T2019 Applications of Inside-Out Vesicle Uptake Assay to Assess the Inhibitory Effects of Various Drugs on Human MRP2 and Rat Mrp2 Transporters

Vina Ketty, Eric Sands, Joanne Bourgea, Chris J. Patten, Charles L. Crespi, Ph.D., and Guangqing Xiao
BD Biosciences - Discovery Labware, Woburn, MA 01801, USA

Abstract

Human MRP2 and rat Mrp2 inside-out vesicles were prepared from *Spodoptera frugiperda* (Sf9) insect cells expressing the corresponding transporters. MRP2 mediated and ATP dependent uptake of ^{3}H-LTC$_4$ was conducted using the rapid filtration system in the absence or presence of MgATP. The inhibitory effects of several HMG-CoA Reductase inhibitors (statins), Ritonavir, Cyclosporin A (CsA), Vinblastine, Vincristine, Benzbromarone, Irinotecan (CPT-11), SN-38, Estradiol-17-β-D-Glucuronide, Methotrexate, and 2-Amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP) were investigated. CsA, Benzbromarone, Atorvastatin Acid, and CPT-11 had significant inhibition to both human MRP2 and rat Mrp2, with IC$_{50}$ values less than 100 µM or close to 100 µM. SN-38, Simvastatin lactone, Vinblastine, and Vincristine also showed inhibition on both human MRP2 and rat Mrp2, although their IC$_{50}$ values were greater than 100 µM. Ritonavir and Pravastatin acid had strong inhibition to rat Mrp2, while exhibiting no inhibitory effects to human MRP2. Methotrexate, PhIP, Cerivastatin acid, and Lovastatin lactone did not affect the transport by human MRP2 and rat Mrp2. In contrast to their lactone forms, CPT-11 and SN-38 in carboxylate form had strong inhibition to both human MRP2 and rat Mrp2. Our study demonstrated that inside-out vesicles are a useful model to examine the interaction between MRP2 and drugs. By using inside-out vesicles, different inhibitory effects of various drugs were observed.

Introduction

Human MRP2 and rat Mrp2 transporters are expressed in liver, kidney, small intestine, and other tissues.[1,2] MRP2 has broad substrate selectivity. It can transport a variety of organic anion compounds.[3-5] However, MRP2 has very different affinity to drugs of similar or close structure. The different inhibitory effects of HMG-CoA Reductase Inhibitors to MRP2 were observed.[6] It has also been found that rat Mrp2 only transported the carboxylate form and not the lactone form of CPT-11 and SN-38.[4,5] Determination of the interaction between compounds and MRP2 transporters is of importance to predict the impact of these compounds on MRP2-mediated transport. The results can be used to predict MRP2 involved drug-drug interactions. In this study, we selected a variety of structurally unrelated compounds and investigated their inhibitory effects on MRP2-mediated uptake of LTC$_4$ into membrane vesicles.

Methods

Preparation of Human MRP2 and Rat Mrp2 Vesicles

Human MRP2 and rat Mrp2 vesicles were prepared from *Sf9* insect cells that express recombinant Human MRP2 or rat Mrp2 cDNA, respectively, using the baculovirus expression system.

Transport Uptake Assay

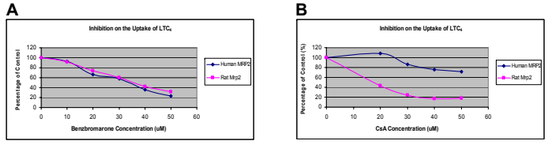
The uptake was carried out using a rapid filtration technique. A 60 µl reaction mixture containing 50 µg vesicles, 0.125 µM LTC$_4$, and 2.5 mM GSH, in uptake buffer (250 mM Sucrose, 10 mM $MgCl_2$, 10 mM Tris/HCl, pH 7.4) was incubated at 37ºC for 5 minutes. For inhibitory studies, test compounds were also preincubated with the vesicles and LTC$_4$. Uptake was started by adding 15 µl of 25 mM MgATP or uptake buffer. The mixture was incubated at 37ºC for an additional 4 minutes. The reaction was stopped by transferring the vesicles to a filter plate and washing the plate with cold washing buffer. The filter plate was dried and radioactivity was measured by scintillation counting.

Data Analysis

ATP-dependent uptake activity was reported as the difference between uptake in the presence and absence of ATP. Percentage of control was reported as the ratio of uptake activity in the presence of inhibitor to the uptake activity in the absence of inhibitor.

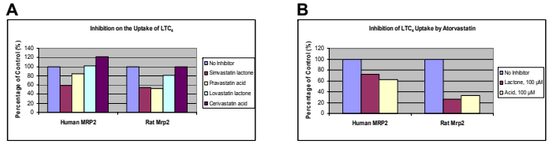
Results

1 Inhibition of LTC$_4$ by Benzbromarone and CsA



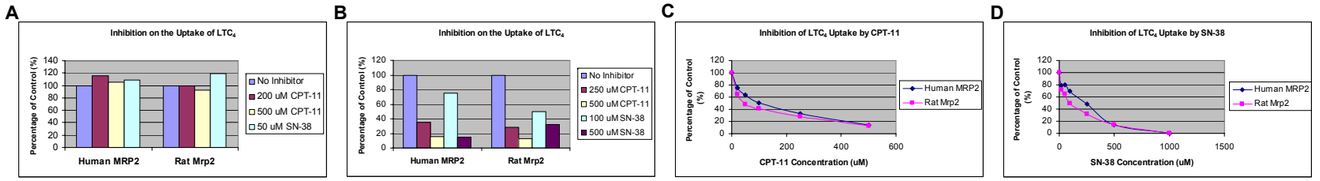
Inhibition of Benzbromarone and CsA on human MRP2 and rat Mrp2. ATP-dependent uptake of LTC$_4$ (100 µM) in human MRP2 and rat Mrp2 vesicles was carried out at 37ºC for 4 minutes in the presence of different concentrations of Benzbromarone (**A**) and CsA (**B**). Benzbromarone and CsA had significant inhibition on both human MRP2 and rat Mrp2. The IC$_{50}$ of Benzbromarone on human MRP2 and rat Mrp2 was 33.6 µM and 37.2 µM, respectively. The IC$_{50}$ of CsA on human MRP2 and rat Mrp2 was >50 µM and 17.2 µM, respectively.

2 Inhibition of LTC$_4$ Uptake by Statins



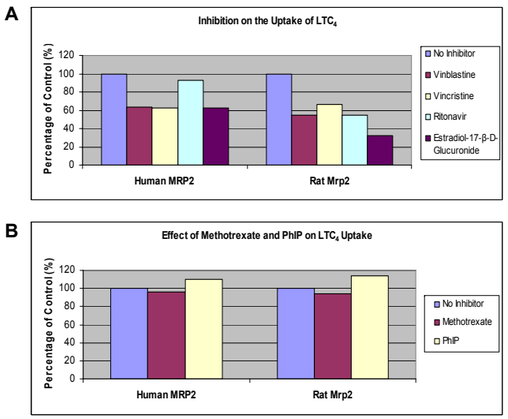
Inhibition of Statins on human MRP2 and rat Mrp2. ATP-dependent uptake of LTC$_4$ (100 nM) in human MRP2 and rat Mrp2 vesicles was carried out at 37ºC for 4 minutes in the presence of different statins at a concentration of 100 µM. (**A**) Simvastatin Lactone and Pravastatin Acid had significant inhibition on both human MRP2 and rat Mrp2, with IC$_{50}$ greater than 100 µM. Lovastatin Lactone only had significant inhibition on rat Mrp2, but not on human MRP2. Cerivastatin Acid had no inhibition on human MRP2 and rat Mrp2. (**B**) Atorvastatin Acid had similar inhibition as Atorvastatin Lactone on human MRP2 as well as on rat Mrp2. IC$_{50}$ values of Atorvastatin Acid and Atorvastatin Lactone on human MRP2 were greater than 100 µM. IC$_{50}$ values of Atorvastatin Acid and Atorvastatin Lactone on rat Mrp2 were less than 100 µM.

3 Effects of Lactone and Carboxylate Forms of CPT-11 and SN-38 to Human MRP2 and Rat Mrp2



Effects of Carboxylate and Lactone Forms of CPT-11 and SN-38 to human MRP2 and rat Mrp2. ATP-dependent uptake of LTC$_4$ in human MRP2 and rat Mrp2 vesicles was carried out at 37ºC for 4 minutes in the absence or presence of CPT-11 and SN-38. (**A**) The lactone form of CPT-11 (200 µM and 500 µM) and SN-38 (50 µM) did not inhibit the uptake of LTC$_4$ by human MRP2 and rat Mrp2. (**B**) The carboxylate form of CPT-11 (250 µM and 500 µM) and SN-38 (100 µM and 500 µM) had significant inhibition on the uptake of LTC$_4$ by human MRP2 and rat Mrp2. (**C**) IC$_{50}$ of Carboxylate CPT-11 on human MRP2 and rat Mrp2 was 100 µM and 48 µM, respectively. (**D**) IC$_{50}$ of Carboxylate SN-38 on human MRP2 and rat Mrp2 is 234 µM and 100 µM, respectively.

4 Effects of other compounds on Human MRP2 and Rat Mrp2



Effects of different compounds on human MRP2 and rat Mrp2. ATP-dependent uptake of LTC$_4$ in human MRP2 and rat Mrp2 vesicles was carried out at 37ºC for 4 minutes in the absence or presence of different compounds. (**A**) Vinblastine, Vincristine, and $E_2$17βG had significant inhibition on both human MRP2 and rat Mrp2. Ritonavir had significant inhibition only on rat Mrp2, but not on human MRP2. (**B**) Methotrexate and PhIP did not inhibit human MRP2 and rat Mrp2. For both **A** and **B**, the concentration of test compounds was 100 µM.

5 IC$_{50}$ Values

Test Compounds	Human MRP2	Rat Mrp2
CsA	>50 µM	17.5 µM
Benzbromarone	33.6 µM	37.2 µM
CPT-11 Carboxylate	100 µM	48 µM
Atorvastatin Acid	>100 µM	<100 µM
Atorvastatin Lactone	>100 µM	<100 µM
$E_2$17βG	>100 µM	<100 µM
SN-38 Carboxylate	234 µM	100 µM
Simvastatin lactone	>100 µM	>100 µM
Vinblastine	>100 µM	>100 µM
Vincristine	>100 µM	>100 µM
Ritonavir	Not significant @ 150 µM	125 µM
Pravastatin Acid	Not significant @ 100 µM	~100 µM
SN-38 Lactone	Not significant @ 50 µM	Not signifcant @ 50 µM
Cerivastatin Acid	Not significant @ 100 µM	Not signifcant @ 100 µM
Lovastatin Lactone	Not significant @ 100 µM	Not signifcant @ 100 µM
PhIP	Not significant @ 100 µM	Not signifcant @ 100 µM
CPT-11 Lactone	Not significant @ 200 µM	Not signifcant @ 200 µM
Methotrexate	Not significant @ 200 µM	Not signifcant @ 200 µM

Significant: p< 0.05

Summary and Conclusions

1. Significant differences were observed on the inhibitory effects of the tested compounds on the uptake of LTC$_4$. Of the tested compounds, CsA and Benzbromarone had the most potent inhibition to both human MRP2 and rat Mrp2.
2. The uptake of LTC$_4$ mediated by human MRP2 and rat Mrp2 was only inhibited by the carboxylate form, but not the lactone form of CPT-11and SN-38. This is consistent with the *in vivo* results that MRP2 can only transport the carboxylate form, but not the lactone form of CPT-11 and SN-38 (5).
3. Differences exist on the inhibitory effects of HMG-CoA Reductase Inhibitors on human MRP2 and rat Mrp2. Both Atorvastatin acid and Atorvastatin lactone had significant inhibition on human MRP2 and rat Mrp2. Cerivastatin acid and Lovastatin lactone did not have inhibitory effects.
4. Generally, the tested compounds had more inhibitory impact on the uptake of LTC$_4$ by rat Mrp2 than that by human MRP2.

References

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