Elucidating Ethnic Variability in Rosuvastatin Pharmacokinetics: A Bioinformatics Approach

Low E.S.H.¹ and Lee E.²

Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore
10 Kent Ridge Road, Singapore 117546

ABSTRACT

Rosuvastatin exhibits ethnic variability in its pharmacokinetic properties and there is strong support for a genetic basis, though actual underlying mechanisms are currently unknown. The objective of this project is to elucidate the important genes and their SNPs that could possibly account for this ethnic variability, and ultimately to shortlist certain SNPs as candidates for further wet-lab research. This project focuses on Asian populations, and employs the use of a bioinformatics approach. BCRP, CYP3A4, OATP-C and MDR1 are the candidate genes examined in this project. Genes with a high number of significant SNPs that are prevalent in the population, determined by predictions from bioinformatics tools, are postulated to be the cause of ethnic variability in rosuvastatin pharmacokinetics, and should be earmarked for further wet-lab validation. SNPs predicted to be significant in OATP-C (rs11045818 and rs11045819) and BCRP (rs2725248) by bioinformatics tools are prime candidates for further research as it has been determined through cross-referencing with gene functional analyses and wet-lab findings that these genes play important roles in rosuvastatin PK, thus potentially the cause of ethnic variability. SNPs in MDR1 and CYP3A4 are regarded as of lower importance as these genes play minor roles in the pharmacokinetics of rosuvastatin.

INTRODUCTION

Background Information

Rosuvastatin is observed to exhibit ethnic variability in its pharmacokinetic (PK) properties (Lee et al., 2005). Such significant population differences are unlikely to be caused by differences in diet, weight etc, and there is strong support for a genetic basis (SNPs located in certain genes) for ethnic variability in rosuvastatin PK (Tirona, 2005), though actual underlying mechanisms remain unknown.

Many statins are metabolized by the cytochrome P450 system (Kim, 2004) Multiple transporters like OATP-C, OATP2B1 and OATP1B3 carry out the hepatic uptake of rosuvastatin. Others such as MDR1, BCRP and MRP2 are responsible for the biliary excretion of rosuvastatin (Kitamura et al., 2008). Currently, actual mechanisms underlying the observed ethnic variability in rosuvastatin PK are unknown, and SNPs located in the above-mentioned genes involved in rosuvastatin PK processes are prime candidates for further research. Understanding the exact PK mechanisms that are responsible for population-specific PK parameters is important as this would account for inter-individual differences in drug

¹ Student
² Senior Lecturer
responsiveness, which may possibly lead to development of clinical drug treatments that will maximise target specificity and minimise the risk of toxicity (Ho and Kim 2005).

The objective of this project is to employ the bioinformatics approach to elucidate the important genes and their SNPs that could possibly account for ethnic variability in rosuvastatin PK, with the focus placed on Asian populations. These SNPs are prime candidates for further wet-lab validations. Candidate genes of interest were narrowed down to 4 and analysed using bioinformatics tools: BCRP, CYP3A4, OATP-C and MDR1. Since the actual genes accounting for ethnic variability in rosuvastatin PK are unknown, the project’s approach would be to use bioinformatics tools to identify genes with a higher number of significant SNPs (that result in change in protein phenotype and function) that are prevalent in a specific population for further validation in the wet-lab, as they are more likely to be responsible for ethnic differences in rosuvastatin PK.

MATERIALS AND METHODS

Materials

Materials employed in this project were bioinformatics tools. Those used are namely F-SNP and an A*STAR-developed tool that is not publicly available yet.

Methods

Both F-SNP and A*STAR tools were used and results cross-referenced to sieve out biologically significant SNPs for each of the gene. For each significant SNP, allelic frequencies were obtained from the dbSNP database. SNPs with higher minor allelic frequencies are more prevalent and play a greater role in population genetic diversity. Genes with a high number of significant SNPs that are prevalent in the population are postulated to be the cause of ethnic variability in rosuvastatin PK.

RESULTS AND DISCUSSION

Results

Table 1. Biologically significant SNPs prevalent in Asian populations predicted by cross-referencing results from F-SNP and A*STAR tool for each gene

<table>
<thead>
<tr>
<th>Candidate Genes</th>
<th>Significant SNPs prevalent in Asian populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCRP</td>
<td>rs2725248</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>rs2242480, rs28988604 and rs6957392</td>
</tr>
<tr>
<td>OATP-C</td>
<td>rs2306283, rs11045818 and rs11045819</td>
</tr>
<tr>
<td>MDR1</td>
<td>rs1202166, rs7787569, rs11975994, rs1128503,</td>
</tr>
<tr>
<td></td>
<td>rs1922244, rs7802773, rs1016794,</td>
</tr>
<tr>
<td></td>
<td>rs2141849, rs1186745, rs6951067, rs4148743,</td>
</tr>
<tr>
<td></td>
<td>rs12154941, rs10274441, rs28746504, rs2373574,</td>
</tr>
<tr>
<td></td>
<td>rs17149864, rs10274623, rs2157930, rs12642844,</td>
</tr>
<tr>
<td></td>
<td>rs12640364, rs4956329, rs4956470, rs6835214,</td>
</tr>
<tr>
<td></td>
<td>rs17418297, rs13146721, rs923307 and rs10014443</td>
</tr>
</tbody>
</table>
Discussion

Based on the results generated by the A*STAR tool and F-SNP, it can be observed that MDR1 had the highest number of significant SNPs that were prevalent in the Asian populations, followed by OATP-C, CYP3A4 and BCRP. Due to its highest number of significant SNPs prevalent in Asian populations, MDR1 would be considered the most plausible cause of rosuvastatin PK’s ethnic variability for further validation. However, bioinformatics tools do not take into account the physiological functional roles of these genes in rosuvastatin PK processes. As such, results generated from bioinformatics tools must be regarded with care and cross-referencing must be done with gene functional analyses and wet-lab experiment results. This is to ensure a more accurate short-listing of appropriate SNPs for further wet-lab research, which is the ultimate aim of this project.

Experimental studies have shown that rosuvastatin is not a substrate for the MDR1 transporter (Cooper et al., 2002; Lee et al., 2005). In light of such findings, it is logical to conclude that the 27 SNPs found in MDR1 are not likely to be the cause of ethnic variability in rosuvastatin PK.

Since expression of OATP-C is limited to the basolateral membrane of hepatocytes, and it has broad substrate specificity (Kim, 2004), polymorphisms in OATP-C are very likely to affect hepatic drug elimination (Marzolini et al., 2004). OATP-C accounts for 43-55% of the total rosuvastatin uptake by hepatocytes (Kitamura et al., 2008), and is therefore essential to the PK processes of rosuvastatin. As shown by Puccetti et al. (2005), polymorphisms in OATP-C affect rosuvastatin PK in humans. This thus indicates that certain polymorphisms located in the gene may be the cause of observed ethnic variability. Results of an experimental study done by Lee et al. (2005) showed that 2 OATP-C SNPs, rs2306283 and rs4149056, were unable to account for the ethnic differences in PK parameters. Notably, SNP rs2306283 was predicted to be significant and prevalent in the Asian populations by the bioinformatics tools used in this project. It is recognised that current wet-lab validations are very limited in scope, and the bioinformatics approach is advantageous in providing new insights on plausible SNPs to be considered for future research. Thus since the SNP rs2306283 has been eliminated as the cause of ethnic variability in PK parameters, the other 2 SNPs in the bioinformatics results for OATP-C, rs11045818 and rs11045819, should be considered as prime candidates for further research in the wet-lab.

CYP3A4 is an important metabolising enzyme that is found in the liver. However, many experimental studies (Cooper et al., 2002) have shown that rosuvastatin is not metabolised through by CYP3A4 (Puccetti et al., 2007) as rosuvastatin undergoes minimal metabolism, and is largely excreted in its unchanged form (Kitamura et al., 2008; Lee et al., 2005). As a result, genetic polymorphisms found in CYP3A4 are less likely to affect rosuvastatin PK (Mangravite et al., 2006) and thus are not plausible causal agents for ethnic variability. Therefore, CYP3A4 SNPs (rs2242480, rs28988604 and rs6957392) generated by bioinformatics tools are not prime candidates for further research.

BCRP mediates the transmembrane transport of rosuvastatin (Tirona, 2005) during biliary excretion. An experimental study by Zhang et al. (2006) for BCRP SNP rs2231142 revealed that this SNP plays a major role in rosuvastatin PK in healthy Chinese males, indicating that the BCRP gene is possibly significant to the mechanism of ethnic differences in rosuvastatin PK. Once again, it is noted that wet-lab elucidations are very limited in scope currently, as only one SNP has been experimentally studied for BCRP so far. As such, the SNP, rs2725248, which is predicted to be significant and prevalent in Asian populations by bioinformatics tools, should be
 earmarked for further validation in the wet-lab. BCRP has already been experimentally proven to play an integral role in rosuvastatin PK and polymorphisms in BCRP would thus have great potential to be the underlying causes of ethnic variability in rosuvastatin PK.

REFERENCES


ACKNOWLEDGEMENTS

I would like to thank Professor Edmund Lee (Department of Pharmacology, Faculty of Medicine, NUS) and Dr James Mah (A*STAR Singapore) for their teaching and guidance.