P-gp. However, our results indicate that only AVA, but not SV, is subject to significant directional transport by human P-gp. Although, it has not previously been reported for structurally related compounds, comparisons between P-gp transport and P-gp inhibition or P-gp-ATPase activity have failed to show a quantitative or qualitative relationships. Results from Polli *et al.* (20) and Scala *et al.* (21) indicate that these surrogate assays show less than 50% qualitative correlation with P-gp efflux assays with a high proportion of both false positive and false negative

predictions. Those studies as well as the results presented in this paper clearly call into question the validity of concluding that statins which inhibit P-gp or alter P-gp-ATPase activity are substrates for P-gp

Given the transport and inhibition studies presented here it is unlikely that P-gp plays a significant role in observed clinical drug interactions for SV. At a dose of 40 mg/day systemic steady state SV levels were <100 nM, well below concentrations that would inhibit P-gp. Considering also that SV is a highly plasma protein bound (96%), it is unlikely that circulating levels of SV would impact P-gp transport of other drugs. This conclusion is further validated by the fact that SV co-administration results in relatively minor (pharmacologically irrelevant) elevations in systemic levels (or AUCs) of other P-gp substrates such as digoxin (22). It is also unlikely that P-gp activity has a significant role in disposition of SV or SVA. In man SV is rapidly and nearly completely absorbed consistent with its relatively high permeability in this study, suggesting that P-gp is not a factor in its oral absorption. Moreover, the finding that SV is not subject to significant P-gp transport suggests that P-gp would also not be a limiting factor in its clearance. Consequently, it is unlikely that P-gp plays a role in increased simvastatin plasma levels observed when co-administered with other agents (7–10). Although P-gp mediated directional transport is observed for its active metabolite SVA, the ratios observed in MDR1 transfected cells are relatively low compared to the conventional P-gp substrates vinblastine, ritonavir, and loperamide. These conventional substrates show high transport ratios (10–20) in human P-gp expressing LLC-PK1 cells (results not shown), but show less than a 2-fold impact on oral and iv drug plasma levels of P-gp substrates (23–25). Therefore it is less likely that the low ratio observed for human P-gp transport of SVA can account for the large increases in SV and SVA plasma levels associated with adverse drug interactions. In the case of AVA the inhibition of P-gp is unlikely to have a significant impact on the disposition of other drugs given its high IC50 relative to systemic plasma levels. This is further verified by results showing clinically insignificant effects of atorvastatin on oral absorption of digoxin with only a 15% increase in digoxin AUC when orally administration with 80mg atorvastatin. However involvement of P-gp in disposition of atorvastatin is more difficult to assess since active uptake of AVA complicate the evaluation of P-gp's role in AVA disposition.

In summary the results presented in this paper do not support P-gp as a significant contributor to adverse drug interaction observed with SV and potentially other statins. Instead, interactions due to inhibition of metabolism, and interactions with hepatic uptake transporters (26,27,28) are more likely to be contributing to drug interactions observed with this class of compounds.

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## REFERENCES

1. T. R. Pedersen, K. Berg, and T. J. Cook. et. al. Safety and tolerability of cholesterol lowering with simvastatin during 5 years in the Scandinavian Survival Study. *Arch. Intern. Med.* **156**:2085–2092 (1996).
2. R. F. Reinoso, A. Sanchez Navarro, M. J. Garcia, and J. R. Prous. Pharmacokinetic interactions of Statins. *Methods Find Exp. Clin. Pharamacol.* **23**:541–566 (2001).
3. M. Igel, T. Sudhop, and K. vonBergman. Metabolism and drug interactions of 3-hydroxy-3methylglutaryl coenzyme A-reductase inhibitors (statins). *Eur. J. Clin. Pharmacol.* **57**:357–364 (2001).
4. P. D. Thompson, P. Clarkson, and R. H. Karas. Statin Associated Myopathy. *JAMA* **289**:1681–1690 (2003).
5. T. Prueksaritanont, B. Ma, and C. Tang. et. al. Metabolic interactions between mibefradil and HMG-CoA reductase inhibitors: an in vitro investigation with human liver preparations. *Br. J. Clin. Pharmacol.* **47**:291–298 (1999).
6. T. Prueksaritanont, C. Tang, Y. Qui, L. Mu, R. Subramanian, and J. H. Lin. Effects of fibrates on metabolism of statins in human hepatocytes. *Drug Metab. Dispos.* **839**:1280–1287 (2002).
7. P. H. Siedlik, S. C. Olson, B. B. Yang, and R. H. Stern. Erythromycin coadministration increases plasma atorvastatin concentrations. *J. Clin. Pharmacol.* **39**:501–504 (1999).
8. E. Wang, C. N. Casciano, R. P. Clement, and W. W. Johnson. HMG-CoA reductase inhibitors (statins) characterized as direct inhibitors of P-glycoprotein. *Pharm. Res.* **18**:800–806 (2001).
9. K. Bogman, A.-K. Peyer, M. Torok, E. Kusters, and J. Drewe. HMG-CoA reductase inhibitors and P-glycoprotein modulation. *Br. J. Pharmacol.* **132**:1183–1192 (2001).
10. T. Sakaeda, K. Takara, and M. Kakumoto. et. al. Simvastatin and lovastatin, but not pravastatin, interact with MDR1. *J. Pharm. Pharmacol.* **54**:419–423 (2002).
11. A. H. Schinkel. The physiological function of drug-transporting P-glycoproteins. *Semin. Cancer Biol.* **8**:161–170 (1997).
12. Z. C. Gatmaitan and I. M. Arias. Structure and function of P-glycoprotein in normal liver and small intestine. *Adv. Pharamcol* **24**:77–97 (1993).
13. F. Thiebaut, T. Tsuruo, H. Hamada, M. M. Gottesman, I. Pastan, and M. C. Willingham. Immunohistochemical localization in normal tissues of different epitopes in the multidrug transport protein P170: evidence for localization in brain capillaries and crossreactivity of one antibody with a muscle protein. *J. Histochem. Cytochem.* **37**:159–164 (1989).
14. C. Cordon-Cardo. O'brien JP, Boccia J, Casals D, Bertino JR, and Melamed MR Expression of multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. *J. Histochem. Cytochem.* **38**:1277–1287 (1990).
15. X. Wu, L. R. Whitfeild, and B. H. Stewart. Atorvastatin transport in Caco-2 cell model: Contribution of P-glycoprotein and Proton-monocarboxylic acid transporter. *Pharm. Res.* **17**:209–215 (2000).
16. M. Yamazaki, W. E. Neway, and T. Ohe. Chen I-Wu, Rowe JF, Hochman JH, Chiba M, and Lin JH. In vitro substrate identification studies for P-glycoprotein-mediated transport: Species difference and predictability of in vivo results. *J. Pharamacol. Exp. Ther.* **296**:723–735 (2001).
17. D. W. Shen, C. Cardarelli, J. Hwang, M. Cornwell, N. Richert, S. Ishii, I. Pastan, and M. M. Gottesman. Multiple drug-resistant human KB carcinoma cells independently selected for high-level resistance to colchicine, adriamycin, or vinblastine show changes in expression of specific proteins. *J. Biol. Chem.* **261**:7762–7770 (1986).
18. A. H. Schinkel, E. Wagenaar, L. VanDeemter, C. A. A. M. Mol, and P. Borst. Absence of mdr1a P-glycoprotein in mice affects tissue distribution and pharmacokinetics of dexamethasone digoxin and cyclosporin A. *J. Clin. Invest.* **96**:1698–1705 (1995).
19. H. Lennernas. Clinical pharmacokinetics of atorvastatin. *Clin. Pharmacokinet.* **42**:1141–1160 (2003).
20. J. W. Polli, S. A. Wring, J. E. Humphreys, L. Huang, J. B. Morgan, L. O. Webster, and C. S. Serabjit-Singh. Rational use of P-glycoprotein assays in drug discovery. *J. Pharmacol. Exp. Ther.* **299**:620–628 (2001).
21. S. Scala, N. Akhmed, U. S. Rao, K. Paull, L. B. Lan, B. Dickstein, J. S. Lee, G. H. Elgemeie, W. D. Stein, and S. E. Bates. P-glycoprotein substrates and antagonists cluster into two distinct groups. *Mol. Pharmacol.* **51**:1024–1033 (1997).

22. Zocor product insert. Physicians Desk Reference 56th edition. 2002; 2219-2223.
23. J. V. Asperen, O. van Tellingen, A. H. Schinkel, and J. H. Beijnen. Comparative pharmacokinetics of vinblastine after a 96-hour continuous infusion in wild-type and mice lacking mdr1a P-glycoprotein. J. Pharmacol. *And Exp. Therap.* **289**:329–333 (1999).
24. R. B. Kim, M. F. Fromm, C. Wandel, B. Leake, A. J. J. Wood, D. M. Roden and G. R. Wilkinson. The drug transporter P-glycoprotein limits oral absorption and brain entry of of HIV-1 protease inhbitors. *J. Clin. Invest.* **101**:289–294 (1998).
25. A. H. Schinkel, E. Wagenaar, C. A. Mol, and L. van Deemter. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J. Clin. Invest.* **97**:2517–2524 (1996).
26. D. Nakai, R. Nakagomi, Y. Furuta, T. Tokui, T. Abe, T. Ikeda, and K. Nishimura. Human liver specific anion transporter, LST-1, mediates uptake of pravastatin by human hepatocytes. *J. Pharmacol. Exp. Therap.* **297**:861–867 (2001).
27. K. Nezasa, K. Higaki, M. Takeuchi, M. Nakano, and M. Koike. Uptake of rosuvastatin by isolated rat hepatocytes: comparison with pravastatin. *Xenobiotica* **33**:379–388 (2003).
28. Y. Shitara, T. Itoh, H. Sato, A. P. Li, and Y. Sugiyama. Inhibition of transporter-mediated hepatic uptake as a mechanism for drug-drug interaction between cerivastatin and cyclosporin A. *J. Pharmacol. Exp. Ther.* **304**:610–616 (2003).