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Transport of statins by multidrug resistance-associated proteins 1 and 5

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ABSTRACT

Statins are widely used in the treatment of hypercholesterolemia but also associated with muscle-related adverse effects. Multidrug resistance-associated protein (MRP) 1 and 5 are expressed in the skeletal muscle, where they may regulate intramuscular levels of their substrates. Here, we investigated the transport of various statins by MRP1 and MRP5 with the vesicular transport assay. Statin concentrations in the vesicles were determined with liquid chromatography tandem mass spectrometry. At 6 μM statin concentration, MRP1 transported both 3R,5S-fluvastatin and 3S,5R-fluvastatin with uptake ratios of 2.6 and 2.0. MRP5 transported 3R,5S-fluvastatin, 3S,5R-fluvastatin, and 10 μM pitavastatin with uptake ratios of 2.9, 3.7, and 2.6, respectively. Atorvastatin was only a weak substrate of MRP5 with an uptake ratio of 1.6 and was therefore not investigated further. In concentration-dependent transport experiments, racemic fluvastatin was transported by MRP1 and MRP5 with apparent affinities (K_m) of 225 μM and 23 μM. Pitavastatin was transported by MRP5 with a K_m value of 433 μM. *In vitro* clearance ($CL_{in\ vitro}$) of fluvastatin was 0.36 μl/min/mg for MRP1, while MRP5 exhibited a $CL_{in\ vitro}$ value of 1.2 μl/min/mg for fluvastatin and 0.21 μl/min/mg for pitavastatin. Pravastatin, rosuvastatin, and simvastatin acid were not transported by MRP1 or MRP5. Atorvastatin and pitavastatin were not transported by MRP1. These data indicate that MRP1 transports fluvastatin, while MRP5 transports both fluvastatin and pitavastatin. Because MRP1 and MRP5 are expressed in the skeletal muscle, they may reduce myocyte exposure to fluvastatin and pitavastatin and protect from muscle toxicity.

1. Introduction

Cardiovascular diseases are among the most common causes of death worldwide (Roth et al., 2020). 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, also called statins, are commonly used for the primary and secondary prevention of cardiovascular diseases (Fig. 1). Statins are effective in reducing low-density lipoprotein cholesterol levels, generally safe, and well-tolerated, with mostly mild adverse effects (Yebyo et al., 2019). However, they can cause musculoskeletal adverse effects ranging from common and mild myalgia to rare and possibly life-threatening rhabdomyolysis (Harper and Jacobson, 2007). Importantly, statin-related myotoxicity is dose- and concentration-dependent (Alfirevic et al., 2014; Neuvonen et al., 2006). Several factors, including drug interactions, pharmacogenetic variability, and advanced age, may increase the systemic statin exposure, leading to elevated statin concentrations in myocytes and skeletal muscle toxicity (Cooper-DeHoff et al., 2022; Lönnberg et al., 2023; Turner and Pirmohamed, 2019).

Solute carrier (SLC) and ATP-binding cassette (ABC) transporters are membrane proteins which have a crucial role in the cellular influx and efflux of drugs, affecting their plasma concentrations and tissue distribution (Galetin et al., 2024; Giacomini et al., 2010; Koepsell, 2013; Vasiliou et al., 2008). Organic anion transporting polypeptides (OATP) 1B1, 1B3, and 2B1 mediate the influx of statins into the liver (Bi et al., 2019; Elsby et al., 2012; Mykkänen et al., 2022, 2024; Niemi et al., 2011). Breast cancer resistance protein (BCRP) and the P-glycoprotein, on the other hand, may restrict the intestinal absorption of statins (Deng et al., 2021; Elsby et al., 2012; Keskitalo., et al., 2009a, 2009b; Lehtisalo et al., 2023; Neuvonen et al., 2006). Several drug transporters are expressed in the skeletal muscle (Knauer et al., 2010). They may participate in regulating statin concentrations in myocytes and, because myotoxicity is concentration-dependent, contribute to statin-related

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myotoxicity.

OATP2B1 is an influx transporter expressed in the skeletal muscle and transports atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin acid (Grube et al., 2006; Ho et al., 2006; Kopplow et al., 2005; Nozawa et al., 2004; Prieto Garcia et al., 2024; Varma et al., 2011; Lehtisalo et al., 2023), but its role in the statin influx in skeletal muscle and myotoxicity is unclear. Additionally, monocarboxylate transporters (MCT) 1 and 4 are expressed in the skeletal muscle, and inhibition of MCT4 by statins has been linked with statin-induced cytotoxicity (Bonen et al., 1998; Kikutani et al., 2016; Kobayashi et al., 2006; Pilegaard et al., 1999). Multidrug resistance-associated protein (MRP) 1, 4, and 5 efflux transporters are highly expressed in the skeletal muscle, unlike other well-characterized ABC efflux transporters such as MRP2, BCRP, and P-gp (Knauer et al., 2010). Their physiological role in skeletal muscle cell is not well-characterized, but they may protect cells from endogenous metabolites and xenobiotics. MRP1 transports organic anions, conjugates, and anticancer drugs (Jedlitschky et al., 1996; Renes et al., 1999), whereas MRP4 and MRP5 transport cyclic nucleotides, antivirals, and anticancer drugs (Chen et al., 2001; Jedlitschky et al., 2000; Sampath et al., 2002; Schuetz et al., 1999). In addition, previous studies suggest that MRP1 and MRP5 may transport various statins (Afrouzian et al., 2018; ; Hoste et al., 2023; Kanamitsu et al., 2017; Knauer et al., 2010; Prieto Garcia et al., 2024). However, the studies have been conducted in different laboratories employing different methods making the comparison between statins challenging.

Statin-related myotoxicity can be life-threatening, but even when mild, the toxicity can be clinically important as it often leads to discontinuation of therapy (Alfirevic et al., 2014; Cooper-DeHoff et al., 2022; Voora et al., 2009). Therefore, we considered it important to investigate how statins are transported by efflux transporters expressed in the skeletal muscle. Previously, we characterized and compared the transport of atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin acid, and four atorvastatin metabolites by six ABC transporters (BCRP, MRP2, MRP3, MRP4, MRP8, P-glycoprotein) (Deng et al., 2021). Here, we investigate the transport of atorvastatin, two fluvastatin enantiomers, pitavastatin, pravastatin, rosuvastatin, and simvastatin acid by MRP1 and MRP5.

2. Materials and methods

2.1. Materials

Atorvastatin, atorvastatin-d5, fluvastatin-d8, pitavastatin-d5, pravastatin, pravastatin-d9, rosuvastatin, rosuvastatin-d6, and simvastatin acid-d6 were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Racemic fluvastatin, 3R,5S-fluvastatin, 3S,5R-fluvastatin, and pitavastatin were obtained from Santa Cruz Biotechnology (Dallas, TX, USA). Simvastatin acid was acquired from SynFine Research (Richmond Hill, Ontario, Canada). MRP1, MRP5, and mock-transfected control vesicles derived from human embryonic kidney 293 cells were purchased from PharmTox (Radboud UMC, Nijmegen, The Netherlands). Solvents used in assays and analytical methods were of analytical quality and purchased from Sigma-Aldrich (St. Louis, MO, USA). Ultrapure water for assays and analyses was purified with Milli-Q water purification system (Merck Millipore, Burlington, MA, USA).

2.2. Vesicular transport assay

The vesicular transport assay was used to investigate statin transport by MRP1 and MRP5 *in vitro*. The assays were carried out with inside-out oriented membrane vesicles over-expressing transporters, and performed as described previously (Deng et al., 2021). In short, membrane vesicles (7.5 μg) were preincubated at 37 °C for 10 min in the reaction buffer containing various concentrations of statin, assay buffer (PharmTox), and 10 mM $MgCl_2$. The transport was initiated by adding prewarmed ATP or AMP (4 mM) to the mixture. Samples were incubated at 37 °C for the indicated time. The reactions were terminated with ice-cold stop buffer (PharmTox) and samples were quickly transferred to a MultiScreenHTS FB Filter Plate 1.0 mm/0.65 mm (Merck KGaA, Darmstadt, Germany). The vesicles were immediately washed three times with the stop buffer and twice with ice-cold wash buffer (40 mM 4-morpholinepropanesulfonic acid/Tris pH 7.0 and 70 mM KCl) and dried with airflow. The accumulated statins were eluted from the

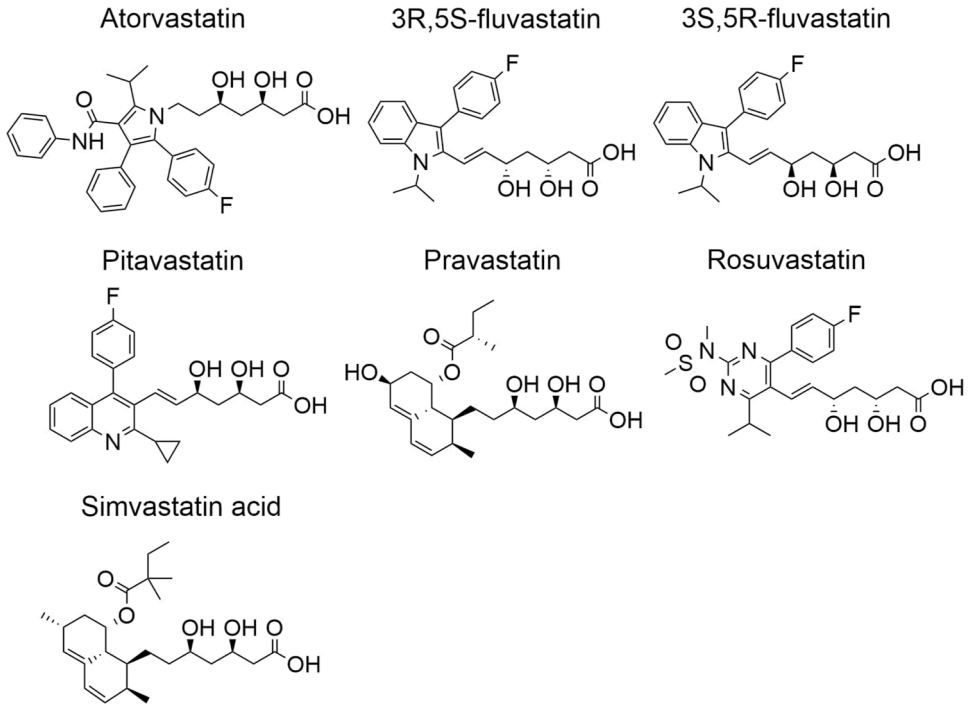

Fig 1. Chemical structures of the investigated statins.

vesicles with the lysis solution (50 % methanol and 25 ng/ml isotope-labeled statin as internal standard). All assays were performed in triplicates on 96-well plates.

The functionality of the membrane vesicles and the vesicular transport assay was confirmed by examining the transport of estradiol-17-glucuronide and 5(6)-carboxy-2′,7′-dichlorofluorescein by MRP1 and MRP5, respectively. Initially, the transport of 10 and 3 μM of atorvastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin acid and 6 μM 3R,5S-fluvastatin and 3S,5R-fluvastatin into MRP1 and MRP5 vesicles were screened with a 10 min incubation time. Statins that exhibited statistically significantly higher transport into the vesicles in the presence of ATP compared to AMP, and had an uptake ratio of 2.0 or higher, were considered as substrates of MRP1 or MRP5, and were studied further.

The time-dependent transport of 10 μM atorvastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin acid and 6 μM fluvastatin enantiomers were investigated with incubation times of 3, 6, and 10 min. Finally, the concentration-dependent transport was determined with statin concentrations ranging from 6 to 150 μM, using an incubation time of 10 min. The appropriate incubation time for the concentration-dependent transport experiments was selected based on time-dependent transport experiments. Additionally, in time-dependent and concentration-dependent transport experiments, statin transport into control vesicles was investigated.

2.3. Analytical methods

Statins were analyzed using a SCIEX 5500 quadrupole linear ion trap liquid chromatography-tandem mass spectrometry system (Sciex, Toronto, Ontario, Canada) with an electrospray ionization ion source. Chromatographic separation utilized a Luna Omega polar C18 column (100 × 2.1 mm, 1.6 μM; Phenomenex, Torrance, CA, USA) and a corresponding guard column. Mobile phases A and B consisted of 5 mM ammonium formate (pH 3.9) and acetonitrile, with a flow rate of 300 μL/min and a column temperature of 40 °C. The mobile phase gradient included a hold at 20 % B for 1 min, a linear increase to 40 % B over 3 min, then to 90 % B over 2 min, and 1 min at 90 % B, followed by re-equilibration to 20 % B. The mass spectrometer operated in multiple reaction monitoring (MRM) mode, with specific ion transitions previously documented (Deng et al., 2021).

2.4. Data and statistical analysis

Data are presented as mean ± standard deviation (SD) or mean with 95 % confidence of interval (CI). ATP-dependent transport was determined by subtracting the statin uptake into vesicles in the presence of AMP from the uptake in the presence of ATP. The uptake ratio of transport was determined by dividing the uptake of statin in the presence of ATP by the uptake in the presence of AMP. Statistical comparisons in the 6 and 10 μM screening and time-dependent transport experiments were carried out using analysis of variance (GraphPad Prism Software version 10.12, San Diego, CA). A P value of below 0.05 was considered statistically significant.

When determining the kinetic parameters of statin transport, we pooled the mean ATP-dependent transport values for each concentration data point from separate experiments. These pooled values were then fitted to the Michaelis-Menten equation Eq. (1), where v represents the velocity of ATP-dependent transport, V_{max} represents the maximum transport rate, [S] represents the substrate concentration, and K_m represents the Michaelis-Menten constant.

$$v = \frac{Vmax\ [S]}{Km + [S]} \quad (1)$$

The *in vitro* clearance ($Cl_{in\ vitro}$) of statins was calculated by using Michaelis-Menten parameters, as described in Eq. (2).

$$CL in\ vitro = \frac{Vmax}{Km} \quad (2)$$

Molecular descriptors were calculated in R version 4.4.2 with RStudio (R Core Team, 2024). The Simplified Molecular Input Line Entry Specifications (SMILES) of the investigated statins were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Molecular descriptors were calculated based on the retrieved SMILES using the rcdk package, which allows the access to the Chemistry development kit (CDK) Java libraries for chemoinformatics (Guha, 2007).

3. Results

3.1. Transport of known substrate of MRP1 and MRP5

The functionality of the vesicles was confirmed using 15 μM estradiol-17-glucuronide as a positive control for MRP1 and 5 μM 5(6)-carboxy-2′,7′-dichlorofluorescein for MRP5. The uptake ratios of these probe substrates in MRP1 and MRP5 were 7.14 and 4.44, respectively, validating both the vesicle functionality and the vesicular transport assay (Supplementary Figure S1).

3.2. Screening for statin efflux transport

MRP1 transported 3R,5S-fluvastatin and 3S,5R-fluvastatin (6 μM) with uptake ratios of 2.6 ± 0.5 and 2.0 ± 0.1, whereas MRP5 transported 3R,5S-fluvastatin, 3S,5R-fluvastatin, and pitavastatin (10 μM) with uptake ratios of 2.9 ± 0.1, 3.7 ± 0.1, and 2.6 ± 1.0, respectively (Fig. 2; Supplementary Table S1). Pravastatin, rosuvastatin, and simvastatin acid were not transported by either MRP1 or MRP5 (Fig. 2; Supplementary Figure S2; Supplementary Tables S1-2). Additionally, atorvastatin and pitavastatin were not transported by MRP1. Atorvastatin uptake by MRP5 in the presence of ATP differed statistically significantly from that in the presence of AMP. However, the uptake ratio was below two (Supplementary Tables S1-2), indicating that atorvastatin is only a weak substrate of MRP5 and was therefore not investigated further.

3.3. Time-dependent transport

The ATP-dependent transport of fluvastatin enantiomers into MRP1 and MRP5 vesicles and pitavastatin into MRP5 vesicles was time-dependent and differed statistically significantly from control vesicles latest at the 10 min time point (Fig. 3; Supplementary Table S3).

3.4. Concentration-dependent transport

To determine the kinetic parameters of statin transport, we next investigated the concentration-dependent transport of fluvastatin into MRP1, MRP5, and control vesicles, and pitavastatin into MRP5 and control vesicles (Fig. 4 and Table 1). Due to the similar levels of transport observed for the fluvastatin enantiomers, racemic fluvastatin was used to study concentration-dependent transport. Fluvastatin was transported into MRP1 and MRP5 vesicles with K_m values of 225 and 23 μM, and with V_{max} values of 81 and 27 pmol/min/mg. The $CL_{in\ vitro}$ values of fluvastatin by MRP1 and MRP5 were 0.36 and 1.2 μl/min/mg. Pitavastatin was transported by MRP5 with a K_m value of 433 μM, a V_{max} of 92 pmol/min/mg and a $CL_{in\ vitro}$ of 0.21 μl/min/mg.

3.5. Molecular descriptors of the investigated statins

According to molecular descriptors, fluvastatin and pitavastatin have smaller total polar surface areas (81 – 90 vs 104 – 148), number of rotatable bonds (8 vs 10 – 13), and number of hydrogen bond acceptors (5 vs 6 – 9) than the rest of the investigated statins (Supplementary Table S4).

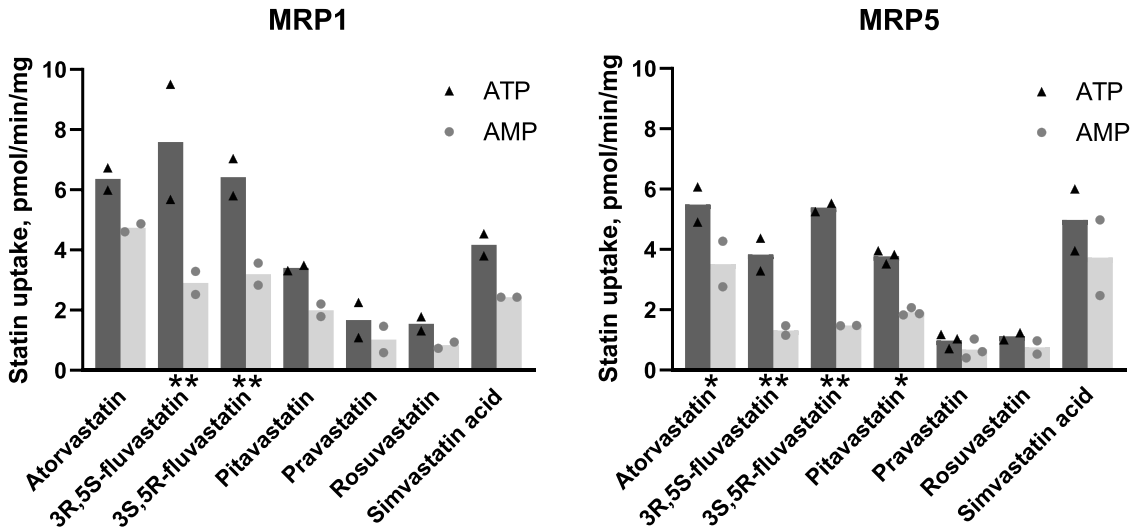

Fig. 2. The uptake of statins into MRP1 and MRP5 vesicles. The statin concentrations were 10 μM, except 6 μM for fluvastatin enantiomers. The incubation time was 10 min and amount of protein 7.5 μg. Dark and light bars represent the mean of two or three experiments in the presence of ATP and AMP, respectively. The means of single experiments are indicated by triangles and circles. * $P < 0.05$, ** $P < 0.01$ compared to AMP.

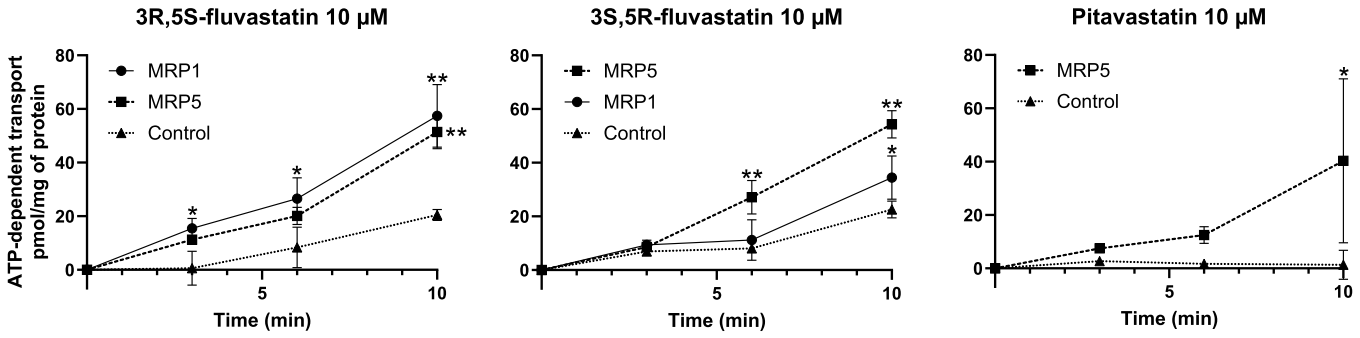

Fig. 3. The time-dependent transport of 6 μM fluvastatin enantiomers into MRP1, MRP5, and control vesicles, and 10 μM pitavastatin into MRP5 and control vesicles. The amount of protein was 7.5 μg. Results were obtained from two (three for 3S,5R-fluvastatin and MRP5) separate experiments with triplicate samples. * $P < 0.05$, ** $P < 0.01$ compared to control vesicles.

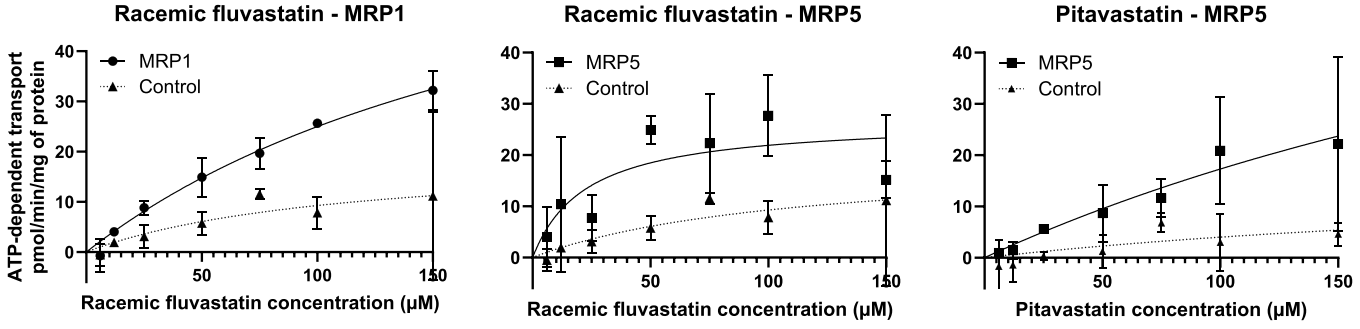

Fig. 4. The concentration-dependent transport of fluvastatin into MRP1, MRP5, and control vesicles, and pitavastatin into MRP5 and control vesicles. The incubation time was 10 min and amount of protein 7.5 μg, except for the transport of fluvastatin by MRP1, which was studied with a protein amount of 11.25 μg. Results were obtained from two to four separate experiments with triplicate samples.

4. Discussion

Skeletal muscle ABC transporters, such as MRP1 and MRP5, may regulate the intracellular concentrations of statins in myocytes and contribute to statin-related myotoxicity. In this study, we investigated the transport of atorvastatin, 3R,5S-fluvastatin, 3S,5R-fluvastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin acid by MRP1 and MRP5 *in vitro* with the vesicular transport assay. Since all the statins were investigated with the same method in a single laboratory, the results are directly comparable between statins and with the MRP4 data from our previous study, which employed the same method in the same laboratory (Deng et al., 2021). Our findings indicate that MRP1 transports fluvastatin, whereas MRP5 transports both fluvastatin and pitavastatin.

In addition to MRP1 and MRP5, MRP4 has been detected in the skeletal muscle (Knauer et al., 2010). In our previous study employing

Table 1
Estimated kinetic parameters of fluvastatin, pitavastatin, and rosuvastatin transport by MRP1, MRP4, and MRP5.

Statin	Transporter	V_{max} (95 % CI) (pmol/min/mg)	K_m (95 % CI) (μM)	$CL_{in\ vitro}$ (μl/min/mg)	Number of experiments
Racemic fluvastatin	MRP1	81.2 (54.1-176)	225 (118-620)	0.361	2-3
Racemic fluvastatin (Deng et al., 2021)	MRP4	54.7 (23.4–4053)	31.8 (0–∞)	1.72	3
Racemic fluvastatin	MRP5	26.9 (18.1-45.0)	22.8 (4.89-91.0)	1.18	3
Pitavastatin	MRP5	92.1 (24.1-∞)	433 (52.4-∞)	0.213	3-4
Rosuvastatin (Deng et al., 2021)	MRP4	12.3 (5.13–∞)	39.3 (0.3–∞)	0.312	3

$CL_{in\ vitro}$, in vitro clearance; K_m, Michaelis-Menten constant; V_{max}, maximum transport rate.

the same methodology, MRP4 transported fluvastatin and rosuvastatin, but not other statins (Deng et al., 2021) (Table 2). The $CL_{in\ vitro}$ values of MRP4 for fluvastatin and rosuvastatin were 1.7 and 0.31 μl/min/mg. In the current study, the $CL_{in\ vitro}$ for MRP1-mediated fluvastatin transport was 0.36 μl/min/mg, while MRP5 transported fluvastatin and pitavastatin with $CL_{in\ vitro}$ values of 1.2 and 0.21 μl/min/mg. In the previous study, the clinically important statin efflux transporter BCRP transported fluvastatin and pitavastatin with substantially higher $CL_{in\ vitro}$ values (16 and 7.3 μl/min/mg) than any of the MRPs investigated in our present and previous study (Deng et al., 2021). While the chemical structures of fluvastatin and pitavastatin are quite similar, the observed K_m and $CL_{in\ vitro}$ values of fluvastatin and pitavastatin in MRP5 vesicles were strikingly different. The nitrogen atom of the quinoline ring in pitavastatin, capable of accepting a hydrogen atom, may contribute to the different transport kinetics in MRP5. Moreover, fluvastatin and pitavastatin have smaller polar surface areas, and less rotatable bonds and hydrogen bond acceptors than other statins. This suggests that the rigid structure and low hydrogen bonding in addition to lipophilicity could be beneficial for the interaction between statins and MRP1 and MRP5. Overall, the absolute protein abundances of MRP1, MRP4, and MRP5 remain unclear, thus restricting the assessment of their relative contributions to statin efflux in the skeletal muscle.

Unlike previous studies, we observed no transport of atorvastatin, pravastatin, or simvastatin acid by MRP1 nor that of rosuvastatin by MRP5. Two studies using HeLa and HEK293 cell accumulation assays suggested that atorvastatin is an MRP1 substrate (Hoste et al., 2023; Knauer et al., 2010). Because atorvastatin is relatively lipophilic (Turner and Pirmohamed, 2019), passive diffusion and non-specific binding of atorvastatin may have masked small ATP-dependent uptake of atorvastatin into MRP1 vesicles in our study. Additionally, Knauer and co-authors showed that rosuvastatin is a good substrate for MRP1 and MRP4, and relatively poor substrate for MRP5 (Knauer et al., 2010). In our study, the uptake of rosuvastatin into MRP1 vesicles in the presence of ATP was 1.9-fold higher than that in the presence of AMP, but the difference was not statistically significant. In a recent study, MRP1 was suggested to transport pravastatin and simvastatin acid in Spodoptera frugiperda (Sf9) membrane vesicles (Prieto Garcia et al., 2024). However, control vesicle and uptake ratio data were not reported, which complicates the interpretation of the results. Nevertheless, another study showed that 1 μM pravastatin was transported in Sf9-MRP1 vesicles with an uptake ratio of 5.0 (Afrouzian et al., 2018).

Instead of whole cell-based methods, we chose to use membrane vesicles as they enable investigating the direct interaction between the drug and transporter, and precise determination of transport kinetics. Moreover, we chose human cell line-derived vesicles since they exhibit higher activity than the insect cell-derived Sf9 vesicles (Sáfár et al., 2021). In addition, post-translational protein modification differs between insect and human cells and the amount of membrane cholesterol is lower in insect cells (Pál et al., 2007). Cholesterol can be loaded to the Sf9 vesicles to increase transporter activity, but this also activates endogenous ABC transporters, possibly SfABCC2 and SfABCC3 (Sjöstedt et al., 2019).

Atorvastatin, fluvastatin, pitavastatin, and simvastatin acid are relatively lipophilic, whereas pravastatin and rosuvastatin are relatively hydrophilic (Neuvonen et al., 2006; Turner and Pirmohamed, 2019). It appears that lipophilic statins are more myotoxic than the hydrophilic

Table 2
Summary of atorvastatin, fluvastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin acid transport by MRP1, MRP4, MRP5, and OATP2B1.

		MRP1 (K_m)	MRP4 (K_m)	MRP5 (K_m)	OATP2B1 (K_m)	References
Atorvastatin	Current study	-	n/a	+	n/a	
	Literature	+	+	-	2.8 μM	(Hoste et al., 2023; Karlgren et al., 2012; Knauer et al., 2010)
Fluvastatin	Current study	225 μM	n/a	23 μM	n/a	
	Literature	n/a	32 μM	n/a	0.7 - 0.8 μM	(Bi et al., 2019; Deng et al., 2021; Kopplow et al., 2005; Noé et al., 2007; Varma et al., 2011)
Pitavastatin	Current study	-	n/a	443 μM	n/a	
	Literature	n/a	+	n/a	1.2 - 25 μM	(Hirano et al., 2006; Kanamitsu et al., 2017; Kanda et al., 2018; Ölander et al., 2016)
Pravastatin	Current study	-	n/a	-	n/a	
	Literature	2.5 - 8.7 μM	+	n/a	+	(Afrouzian et al., 2018; Bi et al., 2019; Kanamitsu et al., 2017; Nozawa et al., 2004; Prieto Garcia et al., 2024; Varma et al., 2011)
Rosuvastatin	Current study	-	n/a	-	n/a	
	Literature	+	21 - 39 μM	+	2.4 - 6.4 μM	(Ho et al., 2006; Deng et al., 2021; Kanda et al., 2018; Kitamura et al., 2008; Knauer et al., 2010; Lehtisalo et al., 2023; Pfeifer et al., 2013)
Simvastatin acid	Current study	-	n/a	-	n/a	
	Literature	179 μM	-	n/a	384 μM	(Deng et al., 2021; Prieto Garcia et al., 2024)

+, transported, but K_m value not available; -, not transported; n/a, not available.

statins *in vitro*. For example, pravastatin and rosuvastatin were less cytotoxic in C2C12 myoblasts than atorvastatin, fluvastatin, and simvastatin acid (Schirris et al., 2015). In addition, in satellite cells isolated from human skeletal muscle biopsies, pravastatin showed lower myotoxicity than atorvastatin, fluvastatin, and simvastatin acid (Skottheim et al., 2008). Since MRP1 and MRP5 are expressed in the skeletal muscle, they may play a protective role against fluvastatin- and pitavastatin-induced muscle toxicity. Supporting this hypothesis, MK-571, dipyridamole, quercetin, and verapamil, known inhibitors of MRP1, MRP4, MRP5, and OATP2B1, have increased the intracellular retention of atorvastatin and rosuvastatin in cultured human skeletal muscle myoblasts (Knauer et al. 2010). Moreover, overexpression of OATP2B1 has elevated atorvastatin and rosuvastatin accumulation and toxicity in myoblasts (Knauer et al., 2010). In addition, coadministration of the MRP1 inhibitor probenecid with rosuvastatin in rats led to increased skeletal muscle toxicity (Dorajoo et al., 2008).

MRP1 and MRP5 expression have been detected in skeletal muscle by western blot analyses (Knauer et al., 2010). It has been estimated that MRP1 expression in skeletal muscle is 60-fold higher than that of MRP5 (Prieto Garcia et al., 2024), but absolute quantitative evidence, such as liquid chromatography-tandem mass spectrometry-based proteomics, are lacking. This absence of quantitative data makes it challenging to estimate the *in vivo* significance of these transporters in humans. MRP1 and MRP5 are also expressed in various other tissues across the body, for example in the brain and the placenta (Meyer zu Schwabedissen et al., 2005; Nies et al., 2004; Stage et al., 2020; St-Pierre et al., 2000). In barrier tissues such as the placenta, efflux transporters may restrict the entry of harmful compounds. Moreover, MRP5 may play a role in intracellular signaling, since it transports cyclic nucleotides (Jedlitschky et al., 2000; Meyer zu Schwabedissen et al., 2005).

Interestingly, MRP5 is also expressed in the human heart (Dazert et al., 2003). The absence of the skeletal muscle influx transporter MCT4 in cardiomyocytes has been suggested to protect against statin-induced cardiomyotoxicity (Sirvent et al., 2005). The presence of efflux transporters like MRP5, might provide additional protection against statin-induced cardiotoxicity by transporting statins out of the cardiomyocytes. In addition to statin-induced muscle toxicity, statins dose-dependently increase the risk of type 2 diabetes (Lotta et al., 2016; Sattar et al., 2010). However, the underlying mechanism of the risk increase is not known. Some studies have reported individual cases of polyneuropathy associated with statin treatment (Law and Rudnica, 2006). As MRP1 is expressed in neurons (Stage et al., 2020), it is tempting to suggest that it might play a protective role against this condition.

The influx and efflux of statins by skeletal muscle transporters can vary due to drug interactions or genetic variability. For example, genetic variability in *SLCO2B1* has been associated with altered rosuvastatin exposure (Lehtisalo et al., 2023). In a recent study, simvastatin acid and pravastatin were found as *in vitro* OATP2B1 and MRP1 substrates (Prieto Garcia et al., 2024). The authors used proteomics-informed physiologically based pharmacokinetic modelling to simulate the effect of 90 % reduced OATP2B1 or MRP1 activity on the statin plasma and tissue exposure. The modelling suggested that the reduced OATP2B1 activity would decrease muscle-to-plasma ratio of simvastatin acid by 8.5-fold, while the reduced MRP1 activity would increase muscle-to-plasma ratio of simvastatin acid by 5-fold. Moreover, the presence of MRP1 was estimated to reduce the muscle simvastatin acid and pravastatin exposure by 13- and 2-fold compared with passive distribution. This model implies that MRP1 may affect the statin concentration in skeletal muscle and thus protect from statin-induced myotoxicity.

5. Conclusion

In conclusion, our present and previous findings demonstrate that MRP1, MRP4, and MRP5, which are expressed in the skeletal muscle, transport fluvastatin *in vitro*, while MRP5 additionally transports pitavastatin, and MRP4 transports rosuvastatin. These data indicate that statins differ in their skeletal muscle efflux transport profiles. Moreover, the data can be utilized to improve prediction of statin-induced adverse effects and to enhance physiologically based pharmacokinetic models. In the future, comprehensive investigations into the expression of skeletal muscle drug transporters and the transport of statins by other skeletal muscle transporters, such as MCTs and OATP2B1, are essential to understand the factors influencing statin concentrations in the skeletal muscle. In addition, linking statin-induced muscle toxicity to transporter expression is important for understanding the transporter-related mechanisms of muscle toxicity. Recognizing and considering the role of transporters in adverse effects becomes crucial in the clinical use of statins to proactively manage and anticipate statin-induced muscle toxicity.

CRediT authorship contribution statement

Suvi-Kukka Tuomi: Writing – original draft, Methodology, Formal analysis, Conceptualization. **Feng Deng:** Writing – review & editing, Supervision, Methodology, Formal analysis, Conceptualization. **Mikko Neuvonen:** Writing – review & editing, Methodology, Formal analysis. **Mikko Niemi:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2025.107070.

Data availability

Data will be made available on request.

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