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Understanding SLCO1B1 and ABCG2 transporter effects on rosuvastatin pharmacokinetics in Whites and Asians

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Understanding SLCO1B1 and ABCG2 transporter effects on rosuvastatin pharmacokinetics in Whites and Asians

by

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DISSERTATION

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Dedicated to

my mom and dad, Peggy and Pei-Kuan,

my husband, Sean,

and all of my family and friends

Whose unconditional support, love and faith

has been the source of my drive

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ABSTRACT

Understanding *SLCO1B1* and *ABCG2* transporter effects on rosuvastatin pharmacokinetics in Whites and Asians

Hsin-Fang Wu

Statins are one of the most commonly prescribed drug classes in the world to treat hyperlipoidemia. Statins inhibit HMG-CoA reductase to block the upstream cholesterol synthesis pathway in the liver. Seven different statins are currently on the market in the US and many exhibit differential drug exposure in different ethnic groups, including pravastatin, rosuvastatin, and simvastatin. In the rosuvastatin drug label, FDA recommends a lower starting dose for Asians due to the observation of 2 fold higher rosuvastatin systemic exposure. The mechanism of differential rosuvastatin exposure between Asians and Whites remains under investigation.

Rosuvastatin is a minimally metabolized drug and with 90% excreted unchanged. According to the Biopharmaceutics Drug Disposition Classification System, it is a class III compound, which suggests that uptake and efflux transporters are necessary for rosuvastatin disposition. Two drug transporters, OATP1B1 (*SLCO1B1*) and BCRP (*ABCG2*), are well described in the literature to play important roles in rosuvastatin absorption, distribution, and elimination. Although previous studies have shown that increase in rosuvastatin exposure was associated with either OATP1B1 or BCRP reduced function variants in both ethnicities, the two-fold higher rosuvastatin AUC in Chinese cannot be explained by either OATP1B1 or BCRP reduced function variants and their allele frequencies alone.

Therefore, we hypothesize that both OATP1B1 and BCRP reduced function variants and their allele frequencies together dictate the rosuvastatin exposure. The

research presented here first evaluated the effect of the major allele of OATP1B1 and BCRP together on rosuvastatin pharmacokinetics in Asian and White healthy volunteers and showed the differential rosuvastatin exposure was mitigated after controlling for both OATP1B1 and BCRP wildtype. Secondly, we evaluated the influence of intestinal absorption on rosuvastatin pharmacokinetics. Our study showed no clinically significant increase in rosuvastatin exposure when the upper GIs are bypassed in obese patients, indicating proximal intestinal absorption might play a minimal role in rosuvastatin pharmacokinetics. Collectively, these studies aim to highlight the potential for personalized medicine of rosuvastatin dosing given the different genetic background that may translate to more effective treatment of hyperlipidemia and obese patients.

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Chapter. 1 Introduction

1.1 Statins

Statins, also known as hydroxymethylglutaryl coenzyme A reductase (HMG-CoA reductase) inhibitors, are the first-line and most commonly prescribed drugs for lowering low-density lipoprotein, LDL cholesterol (LDL-c). Statins are prescribed to patients who are at risk for cardiovascular disease (CVD) and stroke. Studies show statins can lower LDL cholesterol concentrations by an average of 1.8 mmol/l reducing the risk of CVD by 60% and the risk of stroke by 17%.[1] The benefit of taking statins has been objectively proven to outweigh negative risks in large-scale clinical trials for mortality and morbidity. New guidelines released in November 2013 by the American Heart Association broaden the definition of patients at risk for CVD and stroke, and recommend the use of statins for an additional 35 million new patients.[2] It is estimated that one in forty Americans is presently taking a statin drug according to the Center for Disease Control in the US.

Seven statins are available on the market including simvastatin, atorvastatin, rosuvastatin, pravastatin, lovastatin, fluvastatin, and pitavastatin. Rosuvastatin is one of the most potent and commonly prescribed first-line therapies for dyslipidemia and prevention of cardiovascular diseases. It inhibits HMG-CoA reductase, an upstream enzyme that catalyzes the conversion of HMG-CoA to mevalonate, the precursor of cholesterol, and reduces hepatic cholesterol synthesis. Rosuvastatin is a poorly metabolized compound with 90% excreted as unchanged drug. Hepatic clearance of rosuvastatin accounts for about 72 % of total clearance while renal clearance accounts for the remaining of 28%. Peak plasma concentrations were found 3-5 hours post dosing and Area Under the plasma concentration-time Curve (AUC)

measurements were found to be proportional with dose. The terminal elimination half-life is about 19 hours.[3]

Although the benefit of taking statins exceeds the risks, no drug is without side effects, which for rosuvastatin are rare and mostly concentration dependent. Muscle related side effects from statins are the most recognized side effect, including muscle pain, fatigue, and weakness as well as rhabdomyolysis, followed by liver toxicity. Statin-induced side effects are usually dose dependent and can be reversed with statin discontinuation. Rhabdomyolysis is the best recognized and feared statin-induced side effect and occurs when muscles degrade severely, resulting in elevated creatine kinase (CK) levels (more than 10 times the upper detection limit), and can also lead to renal failure and death.[4] A genome wide association study of rosuvastatin efficacy was performed among 6989 patients of European decedent (JUPITAR)[5]. In this trial, single nucleotide polymorphisms (SNPs) at ABCG2 were identified by genome wide association with rosuvastatin in LDL-c reduction while SNPs in OATP1B1 were identified to associate with rosuvastatin induced toxicities[5].

High doses of statins are often started immediately in patients with high CVD risks. If statin-induced side effects are elicited, then the appropriate statin regimen requires titration of statin dosing, starting with a lower dose and gradually increasing the dose over a period of months. Although higher doses of statins correlate with the reduction in LDL-c and increased risk for side effects, marked variability among individuals is commonly observed. To prevent side effects from occurring, consistent clinical monitoring of symptoms is performed. As a result, to better assist rosuvastatin dosing and side effect management, investigation of the contributing factors to rosuvastatin pharmacokinetic variations is warranted.

1.2 Transporter and interethnic variations

Drug transporters expressed throughout the body play an important role in drug absorption, disposition, metabolism, and elimination. Inhibition of these transporters can result in elevated drug systemic exposure that can induce toxic reactions. Previous pharmacokinetic studies have shown that the systemic exposure of rosuvastatin in Japanese was twofold higher than that in Caucasians. To minimize the risk of developing adverse reactions, the FDA recommended a lower starting dose for Asian patients.[6–9] Differences in AUC between Asians and Caucasians can be multifactorial and continues to be investigated. Previous oral and i.v. rosuvastatin pharmacokinetics studies showed that Caucasian demonstrate ~1.7 fold higher hepatic clearance of rosuvastatin than Asians (Table 1-1). Metabolism of rosuvastatin has a negligible effect on rosuvastatin interethnic pharmacokinetic variation since the drug is poorly metabolized and ~90 % excreted unchanged. In the Biopharmaceutics Drug Disposition Classification System (BDDCS)[10], it is predicted that both uptake and efflux transporters would be important for intestinal absorption and hepatic clearance of rosuvastatin.

Table 1-1 Rosuvastatin pharmacokinetics in Japanese and Caucasians.[11]

Race	Gender	N	Dose (mg)	CL/F (L/h)	CL (L/h)	CL_H (L/h)	CL_R (L/h)	V_{ss} (L)	BA (%)	F_h	F_aF_g
Japanese	Male	10	6 (i.v.) 40 (p.o.)	114	31.9	20.3	11.6	67.9	29	0.64	0.45
Caucasian	Male	10	8 (i.v.) 40 (p.o.)	242	48.9	35.3	13.6	134	20	0.47	0.43

CL/F, oral clearance; CL, plasma clearance; CL_H, hepatic clearance; CL_R, renal clearance; V_{ss}, volume of distribution at steady state; BA, bioavailability; F_h, hepatic availability; F_aF_g, absorbed fraction multiplied by gut availability.

C_{max}, CL_R, V_{ss}, and BA are reported values. CL is either the reported value or the value calculated from the formula of Dose/AUC. CL_H is calculated from the formula of CL – CL_R. F_h was calculated using a dispersion model and the parameters blood to plasma ratio, R_B = 0.690, hepatic blood flow, Q_B= 20.7 ml/min/kg, CL, CL_R, and body weight (BW), 77.6 kg reported for Caucasians and 66.6 kg estimated for Japanese, with the assumption that CL_R per BW in Japanese is equal to that in Caucasians. F_aF_g was calculated as BA as a fraction divided by F_h.

Genetic polymorphisms of transporters can significantly alter a drugs' pharmacokinetics and pharmacologic effect. In vitro studies show that rosuvastatin is a hepatic uptake substrate for organic anion transporting polypeptide (OATP) 1B1 and OATP1B3 and a biliary efflux substrate of both P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). In particular, genetic variation and levels of protein expression of OATP1B1 and BCRP have been suggested as important determinants of interindividual variability of the rosuvastatin response.[12–14]

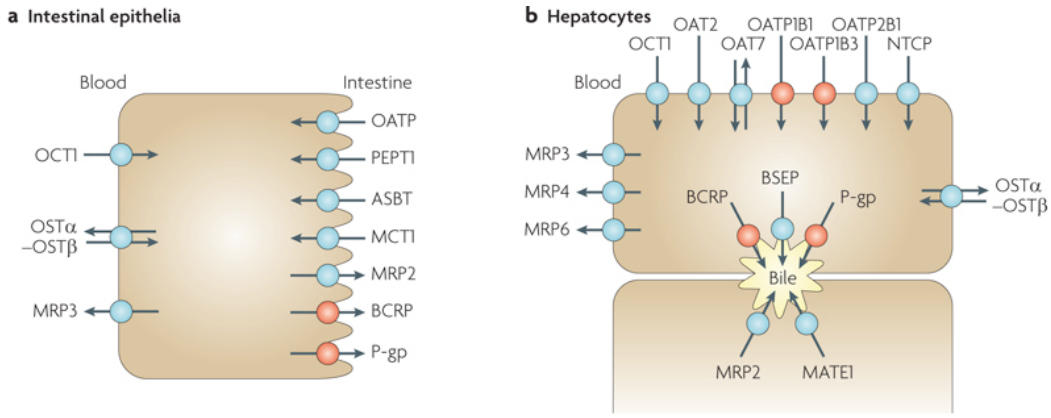

Figure 1-1 Transporter expression in intestinal epithelia and hepatocytes.[15]

OATP1B1 is mainly expressed on the basolateral side of hepatocytes and responsible for taking up substrates into the hepatocyte (Figure 1-1). Several clinical studies have reported that single-nucleotide polymorphisms (SNPs) in the *SLCO1B1* gene significantly alter rosuvastatin AUC in healthy subjects in many different ethnic groups.[6,9,12–14,16] *SLCO1B1* A388>G and T521>C and c.421 C>A are the three most well-described SNPs that together demonstrated increased plasma concentrations of rosuvastatin in a similar effect size in both Asians and Caucasians (Table 1-2). Table 1-3 shows these variants and their allele frequencies in different ethnicity groups, which were calculated based on previous studies. For example, the allele frequency of *SLCO1B1* *5 allele in Whites were calculated to be 14% and 2.4% from two independent studies. However, two studies in Japanese subjects yield contradictory results, one similar to the value found in Chinese (10.3%), while the other value similar to Whites (3.7%). As noted in the table, the reduced function allele frequency of *15 (both c.388A>G and c.521T>C) is much more prevalent in Chinese (14%) than Caucasians (2.7%).[11] Also, the reduced function *ABCG2* c.421C>A variants demonstrates higher allele frequency in Chinese(35%) and Japanese(35%) than Whites (14%) (Table 1-3).

Table 1-2 Reduced variants in both *SLCO1B1* and *ABCG2* results in increased rosuvastatin plasma concentrations

		Plasma Concentration
Uptake (*SLCO1B1*)	Increased function	↓
	Reduced function	↑
Efflux (*ABCG2*)	Increased function	↓
	Reduced function	↑

Table 1-3 *SLCO1B1* and *ABCG2* reduced function variant allele frequencies in different ethnicity groups.

Transporter	Variant alleles	Alteration in function	Allele frequencies (%)		
			White	Chinese	Japanese
SLCO1B1	*5(521T>C)	Reduced	14;2.4	-	0.7
	*15(388A>G & 521T>C)	Reduced	2.7	14	3.7;10.3
ABCG2	421C>A	Reduced	14	35	35

*Modified from Yasuda el al. 2008[17]

Although much effort has been undertaken to demonstrate how genetic polymorphism and allele frequency in *SLCO1B1* might explain the variability observed between Asians and Caucasians, the 2 fold rosuvastatin AUC difference between these two groups was also observed when comparing wild type OATP1B1

(*1a/*1a) in Chinese and White healthy subjects, who resided in the same geographic environment.[11,18,19] Table 1-4 shows the results of the Lee et al. study of rosuvastatin pharmacokinetics in different ethnic groups. Rosuvastatin systemic exposure was still approximately 2 fold higher in Chinese group than in White group after controlling for *SLCO1B1* wildtype(*1a/ *1a or *1a/ *1b).

Table 1-4 Rosuvastatin pharmacokinetics in different OATP1B1 haplotypes.[7]

	Diplotype						
	**1a / *1a*	**1a/ *1b*	**1b/ *1b*	**1a/ *15*	**1b/ *15*	**15/ *15*	**15/ *5*
White(n=36)							
N	8	13	4	5	1	4	1
Frequency	0.22	0.6	0.11	0.14	0.04	0.11	0.03
AUC_{0-96} (ng*h/mL)	183 (152-221)	191 (147-249)	216 (105-445)	214 (122-376)	159	397 (200-789)	499
C_{max} (ng/mL)	18.7 (13.9-25.2)	22.2 (17.5-28.7)	23.7 (13.1-42.8)	23.6 (12.7-43.9)	24.9	54.1 (29.6-98.3)	80.9
Chinese(n=35)							
N	5	12	12	1	5	0	0
Frequency	0.14	0.34	0.34	0.30	0.14		
AUC_{0-96} (ng*h/mL)	538 (260-1110)	466 (386-562)	482 (391-594)	*	577 (380-876)		
C_{max} (ng/mL)	66.6 (31.8-140)	54.4 (44.0-67.3)	54.0 (40.7-71.7)	68.1	77.2 (48.9-122)		

*No value was reported.

BCRP is a member of the ATP-binding cassette (ABC) efflux transporter superfamily (encoded *ABCG2*). BCRP is widely expressed in the small intestine, placenta, and liver and is believed to play an important role in drug disposition.

Recent in vitro studies have shown that rosuvastatin is a substrate of BCRP and that SNPs in the *ABCG2* gene significantly alter rosuvastatin AUC in healthy subjects across different ethnic groups.[20,21] A 1.76-fold higher rosuvastatin AUC associated with *ABCG2* 421C>A was observed in both Finnish[21] and Chinese[20] healthy subjects when controlled for *SLCO1B1* T521>C. Among all the SNPs in *ABCG2* that have been described, G34>A in exon 2 and C421>A in exon 5 are the most prevalent reduced function alleles in Asians, with allele frequency of 20-45% versus about 2% in Caucasians for G34>A and with 25-35% in Asians versus 10% in Caucasians for C421>A.

In addition to transporter function, transporter expression level variations might lead to different magnitudes of increased AUC and different levels of drug toxicity. Tomita et al. had suggested that the ethnic variability in AUC of statins can be better explained when differences in both the allele frequencies of OATP1B1 and BCRP and intrinsic ethnic variability in the activity of OATP1B1 between Japanese and Caucasians were considered.[11] This suggests that other factors, such as lower protein expression level and/or OATP1B1 activity in the liver in Asians, may combine with genetic variances to contribute to the difference observed between Asians and Caucasians. However, a recent study showed no interethnic difference in transporter protein expression in the liver[22]. Also, in the same paper Tomita et al. calculated the F_aF_g and found the value to be similar between Asians and Whites. Therefore, they concluded that absorption of rosuvastatin might not be important for the interethnic difference in rosuvastatin exposure. However, since BCRP is one of the predominant transporters expressed in the intestine and important for rosuvastatin pharmacokinetics, we proposed a study to investigate intestinal absorption further.

Many studies have investigated the effect of hepatic transporters on rosuvastatin pharmacokinetics, but only recently, have a few studies examined the intestinal absorption effect. BCRP-mediated intestinal absorption of rosuvastatin may be substantial for rosuvastatin pharmacokinetics. BCRP protein expression variation may partially explain the two-fold difference observed between Asians and Caucasians. Prediction from a PBPK model based on a drug-drug interaction study with rosuvastatin and eltrombopag, a proven in vitro inhibitor of OATP1B1 and BCRP, suggested that when BCRP in the intestine was taken into account, the simulated plasma concentrations of rosuvastatin were in accord with clinical findings.[23] Furthermore, It has been shown that other transporters, such as P-gp and MRP1 were significantly greater in intestine samples from Chinese than from Caucasian, suggesting a differential expression in transporters in different ethnic groups. Yet, BCRP protein expression was not examined in this study.[24]

Given the importance of OATP1B1 and BCRP for the disposition of rosuvastatin, I hypothesize that the interethnic difference in drug exposure can be attributed to the differences in function of the two transporters. This study evaluated if rosuvastatin interethnic pharmacokinetic variations could be explained by controlling for the major alleles of OATP1B1 and BCRP and also whether intestinal absorption of rosuvastatin affects rosuvastatin pharmacokinetics.

1.3 Challenge in current therapy

Since the 2000 Human Genome project sequenced the entire human genome, personalized medicine has become more available with the potential to transform research as well as treatment. With diverse genetic backgrounds, patients respond to each medication differently. Carbamazepine has been a great example to illustrate

pharmacogenomic effects on patients, where dosing carbamazepine to appropriate subgroup of patients can largely decrease side effects like Steven Johnson's Syndrome(SJS). Carbamazepine is widely used in the world and it is associated with a severe side effect called Steven-Johnson syndrome. More recent research found that a more prevalence HLA subtype in Asians is associated with the higher incident rate of SJS in Asians.[25,26] With the introduction of HLA genotyping prior to dosing carbamazepine in Taiwan, the incidence of SJS from carbamazepine was almost eradicated compared to about 20 cases per year before.[27]

Rosuvastatin pharmacogenomic studies have shown that multiple SNPs can affect rosuvastatin exposure in different ethnic groups. However, there hasn't been a study to holistically look at the clinical effect of the three main SNPs (OATP1B1 c.388, C.521, and *ABCG2* c.421) altogether and how those might translate into dosing guidance. Investigating the pharmacogenomic contribution from these SNPs, might lead the field to be able to streamline identifying the appropriate rosuvastatin dosing regimen, achieving clinical efficacy and avoiding causing side effects during a shorter period than what has been traditionally done.

1.4 Significance

Statins are the largest class of drugs prescribed worldwide for treating hyperlipidemia and reducing the risk of cardiovascular disease (CVD). Statin treatment has been associated with plasma concentration-dependent adverse events such as myopathy or rhabdomyolysis. Although the incidence is very rare, given the extensive usage of statins, these muscle toxicities and side effects are still a major clinical concern. Furthermore, chronic or metabolic disease patients commonly use a combination of other drugs together with statins, which can

increase the risk of developing DDIs.[8] Due to the considerable variability, both in pharmacokinetics and pharmacodynamics of statins, a comprehensive understanding of what contributes to the altered pharmacokinetics of statins is warranted for disease and treatment management. This is the first study to look at both hepatic and intestinal transporter effects on rosuvastatin pharmacokinetics in both healthy and obese patients and both Asian and Caucasian subjects.

1.5 Innovation

Emerging evidence suggests that in addition to OATP1B1, BCRP may also play an important contributing role in statins' pharmacokinetics, which could further explain the ethnic variability observed between Asians and Caucasians. However, only limited studies have characterized BCRP-mediated statin disposition *in vivo*, and the difference in statin pharmacokinetics between Asians and Caucasians is explained poorly by OATP1B1 and BCRP reduced function SNPs and their allele frequencies. This is the first study to investigate both BCRP and OATP1B1 mediated rosuvastatin clearance between Asians and Caucasians. We first conducted a rosuvastatin pharmacokinetic study in a controlled genetic background in healthy volunteers to examine the contribution of all three well-established SNPs to interethnic variation. Next, we conducted a rosuvastatin pharmacokinetic study in obese patients undergoing bariatric surgery as an intestinal absorption model to look at transporter effects in the intestinal absorption of rosuvastatin. Last, we proposed to compare the transporter expression in tissues to test whether transporters expression also plays a role in altered rosuvastatin pharmacokinetics.

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Chapter. 2 Rosuvastatin pharmacokinetics in Asian and White subjects wild-type for both OATP1B1 and BCRP under control and inhibited conditions[1]

2.1 Abstract

The FDA recommends rosuvastatin dosage reductions in Asian patients because pharmacokinetic studies have demonstrated an approximately two-fold increase in median exposure to rosuvastatin in Asian subjects when compared to Caucasian controls. Yet, no explanation for this ethnic difference has been confirmed.

Here we show that rosuvastatin exposure in Asians and Whites does not differ significantly when all subjects are wildtype carriers for both Solute Carrier Organic anion transporter*1B1 *1a* and ATP Binding Cassette Subfamily G Member 2 c.421 transporters in a two-arm, randomized, cross-over rosuvastatin pharmacokinetics study in healthy White and Asian volunteers. For single rosuvastatin doses, $AUC_{0\text{-}48}$ were 92.5(±36.2) and 83.5(±32.2) ng/mL*hr and C_{max} were 10.0(±4.1) and 7.6(±3.0) ng/mL for Asians and Whites, respectively. When transporters were inhibited by intravenous rifampin, rosuvastatin $AUC_{0\text{-}48}$ and C_{max} also showed no ethnic differences. Our study suggests that both *SLCO1B1* and *ABCG2* polymorphisms are better predictors of rosuvastatin exposure than ethnicity alone and could be considered in precision medicine dosing of rosuvastatin.

[1] Modified publication from Wu, HF. *et al.* Rosuvastatin Pharmacokinetics in Asian and White Subjects Wild-type for Both OATP1B1 and BCRP Under Control and Inhibited Conditions. *J. Pharm. Sci. Epub ahead of print on April 3, 2017*

2.2 Introduction

Statins have been utilized worldwide in millions of patients to prevent cardiovascular disease and treat lipid disorders. A number of large clinical trials and post-marketing surveys have demonstrated the substantial health benefit to statin use[1-3]. While adherence to statin therapy is a key factor associated with improved treatment outcomes, it is concerning that as many as 50 % of patients stop treatment within one year of statin initiation[4]. About 62% of former statin users state the reason they stopped their statin was due to side effects, including myopathy and potentially lethal rhabdomyolysis[5]. The onset of adverse events has been associated with elevated statin blood levels[6,7]. Statin-induced myalgias were reported in 10-20% of statin-treated patients and led to treatment discontinuation in 30% of the symptomatic patients[8]. To reduce side effects and achieve an optimal dosing regimen for better adherence to statins in each individual, a holistic understanding of the underlying mechanism is warranted.

FDA recommends that Asian patients initiate rosuvastatin therapy at half of the standard dose for non-Asians. The rosuvastatin drug label states that "Pharmacokinetic studies have demonstrated an approximately 2-fold increase in median exposure to rosuvastatin in Asian subjects when compared to Caucasian controls". The molecular mechanism that leads to differential drug exposure between Asians and Whites remains unknown.

Previous studies have ruled out extrinsic factors including the environment, diet, and variations in body weight as causing the interethnic rosuvastatin exposure differences[9,10]. Rather intrinsic factors of drug absorption, distribution, metabolism, and elimination are suggested to play the major roles, with the hepatic clearance of unchanged drug into the bile believed to be the major route of elimination. Since

rosuvastatin is poorly metabolized and mainly excreted as unchanged drug, rather than via metabolism, the transporting of rosuvastatin into and out of hepatocytes by drug transporters could be playing important roles in the observed interethnic differences.

Our understanding of drug pharmacokinetics has been advanced greatly since the 1990s by recognizing the roles of drug transporters in drug disposition. Drug transporters are expressed throughout the body in different organs and facilitate uptake or efflux of drugs into or out of the body. Rosuvastatin is a hydrophilic molecule, which strongly depends on drug transporters to cross cell membranes and reach its site of action[11]. The effects of hepatic uptake and efflux transporters on the pharmacokinetics and pharmacodynamics of rosuvastatin have been well characterized in the literature[12,13]. Uptake transporters, including organic anion transporting polypeptides (OATP) 1B1, and 1B3, as well as Na+-taurocholate cotransporting polypeptide, facilitate rosuvastatin uptake into hepatocytes, where the drug inhibits HMG-CoA reductase; while efflux transporters, such as breast cancer resistance protein (BCRP), eliminate rosuvastatin into the bile. OATP1B1 is the major hepatic uptake transporter, while BCRP is the major efflux transporter, expressed on the canalicular side of the liver and at the apical border of enterocytes[14,15].

Genetic polymorphisms leading to reduced function in OATP1B1 and BCRP transporters have been shown to affect rosuvastatin pharmacokinetics and its subsequent pharmacologic effects[16]. Due to their abundance and important roles, *SLCO1B1* (the gene encoding OATP1B1)[17,18] and *ABCG2* (the gene encoding BCRP)[19,20] reduced functional polymorphisms and their minor allele frequency has previously been proposed as the cause of interethnic variations in rosuvastatin

pharmacokinetics and drug-drug interactions. The reduced function SNP frequency for both *SLCO1B1* *15 (defined by c.388A>G and c.521T>C) and *ABCG2* c.421C>A are more prevalent in Eastern Asians (14% and 35%, respectively) compared to Whites (2.7% and 14.0%)[21,22]. Another two studies show that at least 2-fold higher rosuvastatin exposure was still observed in Asians compared to Whites residing in the same environment after controlling only for the *SLCO1B1* wildtype[9,10]. Tomita et al. suggested that *SLCO1B1* and *ABCG2* c.421 polymorphisms could not explain the observed plasma concentration variations between Asians and Caucasians[22].Tomita et al. further proposed that in addition to genetic variants, protein expression could be another contributing factor. However, a recent study showed that OATP1B1 protein expression was similar between Asians and Whites[23].

None of the previous clinical studies have prospectively evaluated both wildtype OATP1B1 and BCRP transporters to explain interethnic differences in rosuvastatin systemic exposure. Thus, here we prospectively investigate if interethnic differences in rosuvastatin drug exposure could be mitigated by controlling for both *SLCO1B1* *1a/ *1a or *1a/ *1b together with *ABCG2* c.421 wildtype. Our results could improve treatment adherence by providing a sounder basis for determining the appropriate dosage of rosuvastatin when taken alone or combined with other medications.

2.3 Results

Participant demographics

During recruitment, 39 Asians and 21 Whites were screened. We found 8 eligible healthy volunteers in each ethnic group, who underwent randomization and completed the study. Asian volunteers were mainly Han-Chinese descendants

(87.5%) with only one being Japanese (12.5%). All of the White volunteers were self-reported to be of European decent. The study population averaged 33.8 years old for Asians and 43.1 years old for Whites. Average weights were 63.4 kg for Asians and 68.1 kg for Whites. BMIs were similar, average of 22.3 for Asians and 23.6 for Whites (Table 2-1). The following results are reported based on eight Asian and seven White volunteers because one White volunteer was mistakenly recruited rather than the identified subject with the appropriated genotype data. No statistical differences in these demographics between Asian and White volunteers were observed.

Table 2-1 Demographic of all volunteers.

	White	Asian
N	7	8
Sex		
Male	4	3
Female	3	5
Age (year)	43.1(14.2)*	33.8(9.3)
Weight (kg)	68.1(9.7)	63.4(14.2)
BMI (kg/m^2)	23.6(2.0)	22.3(3.4)
Scr (mg/dL)	0.82(0.11)	0.8(0.2)
AST (Unit/L)	19.0(3.4)	16.6(2.1)
ALT (Unit/L)	19.1(7.3)	15.0(3.2)
LDL-C (mg/dL)	119.1(33.0)	102.1(25.0)
HDL-C (mg/dL)	64.4(16.8)	64.9(14.5)
TC (mg/dL)	196.3(32.5)	184.1(33.2)
TG (md/dL)	69.0(15.1)	84.9(19.5)

*Mean(SD) Scr: Serum Creatinine; AST: Aspartate aminotransferase; ALT: alanine aminotransferase; LDL-C: Low-density lipoprotein cholesterol; HDL-C: High-density lipoprotein cholesterol; TC: Total cholesterol; TG: Triglyceride

Genotype

We screened up to 38 Asian and 28 White healthy volunteers and the enrolled volunteers' genotype results are shown in Table 2-2. We enrolled 8 Asian and 8 White healthy volunteers after target genotypes were identified. Only volunteers with *SLCO1B1**1a/*1a or *1a/*1b and *ABCG2* c.421.CC wildtype was included in our analysis. ROS-02' was mistakenly recruited and studied instead of calling up the ROS-02, who was a wildtype carrier, and his data was excluded from our final calculation because his was found to be a carrier of reduced function *SLCO1B1* *15 allele. In our recruitment, the frequencies of the target alleles, *SLCO1B1* *1a and

ABCG2 c.421CC, were different between Asians (35.9%) and Whites (52.4%). The linkage disequilibrium reported as correlations between the two SNPs, were 0.170 in Asians and -0.25 in Whites for *SLCO1B1* c.388 vs c.521.

Table 2-2 Summary of genotypes in the enrolled healthy volunteers

Ethnicity	Subject ID	*SLCO1B1*	*SLCO1B1*	*ABCG2*
		rs2306283	**rs4149056**	**rs2231142**
White	ROS-01	A/A	T/T	C/C
	*ROS-02’	G/A	C/T	C/C
	ROS-02	A/A	T/T	C/C
	ROS-03	A/A	T/T	C/C
	ROS-04	A/A	T/T	C/C
	ROS-05	A/A	T/T	C/C
	ROS-06	A/A	T/T	C/C
	ROS-07	A/A	T/T	C/C
	ROS-08	A/A	T/T	C/C
Asian	ROSA-01	G/A	T/T	C/C
	ROSA-02	G/A	T/T	C/C
	ROSA-03	A/A	T/T	C/C
	ROSA-04	G/A	T/T	C/C
	ROSA-05	A/A	T/T	C/C
	ROSA-06	A/A	T/T	C/C
	ROSA-07	G/A	T/T	C/C
	ROSA-08	A/A	T/T	C/C

*ROS-02’ was mistakenly studied rather than ROS-02. ROS-02’ was excluded from the final data calculation because of carrying *SLCO1B1* *15 reduced function allele.

All volunteers were homozygous wild-type for *SLCO1B1* c.521TT and *ABCG2* c.421CC. Four Asians were heterozygous for the *SLCO1B1* c.388A>G polymorphism while all the rest was homozygous wildtype. The four subjects heterozygous for the c.388A>G SNP had similar rosuvastatin AUCs (P<0.05) during the control treatment period compared to homozygous subjects (Figure 2-1).

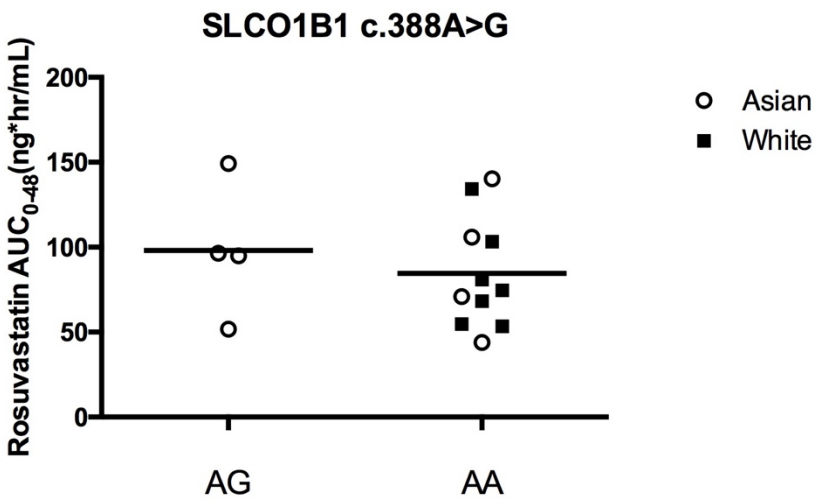

Figure 2-1 Rosuvastatin pharmacokinetics in *SLCO1B1* 388A>G carriers compared with wildtype carriers.

Table 2-3 Pharmacokinetic parameters of rosuvastatin following a 20 mg oral dose of rosuvastatin alone or in combination with rifampin IV

	White (n=7)	Asian (n=8)	Mean ratio (White to Asian)
Rosuvastatin			
Cmax (ng/ml)	7.6 ± 3.0	10.0 ± 4.1	0.76
Tmax (h)	3.0 (1.0-4.0)	3.1 (1.5-4.0)	--
MAT(h)	2.70 ± 0.85	2.25 ± 0.74	1.20
AUC0-∞ (ng·h/ml)	83.5 ± 32.2	92.5 ± 36.2	0.90
AUC0-48 (ng·h/ml)	77.2 ± 31.5	86.2 ± 35.5	0.90
t1/2 (h)	16.2 ± 8.5	15.2 ± 10.5	1.07
CL/F/kg (L/h/kg)	4.01 ± 1.39	3.90 ± 1.25	1.22
Vss/F/kg (L/kg)	59.9 ± 51.7	48.9 ± 37.7	1.22
Rosuvastatin + rifampin			
Cmax (ng/ml)	65.0 ± 32.2[a]	78.1 ± 42.1[a]	0.83
Tmax (h)	1.5 (0.5-2.5)[a]	1.7 (1-3)[b]	--
MAT(h)	1.27 ± 0.50[b]	0.77 ± 0.42[a]	1.65
AUC0-∞ (ng·h/ml)	281.4 ± 73.3[a]	297.2 ± 104.4[a]	0.95
AUC0-48 (ng·h/ml)	278.2 ± 73.2[a]	295.2 ± 102.9[a]	0.94
t1/2 (h)	10.3 ± 3.0	9.0 ± 2.7	1.14
CL/F/kg (L/h/kg)	1.11 ± 0.32[a]	1.21 ± 0.42[a]	0.92
Vss/F/kg (L/kg)	4.90 ± 2.06[c]	5.14± 3.00[b]	0.95

Data were obtained from healthy volunteers in a crossover study design. Values are shown as an arithmetic mean ± SD except for T_{max} where data are given as median and range. AUC, area under the plasma concentration–time curve; C_{max}, maximum plasma concentration; CI, confidence interval; CL/F, oral clearance; MAT, mean absorption time; $t_{1/2}$, terminal half-life; T_{max}, time of observed maximal concentration; V_{ss}/F, oral steady-state volume of distribution.

[a] P<0.001 compared with rosuvastatin alone period. [b] P<0.01 compared with rosuvastatin alone period. [c] P<0.05 compared with rosuvastatin alone period.

Rosuvastatin pharmacokinetics

No ethnic difference in drug exposure was observed when rosuvastatin was administered alone in the control period as reported in Table 2-3. The concentration-

time profiles of rosuvastatin (solid circle) in both ethnic groups are similar as shown in Figure 2-2. Total AUCs were 92.5 (± 36.2) ng*hr/mL for Asians and 83.5 (± 32.2) ng*hr/mL for Whites while the C_{max} were 10.0 (± 4.1) ng/mL for Asians with a T_{max} of 3.1 hours and 7.6(± 3.0) ng/mL with a T_{max} of 3.0 hours for Whites. The oral clearance, CL/F/kg, was calculated to be 3.90(± 1.25) L/hr/kg for Asians and 4.01(± 1.39) L/hr/kg for Whites.

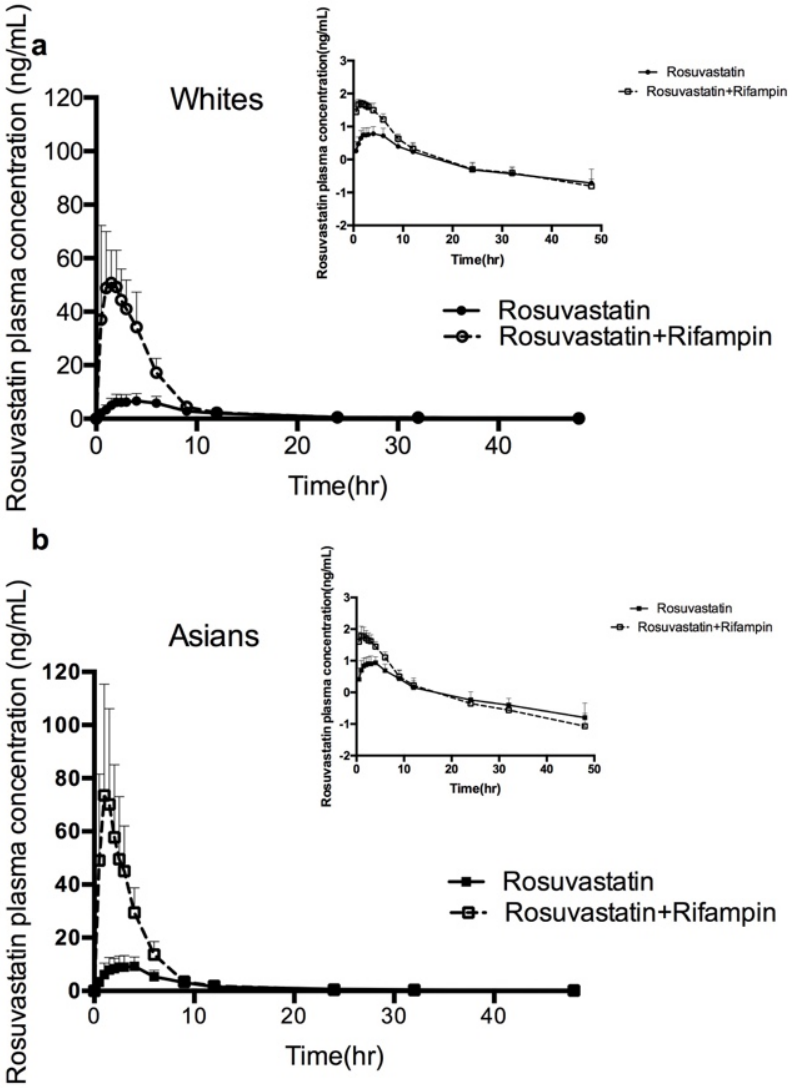

Figure 2-2 Rosuvastatin pharmacokinetics in 8 Asian and 7 White healthy volunteers. Mean plasma concentration of rosuvastatin (± SD) following a single oral 20mg dose of rosuvastatin. The inset depicts the same data on a semi-logarithmic scale. Similar variability in rosuvastatin $AUC_{0-\infty}$ between **(a)** White and **(b)** Asian subjects was noted.

Effect of rifampin on the pharmacokinetics of rosuvastatin

Rifampin is an inhibitor of the uptake transporter OATP1B1 and the efflux transporter BCRP. When rifampin was coadministered intravenously with oral rosuvastatin, as expected, a substantial increase was seen in all of the volunteers with average AUC increasing more than three-fold ($p<0.001$) and C_{max} increasing more than six-fold($p<0.001$) compared to the control period (Figure 2-2 and Table 2-3). A single intravenous dose of rifampin had similar effects on rosuvastatin in both Asians and Whites and no significant differences were observed between Asians and Whites. The effect of rifampin on total AUC and C_{max} of rosuvastatin in each individual is presented in Figure 2-3. Both rosuvastatin mean AUC_{0-48} and C_{max} following a single oral dose of 20 mg rosuvastatin, with and without the administration of rifampin, in White (Figure 2-3a and c) and Asian (Figure 2-3b and d) healthy volunteers increased in the presence of rifampin.

T_{max} values with rifampin were approximately half of that seen in the control period, a reflection of the decreased mean absorption time (MAT). There also was a very marked decline in the volume of distribution of rosuvastatin in the presence of transporter inhibition by rifampin as reflected in the 9-12-fold decrease in V_{ss}/F given in Table 2-3 in both ethnic groups.

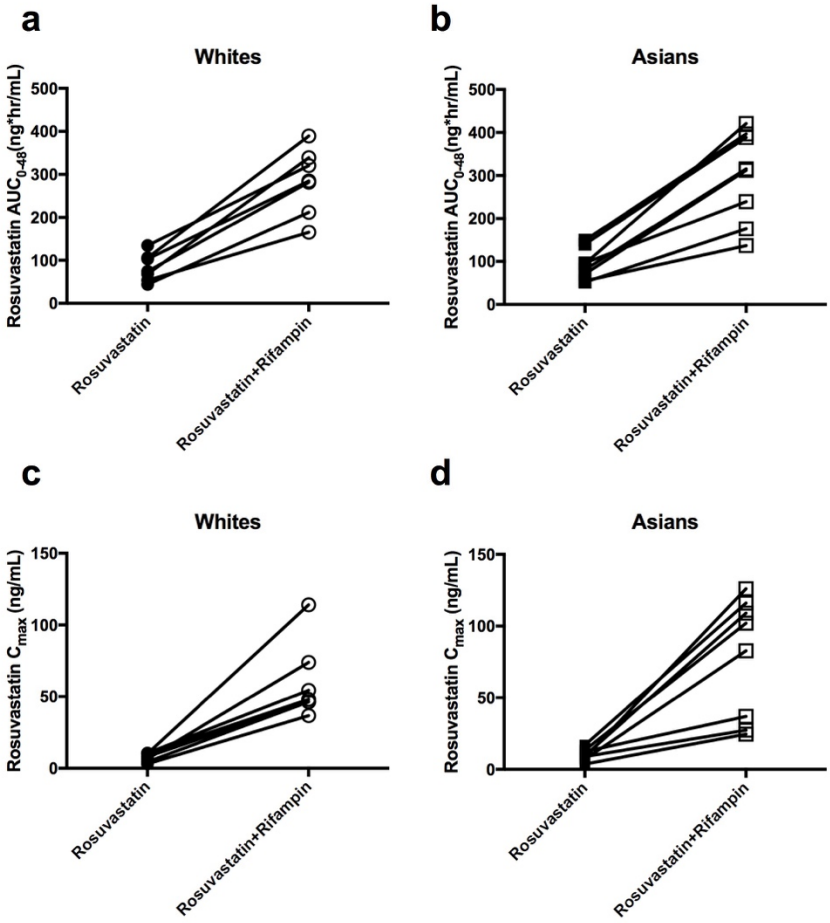

Figure 2-3 The effect of rifampin on the pharmacokinetics of rosuvastatin in White (n=7) and Asian (n=8) healthy volunteers.

Rosuvastatin pharmacokinetics in a *SLCO1B1* *15 carrier

Although mistakenly recruited and excluded from our data set, a Caucasian volunteer with *SLCO1B1* *15 allele (ROS12) was mistakenly studied. The genetic background for ROS-02 was shown in Table 2-2. Due to the reduced function *SLCO1B1* *15 allele, ROS12 demonstrated higher rosuvastatin plasma concentrations compared with wildype: AUC_{0-48} was 112.8 ng*hr/mL and C_{max} was 13.8 ng/mL for rosuvastatin alone period with little change for AUC_{0-48} of 107.9 ng*hr/mL and C_{max} of 18.3 ng/mL in the rifampin inhibition period.

2.4 Discussion

This prospective study demonstrates that the consistently observed two-fold average difference in rosuvastatin drug exposure between Asians and Whites was mitigated after controlling for two drug transporters, *SLCO1B1* *1a and *ABCG2* c.421 wildtype. In our cohort, both Asian and White volunteers exhibited similar rosuvastatin AUC and Cmax, which implicates similar pharmacological effects. In addition, our study result aligns with the previous literature finding of no significant difference in rosuvastatin pharmacokinetics between *SLCO1B1* *1a or *1b carriers. Although the subject numbers were too small to justify statistical comparison(n=4), *SLCO1B1* *1b did not affect the rosuvastatin plasma concentration in our cohort compared with wildtype.

Table 2-4 summarizes the interethnic rosuvastatin pharmacokinetic parameters, C_{max} and AUC, for the studies of Lee et al.[9] at a 40mg rosuvastatin dose between Asians and Whites in subjects with no genotype control and subjects wild-type for *SLCO1B1*; Birmingham et al.[10] at a 20mg rosuvastatin dose in subjects with no genotype control, subjects wild-type for *SLCO1B1* and subjects wildtype for *ABCG2*; and our study at a 20mg rosuvastatin dose in subjects wild type in both *SLCO1B1* and *ABCG2*. The data for our White subjects compare favorably with the previously reported results of Birmingham et al. and dose adjusted results of Lee et al[9,10]. However, in Asian subjects in our study, wild-type for both *SLCO1B1* and *ABCG2*, markedly lower levels are observed than for the two previous reports, but not different than the measurement in Whites wild type in both transporters (Table 2-3). Our results differ from those of Birmingham et al.[10], who briefly reported, but provided no details, which in subjects wildtype for both *SLCO1B1* and *ABCG2* "rosuvastatin $AUC_{(0-t)}$ and C_{max} appeared to be, on average, higher in Japanese and

Chinese compared with Caucasian subjects". When we digitally quantified the data presented in Fig.2a for $AUC_{(0-t)}$ in that paper[10], median values for Chinese and Japanese were 62% and 35% higher, respectively, compared to Caucasians, versus the 11% difference we observed. In a recent study, Wan et al. reported that *ABCG2* c.34AA, with an allele frequency of 12.6% in Chinese, also has a significant effect on rosuvastatin pharmacokinetics in healthy Chinese subjects[24], yielding a mean decrease of 34% in CL/F, although no change was observed for the heterozygous carrier of c.34GA and homozygous carrier of c.34GG. However, the volunteers were not controlled for *ABCG2* c.34 A>G SNP here, since our clinical study proceeded this publication.

Table 2-4 Comparison of the study reported here to other interethnic rosuvastatin pharmacokinetic studies.

		Lee et al. 2005 (40mg Rosuvastatin)		**Birmingham et al. 2015 (20mg Rosuvastatin)**			**Our study (20mg Rosuvastatin)**
Genotype		No control	*SLCO1B1* c.521 TT	No control	*SLCO1B1* c.521 TT	*ABCG2* c.421 CC	*SLCO1B1* c.521 TT & *ABCG2* c.421 CC
C_{max} (ng/mL)	White	25* (21.1-29.6)	18.7* (13.9-25.2)	8.66* (7.15-10.5)	8.2* (6.7-10.0)	7.9* (6.5-9.6)	7.6 (4.6-10.6)
	Asian	59.1 (49.8-70.1)	66.6 (31.8-140)	18.7 (15.5-22.5)	17.4 (14.3-21.0)	15.2 (11.5-20.1)	10.0 (5.6-14.1)
$AUC_{0-\infty}$ (ng*hr/mL)	White	216* (186-252)	183* (152-221)	95.7* (80.0-114)	90.7* (75.8-108.7)	88.8* (73.9-106.7)	83.5 (51.3-115.7)
	Asian	500 (428-583)	538 (260-1110)	179 (150-212)	167.4 (140.8-199.1)	140.9 (108.6-182.6)	92.5 (56.3-128.7)

Our study shows no interethnic differences in rosuvastatin PK in *SLCO1B1* and *ABCG2* wt carriers, while other studies showed 2-fold difference in rosuvastatin PK in either *SLCO1B1* or *ABCG2* wt carriers. * Statistically significant when compared with Whites and Asians.

In our current study, the 90% confidence interval (CI) of $AUC_{0-\infty}$ was within 56.3-128.7 ng*hr/mL, which was lower compared with the range found in the

Birmingham and Lee et al. groups, as shown in Table 2-4. Although we would have expected a similar range, it was not observed in this study. We do note that at least in the Birmingham et al.[10] study the lower levels of the 90%CI for the control genotype subjects fell below that observed in the no control group. Our results might be due to smaller sample size (8 in each group). In the Lee et al. paper, their data was reported on 21 Caucasians and 17 Chinese who were *SLCO1B1* *1a carriers (*ABCG2* was not reported); in the Birmingham et al. paper, the result was reported on 24 Caucasian and 12 Chinese who are *SLCO1B1* *1a carriers (*ABCG2* genotype was not reported in conjunction with *SLCO1B1*)[9,10]. If the diplotype/genotype of *SLCO1B1* and *ABCG2* can explain the racial differences in rosuvastatin exposure, the difference of AUC and C_{max} between Asians and Whites in the Lee et al. and Birmingham et al. studies should become smaller than those in genotype control groups. There were slight decreases for this comparison in the Birmingham et al. data but not for the Lee et al. data. Again, this points out the variances of our results from that previously reported.

Tomita et al.[22] also suggest from their retrospective analysis that *SLCO1B1* c.521T>C and *ABCG2* c.421 C>A polymorphisms cannot explain the observed plasma concentration variations between Asian and Whites, although *SLCO1B1* *1a/*1b were not evaluated.

Previously, Tomita et al.[22] reported that V_{ss} in Asians was approximately half of that found in Whites when no allelic transporter characteristics were quantitated. Here again investigating only wildtype *SLCO1B1* and *ABCG2* subjects, this V_{ss} difference between Asians and Whites was also mitigated in our cohort. Our study provides a further element of precision medicine beyond the previous finding of Lee et al.[9], who demonstrated that the two-fold rosuvastatin AUC difference between

Asian and Whites was still observed when controlling for the *SLCO1B1* allele alone. Our study shows that both *SLCO1B1* *1a, *ABCG2* c.421 play important roles in rosuvastatin drug disposition. This finding is in agreement with the previous pharmacogenetic and pharmacokinetic studies that rosuvastatin pharmacokinetics were susceptible to both *SLCO1B1* and *ABCG2* polymorphisms[10]. However, the results from our prospective study are not consistent with the retrospective analyses of Birmingham et al.[10] and Tomita et al.[22]. Therefore, further studies are needed to confirm the relevance of our finding.

Interethnic differences in statin pharmacokinetics have recently been noted as a general phenomena[10]. Simvastatin acid, atorvastatin, pravastatin, and rosuvastatin were all shown to have higher average AUC and C_{max} levels in Japanese and other Asians compared to Caucasians in healthy volunteer pharmacokinetic studies[10,22,25]. Since atorvastatin[26,27], pravastatin[28,29] and rosuvastatin[10] are substrates of OATP1B1, while atorvastatin[29] and rosuvastatin[29] are known inhibitors of BCRP, we believe that genetic polymorphism leading to interindividual and interethnic pharmacokinetic variations in other statins exposure should be examined in addition to rosuvastatin.

As shown in Table 2-3, concomitant dosing of IV rifampin with oral rosuvastatin markedly increased rosuvastatin exposure, both C_{max}, and AUC, and markedly decreased rosuvastatin CL/F and V_{ss}/F. Interethnic differences in drug-drug interaction with rosuvastatin were previously reported with higher AUC fold increase in non-Asians than Asians in the presence of eltrombopag, an OATP1B1 and BCRP inhibitor[30]. However, that study did not report *SLCO1B1* and *ABCG2* genotype in their results. Here, when *SLCO1B1* and *ABCG2* were inhibited by rifampin, both Asians and Whites with wildtype *SLCO1B1* and *ABCG2* in our study

experienced the same approximate fold increase in rosuvastatin exposure. The interethnic difference in drug-drug interaction was not observed in our study after controlling for *SLCO1B1* and *ABCG2*. A recent study reported no racial difference in liver transporter protein expression[23] and our study further supports the similarity of total protein expression for both OATP1B1 and BCRP between the two groups since the changes in rosuvastatin exposure in the presence of rifampin were similar between Asians (3.2 fold) and Whites (3.4 fold).

Inhibition of OATP1B1 and BCRP by rifampin also markedly affected the rate of rosuvastatin absorption in both Asians and Whites, as reflected by MAT, and resulted in shorter T_{max}. Since OATP1B1 is only expressed in the liver and BCRP is found both in the gut and liver, we believe that BCRP function in the gut also affects interethnic bioavailability. A previous study showed that rifampin can inhibit OATP1B1 and BCRP and when co-dosing rifampin and rosuvastatin, oral rifampin had a bigger effect on rosuvastatin pharmacokinetics than iv rifampin; while no significant difference was noted between p.o and i.v rifampin with pitavastatin. Pitavastatin is an *in vitro* substrate of OATP1B1 and BCRP[31], but when pitavastatin was dosed to *ABCG2* c.421 C>A subjects, the pitavastatin AUC did not differ from wildtype subjects[32,33]. In addition, pitavastatin exhibits high F_aF_g, so intestinal BCRP should have minimal effect on pitavastatin drug exposure. It was not clear as to whether rifampin could inhibit intestinal BCRP in addition to the liver. More prevalent reduced function BCRP in Asians could potentially be an explanation as to why Japanese demonstrate higher rosuvastatin bioavailability (29%) than Caucasians (20%) as cited by Tomita et al.[22]

ROS12, the *SLCO1B1* reduced function carrier, demonstrated similar rosuvastatin exposure at control and treatment period; unlike the wildtype carriers

who experienced about three fold increase in rosuvastatin exposure in the inhibition period, It is interesting to observe this outcome. We suspected that it is because inhibiting a reduced function *SLCO1B1* did not further impair the function of the transporter and therefore did not affect any change in drug exposure. However, given only this very limited subject number, further studies would be needed to confirm this observation.

As seen in Table 2-3, V_{ss}/F decreased more than CL/F after concomitant rifampin dosing, indicating that V_{ss} is markedly decreased as compared to the change in clearance. Since the pharmacokinetic volume term does not relate to any particular space/organ in the body, this marked change could be either protective or deleterious with respect to statin adverse reactions. However, we note that although most side effects of statins are dose dependent, evidence to date shows no increased rates of adverse events in Asian patients taking lower versus higher doses of statins[34-40]. We suggest that it would be useful to investigate differences in myopathy between patients exhibiting decreased function polymorphisms in *SLCO1B1*/*ABCG2* versus wild-type.

2.5 Conclusions

The most recent ACC/AHA Blood Cholesterol Guideline recommends rosuvastatin as one of the two most potent statins to reduce the risk of cardiovascular events in moderate and high-risk patients. And the FDA recommends beginning at a lower starting dose in patients of Asian descent[41]. Yet, our study suggests that about one-third of Asian patients (39%) exhibit wild-type genotype of the important transporters for rosuvastatin disposition. Treating these patients with lower starting doses of rosuvastatin may delay achievement of target goals by weeks

to months (essentially, until the next clinic visit). We recommend that *SLCO1B1* and *ABCG2* polymorphism provide a better prediction for rosuvastatin dosing than ethnicity in order to meet treatment goals in a timely and effective manner. The most important result we found in this study is that we suggest that the 39.6% of Asians who carry wild type *SLCO1B1* *1a and *ABCG2* c.421CC should be prescribed the same dose as Whites instead of lowering the starting dose.

In a similar manner, when treating patients of non-Asian descent with rosuvastatin, clinicians should be aware that many White patients could have reduced-function *SLCO1B1* and *ABCG2* alleles, leading to higher drug exposure. Given that the frequency of the muscle toxicity from statin use is reported higher in real life compared to data from clinical trials these non-Asian patients may be more likely to exhibit statin toxicity and reduced adherence[6]. Here, we found that both *SLCO1B1* *1a and *ABCG2* c.421 alleles should be considered when examining interethnic rosuvastatin exposure differences. However, since our prospective study results contradict previous retrospective analyses and we did not include a no control group for comparison, further studies are needed to confirm this finding.

2.6 Materials and Methods

Study design

We conducted an investigator-initiated, prospective, two-arm, crossover, randomized, controlled trial to evaluate the pharmacogenomic effect of drug transporters on rosuvastatin pharmacokinetics in Asian and White healthy volunteers. Recruitment was from the general public in the San Francisco/Bay area from November 2014 to July 2015. Each participant provided written informed consent.

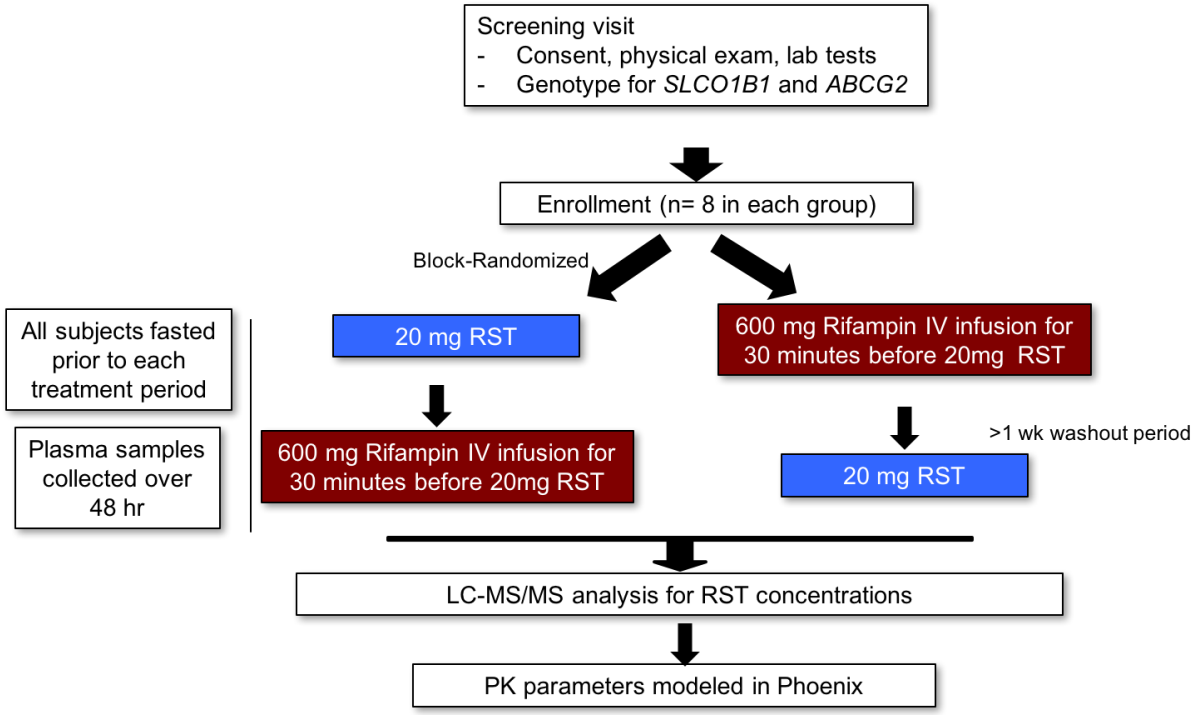

Figure 2-4 Healthy volunteer clinical study design summary

As depicted in Figure 2-4, subjects were block randomly assigned to receive either an oral 20mg rosuvastatin (RST) tablet (Crestor®, AstraZeneca, Wilmington, DE) first or an oral 20mg rosuvastatin tablet immediately following a 30-min intravenous infusion of rifampin (Rifadin®, Sanofi-Aventis, Bridgewater, NJ) 600 mg in 10 ml sterile normal saline at a rate of 20 mg/min. The two periods were separated by at least a 7-day washout and all subjects completed both periods. To eliminate a food effect, subjects fasted from 8 hours prior to rosuvastatin dosing to 3 hours post dosing and standardized meals were provided. Venous blood samples (8 mL each) were collected into K3-EDTA tubes at t=0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 9, 12, 24, 32, 48 hours post dosing. Blood was centrifuged within 30 min at 4 °C and aliquot plasma samples were stored at -80 °C until bioanalysis.

Study subjects

Eight Asians and eight Whites, non-smoking, healthy volunteers, male and female, between the ages of 18-65 were enrolled. Eligibility was determined by medical history, physical examination, and clinical laboratory evaluation in a screening visit. Ethnicity was self-reported by the volunteers for both parents and all four grandparents; only European and East Asian descendants were studied. Since previous studies both in Asians and Whites showed little pharmacokinetic differences between *SLCO1B1* * 1a and *1b allele[9], we enrolled the volunteers carrying either *SLCO1B1* *1a/*1a or *1a/ *1b allele and *ABCG2* c.421CC genotype. Pre-menopausal females were tested for pregnancy before and during study enrollment and maintained adequate birth control independent of hormonal contraceptive use during the study. Subjects with known allergies to the study medications and a history of rhabdomyolysis, gastrointestinal bleeding, peptic ulcer disease, and drug-related myalgia were excluded. Subjects abstained from caffeinated drinks, alcohol, herbal tea, and grapefruit one day prior to the study.

Genotyping of *SLCO1B1* and *ABCG2* polymorphisms

DNA extraction from blood samples and sequencing was conducted by the UCSF Genomics Core Lab (San Francisco, CA). All sample genotyping was carried out in a blinded fashion with use of coded ID samples. Regions containing *SLCO1B1* c.388A>G, *SLCO1B1* c.521T>C and *ABCG2* c.421C>A were amplified using the following primers (Primer3 algorithm) on a 9700 thermal cycler (Applied Biosystems) with a touchdown PCR method:

SLCO1B1 rs2306283_F: 5'-AAACACATGCTGGGAAATTGAC-3'

SLCO1B1 rs2306283_R: 5'-TCATCCAGTTCAGATGGACAAA-3'

SLCO1B1 rs4149056_F: 5'- GCAGCATAAGAATGGACTAATACACC-3'

SLCO1B1 rs4149056_R : 5'-TCGCATGTGTGCTTAGAAAGAC-3'

ABCG2 rs2231142_F: 5'- TCATTGTTATGGAAAGCAACCA-3'

ABCG2 rs2231142_R: 5'- GGCAAATCCTTGTATGAAGCAG-3'

The PCR products were cleaned up and sequenced with the BigDye Terminator reagent (Applied Biosystems). The sequence data were viewed and analyzed with the Sequencher program (GeneCodes).

Study end points

Primary end points were rosuvastatin systemic exposure measured as area under the curve (AUC) from 0 to 48 hours ($AUC_{0\text{-}48}$) and 0 to infinity ($AUC_{0\text{-}\infty}$). Secondary outcomes were rosuvastatin peak plasma concentration, C_{max}, time to peak concentration, T_{max}, mean absorption time (MAT) and volume of distribution at steady state divided by bioavailability (V_{ss}/F).

Study oversight

The study was approved by the Committee on Human Research of the University of California, San Francisco and conducted at the Clinical & Translational Science Institute's Clinical Research Center in compliance with the principles of the Declaration of Helsinki. This study was registered on the US National Institutes of Health Clinical Trials Database (NCT02215174; https://clinicaltrials.gov/ct2/show/NCT02215174 .)

Plasma sample bioanalysis

Rosuvastatin concentrations were measured using a high-pressure liquid chromatography-tandem mass spectrometry method. The system consisted of QTrap 5500(AB Sciex, Redwood City, CA) with Shimadzu HPLC using electrospray ionization in the positive mode. Rosuvastatin and the internal standard, rosuvastatin-

d3, were separated on a Kinetex C8 50x2.1mm column at ambient temperature. The mobile phase was a combination of (A) water and (B) acetonitrile both with 0.1% formic acid. The gradient ran from 15% to 95% for 1 minute. Ion detection was performed in the multiple reaction monitoring mode with Q1→Q3 transitions for rosuvastatin of 482.1 →258.2 m/z, and rosuvastatin-d3 of 485.1-->261.2 m/z. Plasma samples, calibration curves, and quality control (QCs) samples were prepared in the same way. The rosuvastatin method had a final LLOQ of 0.015ng/ml and ULOQ of 100ng/ml. The mean concentrations of QCs were within 15% of nominal concentrations and with coefficients of variation <15%.

Pharmacokinetic analysis

Rosuvastatin pharmacokinetic parameters were estimated from plasma concentration data by noncompartmental analysis using Phoenix® WinNonlin® (Pharsight, Mountain View, CA). The terminal rate constant (λ_z) was estimated by linear regression of the terminal phase of the log plasma concentration-time curve. $AUC_{0\text{-}48}$ was calculated by the linear up /logarithmic down trapezoidal method. Summation of $AUC_{0\text{-}48}$ and the concentration at the last measured point divided by λ_z yielded $AUC_{0\text{-}\infty}$. Rosuvastatin T_{max} and C_{max} were obtained directly from observed data. Oral clearance (CL/F) was calculated as dose/ $AUC_{0\text{-}\infty}$. MAT was estimated as the reciprocal of the first-order absorption rate constant after the data were fit to a 2 compartment model with absorption from the gut compartment using Phoenix® WinNonlin®. Oral volume of distribution (V_{ss}/F) was calculated as previously described[26] as the ratio of the Area Under the first Moment Curve ($AUMC_{0\text{-}\infty}$) divided by $AUC_{0\text{-}\infty}$, multiplied by CL/F, then subtracting MAT.

Statistical analysis

Using a paired t-test and prior data[26], the sample size was sufficient to detect a 50% difference in AUC_{0-48} between the two arms with a statistical power of 80%, alpha = 0.05, and standard deviation of 40%. Pharmacokinetic parameters were analyzed for differences between the two treatment periods by the paired t-test, except for Tmax where a Wilcoxon matched pair test was used.

2.7 Acknowledgements

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Chapter. 3 Rosuvastatin systemic exposure in morbidly obese patients with *SLCO1B1**1a and *ABCG2* c.421CC post bariatric surgery in the US and Taiwan

3.1 Abstract

Rosuvastatin is one of the most commonly prescribed drugs in morbidly obese patients undergoing bariatric surgery for weight loss. The effect of bariatric surgery on rosuvastatin pharmacokinetics has not been investigated. Since rosuvastatin is a poorly metabolized drug and primary dependent on drug transporters for disposition, Roux-en-Y gastric bypass surgery can also serve as an appropriate model to study rosuvastatin intestinal absorption in human.

Here we show that rosuvastatin pharmacokinetics were not significantly different post roux-en-Y gastric bypass surgery when all subjects are wildtype carriers for both Solute Carrier Organic Anion transporter*1B1 *1a* and ATP Binding Cassette Subfamily G Member 2 c.421 transporters in a randomized, two-period rosuvastatin pharmacokinetics study in morbidly obese patients. The AUC_{0-48} values were 83.1 (±63.0) ng*hr/ml pre-surgery and 109.1 (±60.1) ng*hr/ml post-surgery. We recommend no clinical dose adjustment in this population.

3.2 Introduction

Prevalence of morbid obesity has increased markedly in recent years and has become a healthcare burden. Approximately 1 in 3 adults meet the National Institutes of Health (NIH) definition of obesity (body mass index [BMI] of ≥30 kg/m^2) and about 1 in 20 adults meet the criteria for morbid obesity (BMI of ≥40 kg/m^2) in America.[1,2] Morbid obesity is not only a high-risk factor for type 2 diabetes, cardiovascular disease, hypertension, non-alcoholic fatty liver disease and other health problems, but also causes numerous comorbidities, with marked outcome improvement after weight loss.

Bariatric surgery has proven to be the most efficient and sustainable surgical procedure to achieve weight loss and reduce comorbidities. The number of bariatric surgeries performed in 2009 was approximately 220,000 according to the American Society for Metabolic and Bariatric Surgery.[3,4] The most commonly performed and effective bariatric surgery in the United States as of 2015 was roux-en-Y gastric bypass surgery (RYGB), with an average 67% excess body weight loss at three years across studies, although the popularity of RYGB is decreasing over time due to newer surgery techniques.[5,6] RYGB is both a restrictive and a malabsorptive procedure to achieve weight loss that entails the creation of a small stomach pouch from the upper stomach and creating a bypass of the lower part of the stomach, duodenum, and proximal jejunum. In this way, the size of the stomach is decreased, markedly restricting food absorbtion. The distal jejunal limb becomes the Roux limb and is anastomosed to the new gastric pouch. The proximal biliopancreatic limb of the jejunum, which secrets gastric and biliopancreatic juices, is joined to the Roux limb somewhere from 80 to 120 cm beyond the gastrojejunal anastomosis. This altered jejunal anatomy facilitates malabsorption by preventing food from contacting

intestinal surfaces for absorption and the mixing of food and digestive enzymes as food passes through the Roux limb.[7–9]

As a result of the altered anatomy, malabsorption of nutrients and vitamins occurs post-RYGB surgery. Consequentially, drug absorption, distribution, and clearance might also be affected, independent of weight loss. Restriction of gastric volume has been shown to reduce gastric emptying time and may further lead to an increase in gastric pH, which could affect the solubility of orally administered drugs.[10–13] In parallel, decreases in intestinal surface area and altered intestinal anatomy may lead to permanent changes in the rate and/or extent of absorption, the influence of active membrane transporters, intestinal metabolism, first-pass liver effect, as well as hepatic clearance. A recent study with atorvastatin demonstrated pharmacokinetic alteration in obese patients after undergoing RYGB surgery, recommending dose titration.[14]

Rosuvastatin is one of the most potent and commonly used statins. Based on the Biopharmaceutical Drug Disposition Classification System(BDDCS), which classifies drugs by the extent of metabolism and solubility, rosuvastatin is a class III compound, which suggests uptake and efflux transporters are both essential for rosuvastatin absorption, distribution, and elimination. Metabolism plays a minor role in the elimination for rosuvastatin, with 76.8% of rosuvastatin eliminated as unchanged drug.[15] Hepatic clearance of rosuvastatin accounts for 72.5% of the total clearance[16], presumably primarily via biliary excretion. With high solubility and hydrophilicity, rosuvastatin depends on drug transporters to enter and exit intestinal epithelial cells for absorption and hepatocytes for elimination. Previous studies, have shown and implied from the results presented in Chapter 2, that polymorphisms resulting in reduced function of solute carrier organic anion transporter family

member 1B1(*SLCO1B1*, the gene encoded for OATP1B1) and of ATP-binding cassette subfamily G member 2 (*ABCG2*, the gene encoded for BCRP), give increased rosuvastatin exposure in healthy volunteers by 2-fold. OATP1B1 is a dominant hepatic uptake transporter expressed on the basolateral membrane of the hepatocyte while BCRP is an efflux transporter expressed both on the apical membrane of the intestinal epithelial cell as well as the apical membrane of hepatocytes.[17–19] OATP1B1 is responsible for hepatic uptake of rosuvastatin while BCRP results in efflux of rosuvastatin into bile. Sugiyama and coworkers have carried out simulations showing that inhibition or reduced function in OATP1B1 will cause marked increases in a substrate systemic exposure but only minor increase in liver exposure. In contrast, reduced function of a hepatic efflux transporter will have the opposite effect, that is, increasing drug liver exposure but not systemic exposure[20]. Considerable efforts have been made to investigate the transporter effects on hepatic clearance of rosuvastatin, but few studies have examined the transporter effects on the intestinal absorption of rosuvastatin. Johson et al., in a paper published early this year showed a 2-fold decrease in rosuvastatin exposure when inhibiting the intestinal uptake transporter, OATP2B1, implicating the importance of intestinal absorption in rosuvastatin pharmacokinetics.[21]

Although there is a general understanding of malabsorption caused by RYGB surgery, changes in drug pharmacokinetics and dynamics remain drug specific, and no common trend has been found.[22] Given that the nature of RYGB is to bypass the proximal intestine, here we test whether RYGB surgery has an effect on rosuvastatin intestinal absorption in obese patients and whether rosuvastatin dosing should be re-titrated in obese patients post-RYGB as a result. To minimize pharmacogenomic effects due to reduced function polymorphisms, we genotyped patients for *SLCO1B1*

c.388 and c.521 and *ABCG2* c.421.[16,23-27] We only enrolled patients with wildtype *SLCO1B1* (*1a) in our cohort but placed no restriction with respect to *ABCG2* c.421. However, 8 of our 9 subjects were *ABCG2* c.421CC and one a heterozygote c.421CA.

3.3 Results

Participant demographics

During recruitment 23 patients were screened; 15 patients were found with the target gene, *SLCO1B1* *1a, of which 8 Caucasians and 1 Asian patient (Patient 8) at UCSF completed this study in the US. Three eligible patients ultimately chose alternate surgical procedures rather than RYGB, while the remaining 3 eligible patients discontinued participation due to conflicts in scheduling or concerns from family members. All of the patients self-reported their ethnicity: for those completing the study 8 were of European descent and 1 was East Asian descent. The data from the Asian patients in Taiwan will be reported separately after the Taiwan site completes recruitment in another year. The US study population averaged 43.6 years old with an age range of 30-63 years. Before and after surgery, average BMI was 44.9 (±3.8) kg/m^2 and 37.4 (±4.7) kg/m^2, while weight was 121.4 (±9.7) kg and 97.0 (±7.2) kg, respectively, as shown in Table 3-1. Most patients are female (88.9 %) with only one male participant. Common comorbidities shared by more than three patients are type 2 diabetes, obstructive sleep apnea, and hypertension.

Table 3-1 Demographics of all US volunteers studied.

Patient number	Sex	Age (years)	Weight (kg)		BMI (kg/m^2)		*SLCO1B1*	*ABCG2* c.421	Post period time from surgery (weeks)
			Pre	Post	Pre	Post			
1	F	50	136.5	115.7	48.6	41.2	*1a/ *1a	CC	14
2	F	47	131.9	104.3	44.2	35.0	*1a/ *5	CC	14
3	F	43	125.4	99.3	48.9	38.8	*1a/ *1a	CC	12
4	F	30	128	98.0	41	31.2	*1a/ *1a	CC	14
5	F	33	128.8	104.3	50.3	43.4	*1a/ *1a	CC	12
6	F	63	115.8	93.9	46.0	37.1	*1a/ *1b	CC	12
7	F	47	109.6	82.6	44.2	41.2	*1a/ *1b	CC	12
8	F	34	105.9	92.7	45.6	41.2	*1a/ *1b	CC	13
9	M	52	125.6	100.7	38.6	31.0	*1a/ *1b	CA	12
Mean		43.6	121.4	97.0	44.9	37.4			12.6
SD		11.1	9.7	7.2	3.8	4.7			0.9

BMI, body mass index; F, female; M, male; SD, standard deviation

Genotype

The frequencies of *SLCO1B1* and *ABCG2* are summarized in Table 3-2. Only volunteers with *SLCO1B1**1a wildtype were included in our analysis, and 4 out of 9 patients carried *1a/ *1a, 4 out of 9 patients carry the *1a/ *1b allele, and 1 out of 9 patients carried the *1a / *5 allele. In our cohort, we also genotyped for *ABCG2* c.421 C>A, and found only one patient (#9) to carry the c.421 C>A minor allele, while the others all carried wild type for *ABCG2* c.421. The sample size is too small to draw a conclusion, but the heterozygous *SLCO1B1* c.388A>G did not appear to affect the plasma concentration compared to homozygous wildtype, as we also showed in Chapter 2.

Table 3-2 Genotyping frequency of *SLCO1B1* and *ABCG2*

Allele	Number of patients
***SLCO1B1* c.521**	
TT	8
TC	1
CC	0
***SLCO1B1* c.388**	
AA	5
AG	4
GG	0
***ABCG2* c.421**	
CC	8
CA	1
AA	0

Pharmacokinetics of rosuvastatin

No statically significant rosuvastatin pharmacokinetic differences were observed when rosuvastatin was administered pre- and post-RYGB surgery as reported in Table 3-3 when only measured values are considered. The $AUC_{0\text{-}48}$ (±SD) were 89.6 (±62.1) ng*hr/ml during the pre-surgery phase and 102.7 (±59.4) ng*hr/ml during the post-surgery phase. The C_{max} values were 10.1 (±7.2) ng/ml pre-surgery and 10.9 (±5.7) ng/ml post-surgery. Marked increases (>50%) in $AUC_{0\text{-}48}$ to $AUC_{0\text{-}\infty}$ were seen in patients 1, 5, and 6, while a marked decrease (<50%) was only seen in Patient 9, but overall the mean $AUC_{0\text{-}48}$ to $AUC_{0\text{-}\infty}$ increased only 15%.

Table 3-3 Pharmacokinetic parameters of rosuvastatin following a 20mg oral dose of rosuvastatin pre- and post- Roux-en-Y gastric bypass surgery.

Patient number	C_{max} (ng/ml)		AUC_{0-48} (ng·hr/ml)		Ratio	$t_{1/2}$ (h)	
	Pre	Post	Pre	Post		Pre	Post
1	2.3	6.7	24.2	73.5	3.04	15.8	12.9
2	25.7	15.4	215.1	163.6	0.76	10.1	32.1
3	3.0	3.5	33.7	40.0	1.19	22.5	9.7
4	11.4	19.0	99.4	102.8	1.03	12.3	17.2
5	15.5	15.0	123.9	224.2	1.81	8.8	51.4
6	7.7	17.1	57.1	116.9	2.05	16.5	33.9
7	8.4	7.7	68.5	94.7	1.38	4.7	12.6
8	5.9	6.3	42.8	57.0	1.33	6.2	12.0
9	11.3	7.2	141.7	51.7	0.36	13.2	16.7
Mean	10.1	10.9	89.6	102.7	1.44	12.2	22.1
SD	7.2	5.7	62.1	59.4	0.79	5.5	14.1

Values are shown as arithmetic mean ± SD; C_{max}, maximum plasma concentration; AUC_{0-48}, Area Under the Curve from 0 to 48 hr; $t_{1/2}$, terminal half-life.

Since Patient 9, the only male studied, carried the *ABCG2*.c421 reduced variant, we excluded his data from the group and presented in Table 3-4 the data only from the 8 female wildtype carriers. The concentration-time profiles of rosuvastatin in both periods are shown in Figure 3-1. The AUC_{0-48} values were 83.1(±63.0) and 109.1(±60.1) ng*hr/ml for pre and post-surgery period respectively; while $AUC_{0-\infty}$ values were 88.0 (±63.0) and 146.1 (±114.0) ng*hr/ml. The increase in the mean $AUC_{0-\infty}$ ratio from 1.57 (±0.72) to 1.77 (±0.86) results primarily due the marked increase in Patient 5 in whom a 51.4 hr half-life was calculated, a value which is longer than the sampling interval and thus must be viewed with suspect. This increase is also reflected in MRT. The C_{max} mean values were 10.0 (±7.7) ng/ml pre-surgery with a T_{max} of 4.0 hours and 11.3 (±5.9) ng/ml post-surgery with a T_{max} of

3.7 hours. None of the pharmacokinetic parameters post-surgery were statistically different from the pre-surgery values when evaluating all 9 patients or the 8 female patients in Table 3-3 and 3-4, using the Wilcoxon signed rank test. RYGB surgery had no effect on delayed or prolonged absorption as shown by similar T_{max} values.

Table 3-4 Pharmacokinetic parameters of rosuvastatin following a 20mg oral dose of rosuvastatin pre- and post- Roux-en-Y gastric bypass surgery in *ABCG2*c.421 CC wildtype carriers.

Patient#	C_{max} (ng/ml)		AUC_{0-48} (ng·hr/ml)		Ratio	$AUC_{0-\infty}$ (ng·hr/ml)		Ratio	$t_{1/2}$ (h)		MRT (h)	
	Pre	Post	Pre	Post		Pre	Post		Pre	Post	Pre	Post
1	2.3	6.7	24.2	73.5	3.04	27.0	79.1	2.93	15.8	12.9	20.4	16.6
2	25.7	15.4	215.1	163.6	0.76	220.5	219.8	1.00	10.1	32.1	12.9	34.2
3	3.0	3.5	33.7	40.0	1.19	41.3	41.4	1.00	22.5	9.7	26.5	14.6
4	11.4	19.0	99.4	102.8	1.03	104.3	118.0	1.13	12.3	17.2	13.6	13.5
5	15.5	15.0	123.9	224.2	1.81	127.6	390.9	3.06	8.8	51.4	11.8	60.9
6	7.7	17.1	57.1	116.9	2.05	68.7	156.0	2.27	16.5	33.9	24.4	33.8
7	8.4	7.7	68.5	94.7	1.38	68.6	104.5	1.52	4.7	12.6	8.1	17.2
8	5.9	6.3	42.8	57.0	1.33	46.1	59.1	1.28	6.2	12.0	8.9	11.7
Mean	10.0	11.3	83.1	109.1	1.57	88.0	146.1	1.77	12.1	22.7	15.8	25.3
SD	7.7	5.9	63.0	60.1	0.72	63.0	114.0	0.86	5.9	14.9	7.0	16.9

Values are shown as arithmetic mean ± SD; C_{max}, maximum plasma concentration; AUC_{0-48}, Area Under the Curve from 0 to 48 hr; $AUC_{0-\infty}$, Area Under the Curve extrapolated to infinite using the measured terminal half-life; $t_{1/2}$, terminal half-life; MRT, mean residence time.

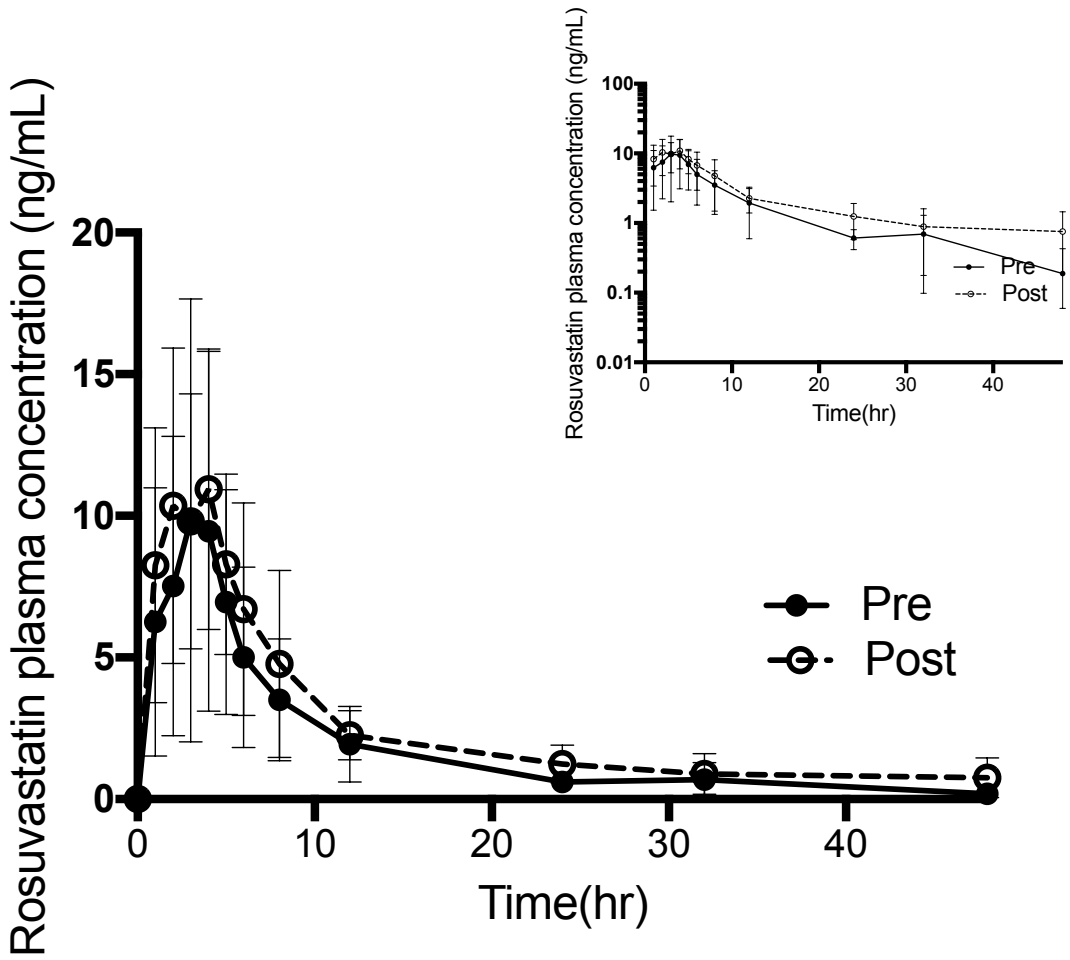

Figure 3-1 The rosuvastatin pharmacokinetics in 8 morbidly obese patients pre- and post-RYGB surgery. Mean plasma concentration of rosuvastatin (± SD) following single oral 20mg dose of rosuvastatin. The inset depicts the same data on a semi-logarithmic scale.

Individual change in AUC_{0-48} and C_{max} before and after the surgery are shown in Figure 3-2.

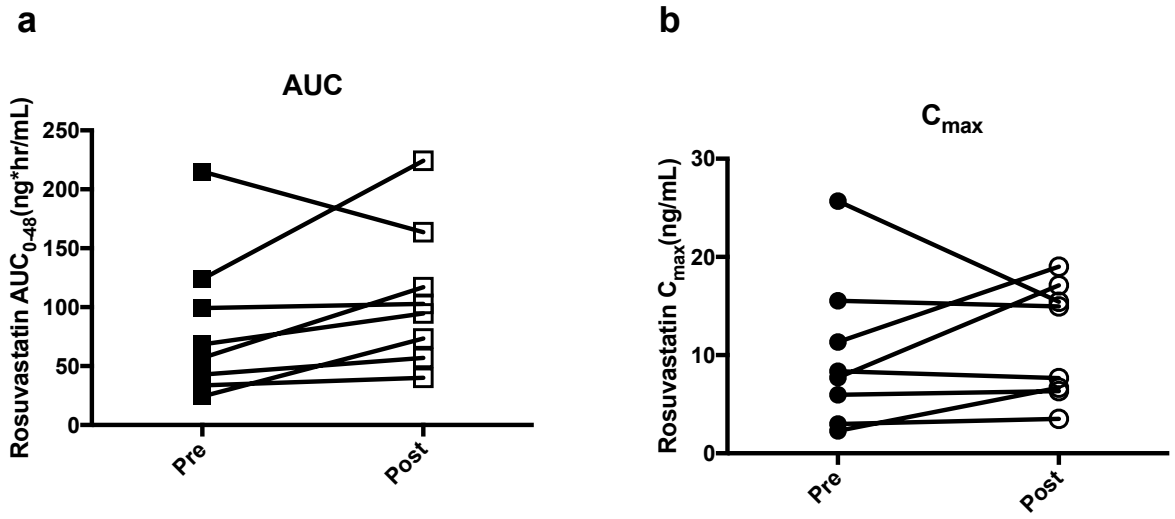

Figure 3-2 The effect of RYGB on the pharmacokinetics of rosuvastatin in morbidly obese patients(n=8). Rosuvastatin (a) mean AUC_{0-48} and (b) C_{max} were similar before and after RYGB following a single oral dose of 20 mg rosuvastatin.

Pharmacokinetics of rosuvastatin in the Asian patient in Taiwan

Recruitment of study subjects in Taiwan has taken a longer time than what we expected. We have only managed to identify one eligible Asian patient out of 6 screened in Taiwan. Most Asian patients have sleeve surgery, an alternative bariatric surgery recommended for patients with less BMI, as we found in Taiwan. As a result, we have submitted an IRB modification to include the sleeve patients into the study cohort and resumed study recruitment in Taiwan as of Jan 2017.

3.4 Discussion

Over all, RYGB appears to have no clinically significant effect on rosuvastatin pharmacokinetics, when measured exposure levels are compared pre- and post-surgery in morbidly obese patients, although when $AUC_{0-\infty}$ values are compared, the mean exposure increased on average to 77%, but this increase is driven primarily due to the very long 51 hr half-life determined in one patient post-surgery. To our understanding, this is the first study to examine the RYGB effect on statin pharmacokinetics with adequate pharmacogenetic controls. We conducted the rosuvastatin pharmacokinetic study before and 3 month after RYGB surgery with standardized and comparable surgical techniques. We believed that enrolling *SLCO1B1* wildtype carriers, using patients as their own controls and standardizing the surgical techniques could minimize the potential inter-individual variabilities in rosuvastatin pharmacokinetics.

RYGB surgery results in physiological alterations and possibly impacts oral drug absorption in the following ways: reduced gastric volume, bypass of the duodenum and proximal jejunum, dissociation of bile salt delivery and weight loss.[28–34] Reduced gastric volume often leads to increased gastric and gastrointestinal pH. However, rosuvastatin is a highly soluble drug (BDDCS class III). Also, rosuvastatin is a hydrophilic drug with minimal passive intestinal permeability. Therefore, the drug primarily depends on transporters to cross the intestinal cell membrane for absorption. A previous study showed that OATP2B1 expressed on the epithelial cell membrane mediates rosuvastatin intestinal absorption and that the activity of OATP2B1-mediated uptake of rosuvastatin is not pH sensitive.[35] Therefore, changes in pH resulting from altered intestinal anatomy might not have much effect on rosuvastatin absorption. Our finding of no clinically significant change

in rosuvastatin exposure aligns with the previous speculation. Also, it's well established that delayed gastric emptying is often observed post RYGB. However, we observed no significant change in rosuvastatin exposure nor T_{max}.

Second, our finding that bypassing the duodenum and proximal jejunum(50 cm) had no clinically significant effect on rosuvastatin exposure suggests that the main absorption site of rosuvastatin could potentially be in the latter part of the jejunum (>50cm) intestine, which could compensate the reduced in absorption from the proximal jejunum. Gkotsina et al. compared levothyroxine(LT4) pharmacokinetics in sleeve, RYGB, and biliopancreatic diversion surgeries, bypassing different parts of the GI tracts and concluded that the stomach, the duodenum, and the upper part of the jejunum were not sites for LT4 absorption.[36] Our study implies a similar result.

Dissociation of bile salt delivery to the intestinal tract, which results in decreased drug absorption for compounds requiring solubilization with bile salts, is also common in post RYGB patients with altered anatomy.[29,30] Due to the hydrophilicity and high solubility of rosuvastatin, it appears that bile salt secretion plays a minimal role in rosuvastatin absorption. It appears that the rate and extent of rosuvastatin absorption post-RYGB are comparable with that pre-RYGB.

Weight loss from RYGB often results in decreased inflammation and total body fat composition, leading to a change in drug metabolism and distribution. However, rosuvastatin is a low lipophilicity drug and we believe that change in fat composition would have minor effects on rosuvastatin distribution, hence, no clinically significant change in rosuvastatin measured oral clearance nor MRT were observed in our study.

In a recent study, Drozdzik et al.[38] examined the transporter protein expression along the entire length of intestine and found differential transporter

expressions in the duodenum, jejunum, ileum and colon. Therefore, bypassing the intestinal segment could omit the main portion of where the transporter important for rosuvastatin absorption is expressed. However, we observed no evidence of change in rosuvastatin exposure resulting from bypassing the stomach, duodenum, and proximal jejunum. Furthermore, Miyauchi et al.[39] examined transporter protein expression in the intestine in obese patients and found the transporters, such as *ABCG2* levels in the intestine were similar to healthy subjects. As shown below, comparision of rosuvastatin pharmacokinetics were similar between healthy volunteers[40] (as reported in Chapter 2) and obese patients pre- and post-RYGB sugery.

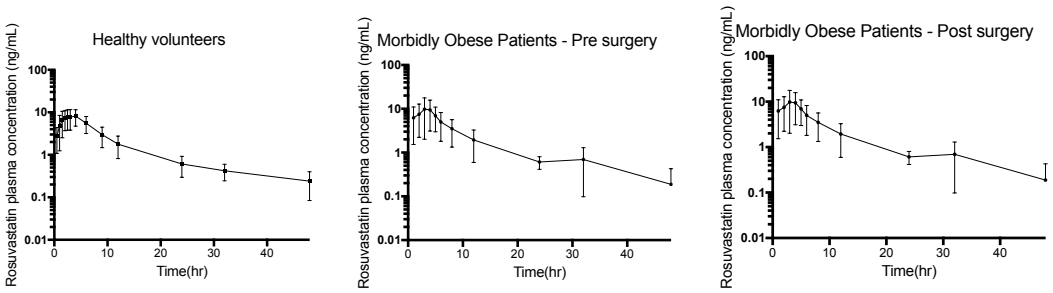

Figure 3-3 Rosuvastatin pharmacokinetics in healthy volunteers and morbidly obese patients wildtype for *SLCO1B1* and *ABCG2*.

In Fig.3-3, we compare the mean rosuvastatin plasma concentrations we reported in Chapter 2 for 7 Whites and 8 Asians wildtype for OATP1B1 and BCRP with the 7 Whites and 1 Asian reported here pre- and post- RYGB surgery. As we reported in Chapter 2, when only wild-type subjects are studied, we see no clinically significant differences in rosuvastatin exposure, and here we show no obvious difference in exposure between healthy volunteers and morbidly obese patients pre- and post- RYGB surgery. We also note that the one Asian patient studied here (Patient 8) exhibited exposure measurements that were at the lower end of the range

in Asian healthy volunteers (between the 6th and 7th lowest AUC measurements of the 8 healthy Asians). Thus, we report here a 9th Asian subject that did not differ in exposure from Whites when controlling for only subjects wildtype for OATP1B1 and BCRP. However, we do note that the standard deviations in the patients are almost double those in healthy volunteers, which is not unexpected.

It may also be useful to compare our results here for rosuvastatin with the previous report of Skotteim et al.[14] of the effects following gastric bypass surgery for atorvastatin. These investigators studied 12 morbidly obese patients undergoing RYGB surgery and reported overall no significant difference in atorvastatin exposure post-surgery (1.2 fold post/pre ratio). However, they recommended that individual patients be retitrated for atorvastatin post-surgery since they found that the three patients exhibiting the highest exposure levels of atorvastatin acid pre-surgery experienced an average 60 ± 7% decrease in exposure post-surgery, while the three patients exhibiting the lowest exposure pre-surgery experienced a 106 ± 30% increase in exposure post-surgery. As seen in Fig. 3-2, we did not observe such consistent changes in exposure post/pre-surgery. Skotteim et al.[14] reported a 35 fold variability in atorvastatin exposure pre-surgery and 6-7 fold variability post-surgery, while we only observed a similar variability in rosuvastatin exposure pre-surgery (8-9 fold) and post-surgery(5-6 fold) in a OATP1B1 and BCRP wildtype controlled group. As we noted above the coefficient of variation(SD/mean=CV) in our obese patients pre-surgery was about twice (76% vs. 41%) that observed in healthy volunteers. Similarly, the dose normalized CV in the 12 obese RYGB patients studied by Skotteim et al.[14] pre-surgery was about twice that we observed in 11 healthy volunteers[41] (75% vs. 35%). Post-surgery CV decreased in both studies (atorvastatin 33%[14] vs. rosuvastatin 55%).

The same research group had also reported a 2 fold increase in atorvastatin exposure in obese patients post biliopancreatic diversion with duodenal switch[41], another bariatric surgery procedure that bypasses a greater length of intestine. One would suspect a greater change for atorvastatin following extensive removal of upper intestinal tissue in bariatric surgery since atorvastatin is a substrate for intestinal CYP3A enzymes. However, any consideration of dosage adjustment based only on atorvastatin acid systemic concentrations is highly suspect since as we have shown[41] the 2-hydroxy atorvastatin acid metabolite is equipotent with the parent acid compound as an HMG-CoA reductase inhibitor and reaches systemic concentrations in humans comparable to those observed for the parent atorvastatin acid. Since rosuvastatin is only minimally a substrate for metabolic elimination, the same issues are not relevant here.

Limitations of our study include the limited sample size and the inability to explore the underlying mechanism (e.g. OATP2B1 or *ABCG2* function and expression) of intestinal absorption. Also, interindividual rosuvastatin exposure variability is commonly observed in pharmacokinetic studies. Rosuvastatin AUC varies about 8-fold in our cohort, even after controlling for the *SLCO1B1* *1 allele and *ABCG2* c.421 CC, which could result from other transporters playing a role in rosuvastatin pharmacokinetics, P-gp[43] and NTCP[44], all of which have been proposed to be responsible for rosuvastatin transport. Furthermore, comorbidities in different patients could potentially be another caveat. Interindividual inflammation in the intestine and recovery time post-surgery could be another factor affecting rosuvastatin pharmacokinetics. However, we conducted the post-surgery study period at least three months post-surgery, which is believed to be sufficient recovery time to allow solid food and drug intake by the standard of care.

3.5 Conclusions

A non-significant increase in rosuvastatin systemic exposure was seen in morbidly obese patients carrying OATP1B1*1a and *ABCG2* c.421 CC wildtype post bariatric surgery. Morbidly obese patients undergoing RYGB surgery still heavily rely on multiple medications, such as statins, for their comorbidities, even though post-RYGB, those conditions are much improved. Statins are one of the most commonly prescribed drug classes in obese patients to lower cholesterol and treat hyperlipidemia. Our study is the first to demonstrate that RYGB appears to have no clinically significant effect on rosuvastatin pharmacokinetics. Thus, rosuvastatin may be a preferred statin for morbidly obese patients potentially considering RYGB surgery.

The RYGB effect on drug pharmacokinetic change remains highly drug specific and it is difficult to predict which direction drug exposure will be altered, depending on various factors including physical and chemical characteristics of drugs, transporter effects and metabolizing enzymes. RYGB could also result in high inter-individual variability in drug absorption, disposition, and elimination. Therefore, comprehensive drug pharmacokinetic studies are still of necessity before any dose adjustment is adopted and generalized.

Here we recommend based on data only in 8 bariatric surgery patients wild-type for both OATP1B1 and BCRP that clinically significant change in rosuvastatin are not expected as a result of RYGB. However, the result must be considered with caution, and retitration may be the appropriate approach considering the significant decrease in exposure we observed in male patient 9 who was not wild-type in BCRP.

3.6 Materials and Methods

Study design

We conducted an investigator-initiated, prospective, controlled trial to evaluate the Roux-en-Y gastric bypass surgery effect on rosuvastatin pharmacokinetics in morbidly obese patients. Recruitment was from the Bariatric Surgery Center at University of California, San Francisco from March 2015 to December 2016. Each participant provided written informed consent.

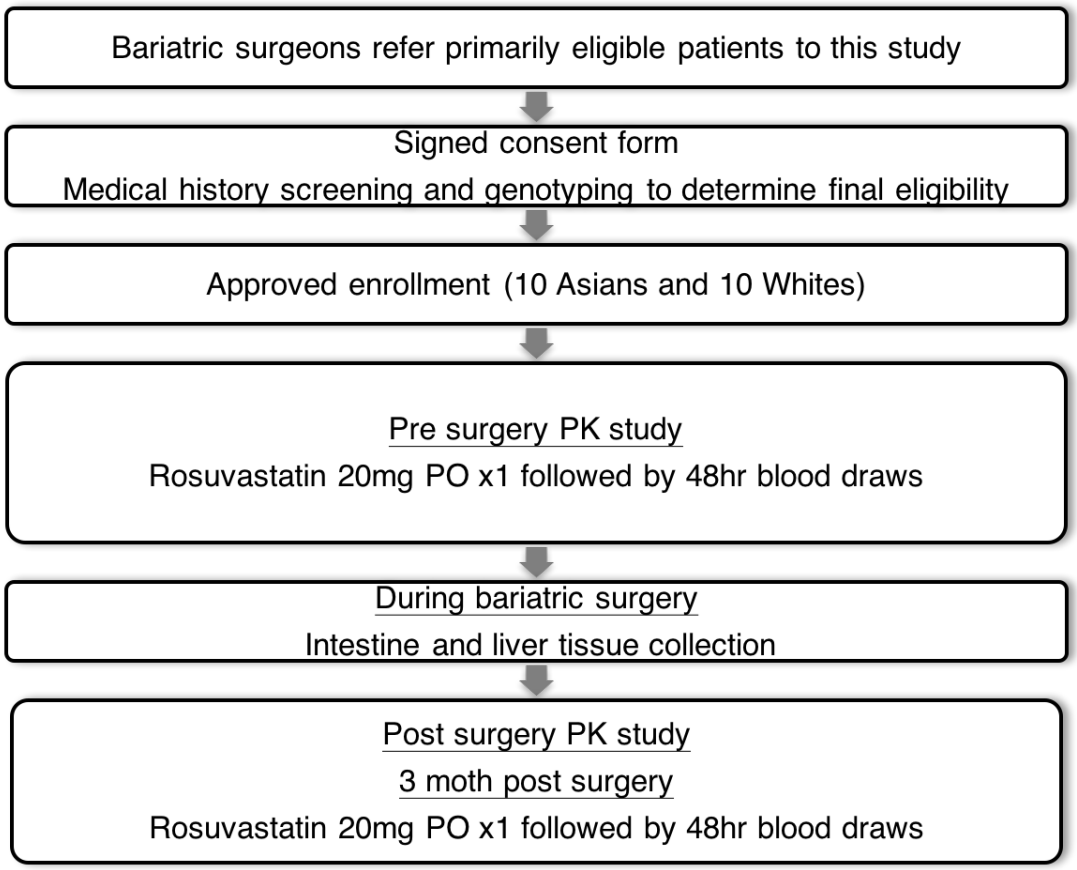

Figure 3-4 Rosuvastatin pharmacokinetic clinical study design flow chart

Subjects were assigned to receive an oral 20mg rosuvastatin tablet (Crestor®, AstraZeneca, Wilmington, DE) before and after their RYGB surgeries (Figure 3-4). The two periods were separated by at least a 3-month recovery period to allow solid food/drug intake after RYGB surgery and all subjects completed both periods. To

eliminate a food effect, subjects fasted from 8 hours prior to rosuvastatin dosing to 3 hours post dosing. Standardized liquid diet meals for the pre-surgery period and a standardized diet for the post-surgery period were provided. Venous blood samples (8 mL each) were collected at t=0, 1, 2, 3, 4, 5, 6, 8, 12, 24, 32, 48 hours post dosing into K3-EDTA tubes (Figure 3-5). Blood was centrifuged within 30 min at 4°C and aliquot plasma samples were stored at -80°C until bioanalysis. Surplus intestinal tissue and liver biopsy were taken under consent for all 9 patients reported here. Proteomic analysis and quantification of intestinal and hepatic tissue will be undertaken when the Taiwan studies are completed.

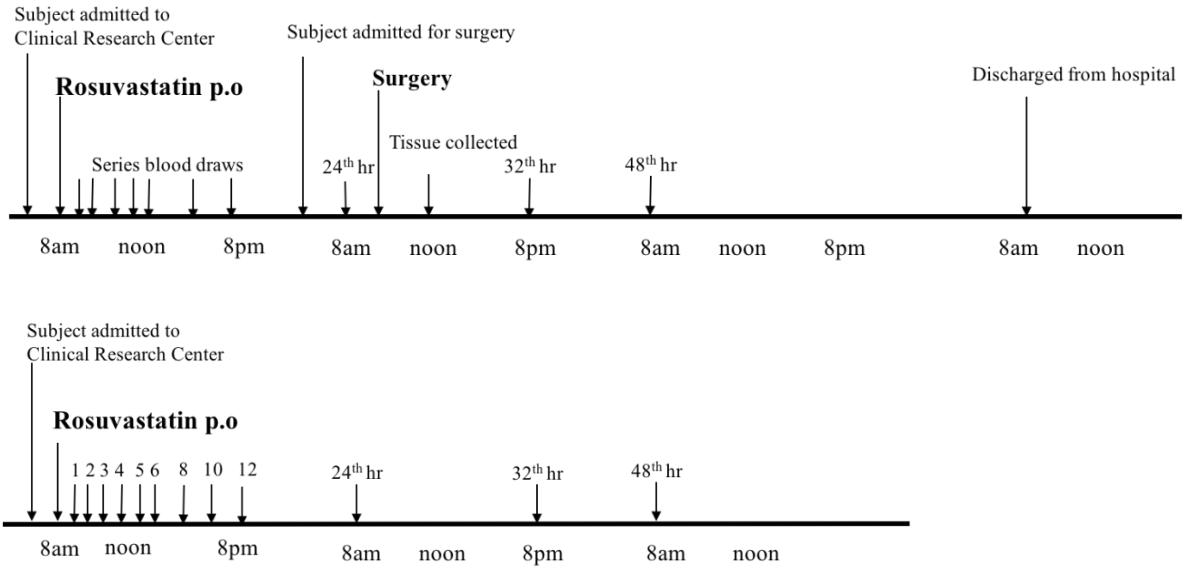

Figure 3-5 Rosuvastatin pharmacokinetic pre- and post-surgery period summary

The study was approved by the Committee on Human Research at the University of California San Francisco(UCSF), E-Da Hospital and China Medical University Hospital in Taiwan. The study was completed at the Clinical and Translational Science Institute‘s Clinical Research Center at UCSF in compliance with the principles of the Declaration of Helsinki. This study was registered on the US

National Institutes of Health Clinical Trials Database (NCT02215174; https://clinicaltrials.gov/show/NCT02215174)

Patients

Eight female and one male patients, between the ages of 18-65, who were undergoing RYGB surgery were enrolled. Eligibility was determined by medical history, genotyping, and clinical laboratory evaluation in a screening visit. Ethnicity for both parents and all four grandparents was self-reported by the volunteers. All subjects whose results are reported here carried *SLCO1B1**1a and although not restricted the 8 female patients carried *ABCG2* c.421CC genotype. Subjects with known allergies to the study medications and a history of rhabdomyolysis, drug-related myalgia, severe liver and kidney dysfunction, hepatitis B, hepatitis C, and cancer were excluded. Subjects abstained from caffeinated drinks, alcohol, herbal tea, and grapefruit one day prior to the study. Medications known to have a drug-drug interaction with rosuvastatin were stopped three days prior to the study days. On the study days, the patient's current medications were not taken until 3-hour post rosuvastatin dosing.

Genotyping of *SLCO1B1* and *ABCG2* polymorphisms

DNA extraction from buccal swap samples was conducted using Buccal Swab DNA Mini Kit according to the manufacturer instruction (Geneaid, Taiwan)**.** To summarize, the swab was firmly scraped against the inside of each cheek between 15-20 times. Cell samples were dissolved into the sample preparation buffer and incubated for 20 min at 60˚C. Cells were lysed by the lysis buffer with carrier RNA and incubated for another 10 min. DNA was precipitated by adding absolute ethanol and binding to GD column membrane after flowing through the column provided by the manufacturer. The DNA binding membranes were washed twice with the wash

buffer and eluted after incubation with nuclease free water (ThermoFisher, Waltham, MA) for 3 min. DNA concentrations were verified by Nanodrop ND-1000 spectrophotometer (ThermoFisher, Waltham, MA).

Regions containing *SLCO1B1* c.388A>G, *SLCO1B1* c.521T>C and *ABCG2* c.421C>A were amplified using the following primers (Primer3 algorithm) on a 9700 thermal cycler (Applied Biosystems) with a touchdown PCR method. After the PCR, products were cleaned-up and sent to and sequenced by MCLAB (South San Francisco, CA).

SLCO1B1 rs2306283_F: 5'-AAACACATGCTGGGAAATTGAC-3'

SLCO1B1 rs2306283_R: 5'-TCATCCAGTTCAGATGGACAAA-3'

SLCO1B1 rs4149056_F: 5'- GCAGCATAAGAATGGACTAATACACC-3'

SLCO1B1 rs4149056_R : 5'-TCGCATGTGTGCTTAGAAAGAC-3'

ABCG2 rs2231142_F: 5'- TCATTGTTATGGAAAGCAACCA-3'

ABCG2 rs2231142_R: 5'- GGCAAATCCTTGTATGAAGCAG-3'

Study endpoints

Primary end points were rosuvastatin systemic exposure measured as area under the curve (AUC) from 0 to 48 hours ($AUC_{0\text{-}48}$) and 0 to infinity ($AUC_{0-\infty}$). Secondary outcomes were rosuvastatin peak plasma concentration, C_{max}, time to peak concentration, T_{max} as observed from concentration-time curve plots.

Surgical procedure

Gastric bypass was performed by three surgeons with a standardize procedure at UCSF and for the one patient in Taiwan by the surgeon following the same procedure. A 30mL gastric pouch was recreated and anastomosed to a 100cm Roux limb in an antecolic, antegastric fashion. The biliopancreatic limb (measured from the ligament of Treitz to the jejunojejunostomy) was about 50cm.

Plasma sample bioanalysis

Rosuvastatin concentrations were measured using a high-pressure liquid chromatography-tandem mass spectrometry method. The system consisted of API 5000 (AB Sciex, Redwood City, CA) with Shimadzu Prominence UFLC XR system using electrospray ionization in the positive mode. Rosuvastatin and the internal standard, carbamazepine, were separated on a XTerra RP18 3.5 µm 4.6x100 mm column with a guard column (Waters, Milford, MA) at 40˚C. The mobile phase was a combination of (A) double distilled water and (B) acetonitrile both with 0.1% formic acid. The gradient ran from 50% to 95% for 3.5 minutes. Ion detection was performed in the multiple reaction monitoring mode with Q1→Q3 transitions for rosuvastatin of 482.4→258.3 m/z, and carbamazepine of 237.0→194.3 m/z. Plasma samples, calibration curves, and quality controls (QCs) samples were prepared in the same way. The rosuvastatin method had a final lowest limit of quantitative concentration of 0.1 nM and upper limit of quantitative concentration of 1000 nM. The mean concentrations of QCs were within 15% of nominal concentrations and with coefficients of variation <15%.

Pharmacokinetic analysis

Rosuvastatin pharmacokinetic parameters were calculated from plasma concentration data by noncompartmental analysis using Phoenix® WinNonlin® (Pharsight, Mountain View, CA). The terminal rate constant (λ_z) was estimated by linear regression of the terminal phase of the log plasma concentration-time curve. $AUC_{0\text{-}48}$ was calculated by the linear up/logarithmic down trapezoidal method. $AUC_{0-\infty}$ was calculated by summation of AUC_{0-48} and the concentration at the last measured point divided by λ_z. Rosuvastatin T_{max} and C_{max} were obtained directly from observed data. MRT was calculated as area under the moment curve multiplied

by dose and divided by $AUC_{0-\infty}{}^2$ as previously reported.[41] The patient weights before and after RYGB surgery were not considered in calculating the pharmacokinetic parameters.

Statistical analysis

Using a paired t-test, the sample size was sufficient to detect a 20% difference in AUC_{0-48} between the two arms with a statistical power of 80%, alpha = 0.05, and standard deviation of 40%. Pharmacokinetic parameters were analyzed for differences between the two treatment periods by the paired t-test, except for AUC_{0-48}, $AUC_{0-\infty}$, and T_{max} where a Wilcoxon signed rank test was used.

3.7 Acknowledgements

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Chapter. 4 Conclusions

Statins are among the most commonly prescribed drug class in the United States. The introduction of statins has greatly improved LDL-c control over the past decades and led to reduction in cardiovascular disease. A recent review concluded that among 10,000 people taking statins, about 1000 heart attack or stroke cases are prevented and that for each 1 mmol/L reduction in LDL cholesterol using statin therapy after the first year, the risk of coronary deaths and CVD, such as heart attacks, strokes and coronary bypass procedures, is reduced by approximately 25%[1]. However, with the wide administering of statins, it is critical to determine the high-risk and low-risk patient group to statin side effects. The pharmacologic effect in the body are driven by pharmacokinetics, where factors affecting drug absorption, metabolism, distribution and elimination might negatively impact the utility of a drug either through sub-therapeutic drug exposure or toxicity. In addition, a recent NIH survey showed clinical studies in drug development have under-represented the minority groups in the US, as 98% of the clinical studies are conducted and reported in Caucasians. The clinical outcome from Caucasian was applied to different race group without further examining interethnic differences, potentially leading to high pharmacological variations. Therefore, it is the goal of this study to evaluate the contributing transporter effects to rosuvastatin pharmacokinetics in different ethnic groups, hopefully leading to a better dosing regimen and achieving therapeutical efficacy and safety.

As described in Chapters 2 and 3, OATP1B1 and BCRP are the two most well-studied transporters important for rosuvastatin pharmacokinetics. Reduced function in these two transporters often leads to clinically significant drug exposure

alterations and the need for dose adjustment accordingly. In Chapter 2, we evaluated OATP1B1 and BCRP contributions in altering rosuvastatin pharmacokinetics in both Asians and Caucasians. After genetically controlling for the two major transporters, we saw little interethnic variations in rosuvastatin pharmacokinetics, suggesting together OATP1B1 and BCRP play important roles in rosuvastatin pharmacokinetics. The two-fold AUC difference previously observed in Asians vs. Whites, leading to the recommendation of lower doses in Asians, was mitigated after controlling for the same *SLCO1B1* and *ABCG2* c.421 genotype. As a result, current FDA recommendation for half of the dose in Asians might result in sub-therapeutic effect in the wildtype OATP1B1 and *ABCG2*c.421 carriers in Asians.

In Chapter 3, we further investigated the intestinal absorption effect on rosuvastatin in Asians (Taiwan) and Caucasians (USA) in *SLCO1B1* and *ABCG2* c.421 wildtype carriers and completed the Caucasian arm. In our cohort, which consisted of 7 White and 1 Asian obese patients, no clinically significant change in rosuvastatin exposure post-RYGB surgery was noted, which suggests that the absorption in distal jejunum could compensate the bypassing duodenum and proximal jejunum for rosuvastatin absorption. Aligned with previous study by Skotteim et al., here we showed RYGB has no clinically significant effect on rosuvastatin pharmacokinetics. We recommend that rosuvastatin may be a preferred regimen for morbidly obese patients considering RYGB because no significant change in rosuvastatin exposure was observed. However, given the small sample size, further confirmatory studies will be warranted.

In conclusion, our studies shed light on the personalized dosing of rosuvastatin by characterizing two primary drug transporters, *SLCO1B1* and *ABCG2*. By genotyping the two genes, rosuvastatin pharmacokinetic variation could be

further reduced, and dose regimen could be approximated to achieve a target therapeutic effect in a more efficient manner, despite the previously reported ethnic differences. Also, for those who carry polymorphisms in those two transporters, it is critical to be aware of their higher rosuvastatin exposure. Therefore, a lower dose would be recommended in these subgroups for a better drug toxicity and disease management. We also showed that the rosuvastatin pharmacokinetics were similar between healthy and disease (morbidly obese) state, which should allow research studies carried out in healthy volunteers to be extrapolated to the obese patients. Last, we demonstrated that rosuvastatin may be a preferred statin for obese patients undergoing RYGB because no dose adjustment nor re-titration should be required.

4.1 Reference

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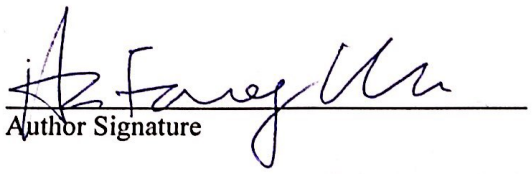

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