The impact of *SLCO1B1* polymorphisms on the plasma concentration of lopinavir and ritonavir in HIV-infected men

Fabiana B. Kohlrausch, Rita de Cássia Estrela, Paulo F. Barroso¹ & Guilherme Suarez-Kurtz

Divisão de Farmacologia, Coordenação de Pesquisa, Instituto Nacional de Câncer and ¹Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Correspondence

Professor Guilherme Suarez-Kurtz,
Coordenação de Pesquisa, Instituto
Nacional de Câncer, Rua André Cavalcanti
37, Rio de Janeiro 21230-050, Brazil.
Tel: +55 21 3233 1310
Fax: +55 21 3233 1340
E-mail: kurtz@inca.gov.br

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- There is large interindividual variability in the pharmacokinetics of protease inhibitors (PIs) among human immunodeficiency virus (HIV)-infected individuals under highly active antiretroviral therapy.
- Protease inhibitor have been recently reported to be substrates of the *SLCO1B1*/OATP1 drug transporter.
- A single nucleotide polymorphism (SNP) in the *SLCO1B1* gene (521T→C) was associated with plasma levels of lopinavir in HIV-infected individuals.

WHAT THIS STUDY ADDS

- Data on the impact of three *SLCO1B1* SNPs (521T→C, 388A→G, 463C→A) on the trough plasma concentration of lopinavir and ritonavir in a cohort of 99 adult HIV-infected Brazilian men under stable highly active antiretroviral therapy.
- Evidence that carriers of the 521C allele display significantly higher lopinavir, but not ritonavir plasma concentrations relative to the wild-type TT genotype.
- No effect of either 388A→G or 463C→A SNPs on lopinavir or ritonavir plasma concentrations.
- Further studies are required to confirm the clinical significance of the association between the *SLCO1B1* 521T→C polymorphism and lopinavir pharmacokinetics.

AIMS

To investigate possible associations between three *SLCO1B1* single nucleotide polymorphisms (388A→G, 463C→A, 521T→C) and lopinavir/ritonavir plasma concentrations.

METHODS

The study included 99 human immunodeficiency virus-infected men on stable highly active antiretroviral therapy containing lopinavir/ritonavir. Trough concentrations of lopinavir and ritonavir in plasma were quantified using liquid chromatography–tandem mass spectrometry. Genotyping of *SLCO1B1* 388A→G, 463C→A and 521T→C polymorphisms was performed by allelic discrimination using real-time polymerase chain reaction.

RESULTS

The trough concentration of lopinavir in plasma is significantly associated with *SLCO1B1* 521T→C genotypes ($P = 0.03$). There is a significant trend for increasing concentrations of lopinavir from TT to TC to CC genotypes ($P = 0.02$). Carriers of the 521C allele display significantly higher lopinavir plasma concentrations relative to the wild-type TT genotype ($P = 0.03$).

CONCLUSIONS

Reduced uptake of lopinavir by hepatocytes in carriers of the 521C allele may account for these results, but further studies to confirm the clinical importance of *SLCO1B1* polymorphisms in lopinavir pharmacokinetics are warranted.

Introduction

Despite the remarkable success of highly active antiretroviral therapy (HAART) in reducing human immunodeficiency virus (HIV) morbidity and mortality worldwide, considerable interindividual variability in patient outcome is still observed. Part of this variability has been associated with pharmacogenetic factors affecting drug disposition and response [1, 2]. Regarding protease inhibitors (PIs), which are an integral component of HAART and the focus of the present communication, pharmacogenetic studies have focused mainly on *CYP3A* and *ABCB1* polymorphisms, with conflicting results [3–5]. Novel pharmacogenetic targets modulating the pharmacokinetics of PIs have been recently identified within the organic anion transporting polypeptide (OATP/*SLCO*) family. Kwan *et al.* [6] reported that lopinavir and other PIs are substrates of *SLCO1B1* and *SLCO1A2*. Shallcross *et al.* [7] observed that a relatively common single nucleotide polymorphism (SNP) in the *SLCO1B1* gene, namely 521T→C [8, 9], is associated with the plasma levels of lopinavir in HIV-infected individuals under HAART. The availability of plasma concentration data for lopinavir and ritonavir from a cohort of HIV-infected men under stable HAART, previously enrolled in a pharmacogenetics study of *ABCB1* polymorphisms [10], provided the opportunity to seek independent confirmation of the results reported by Shallcross *et al.* [7] and to extend their observations in two directions: first, by examining two other *SLCO1B1* SNPs (388A→G, 463C→A), and second, by exploring the association of the *SLCO1B1* polymorphisms with ritonavir plasma concentrations.

Methods

The present analyses are based on data from 99 HIV-infected men on stable HAART therapy containing lopinavir/ritonavir for at least 4 weeks, recruited at the Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro, Brazil. Details of the study protocol have been previously published [10]. The study was approved by the hospital Ethics Committee and each subject provided written informed consent. The subjects were categorized according to the Brazilian Census, which relies on self-perception of ‘race/colour’ as *branco* (white, *n* = 47), *pardo* (‘brown’ = 42) and *preto* (black, *n* = 10). The trough (predosing) concentrations of lopinavir and ritonavir in plasma were quantified using liquid chromatography–tandem mass spectrometry [10]. Genotyping of *SLCO1B1* 388A→G, 463C→A and 521T→C polymorphisms was performed by allelic discrimination using real-time polymerase chain reaction TaqMan® assays (Applied Biosystems, Foster city, CA, USA). For the SNPs 388A→G (rs2306283) and 521T→C (rs4149056) validated assays were purchased from Applied Biosystems, whereas a custom assay for the 463C→A (rs11045819) was devel-

Table 1

Allele and genotype frequencies of *SLCO1B1* polymorphisms in 99 HIV-infected Brazilian men

Polymorphism	Genotype			Allele	
388A→G	GG	GA	AA	G	A
	31 (0.32)	41 (0.42)	26 (0.26)	0.53	0.47
463C→A	AA	CA	CC	A	C
	3 (0.03)	19 (0.20)	74 (0.77)	0.13	0.87
521T→C	CC	TC	TT	C	T
	3 (0.03)	22 (0.22)	73 (0.75)	0.14	0.86

oped with primers and probes as follows: forward primer 5'-ACATCGACCTTATCCACTTGTTTAATTAATCA-3', reverse primer 5'-AATGTATACATTTTAGAAGACTTTTACTGTCAAT-3'; probe 463A 5'-CACTATCTCAGTTGATGCT, and probe 463C 5'-ACTATCTCAGGTGATGCT-3'.

Results

The allele frequency and genotype distribution of the *SLCO1B1* polymorphisms in the study population are shown in Table 1. The genotype frequencies at each locus did not deviate from expected Hardy–Weinberg proportions. We observed no statistically significant association (Kruskall–Walis test) between the trough concentrations of lopinavir or ritonavir in plasma and either 388A→G or 463C→A polymorphisms (data not shown). However, the distribution of the trough concentrations of lopinavir (Figure 1), but not ritonavir, was statistically associated with the 521T→C genotypes (Kruskal–Wallis test, *P* = 0.03). A significant trend for increasing concentrations of lopinavir from TT (median 6133 ng ml⁻¹; 95% confidence interval 5502, 6977) to TC (7094; 6219, 8143) to CC (9123; 7604, 13 355) genotypes was observed (Jonckheere–Terpstra test, *P* = 0.02). The small number (*n* = 3) of individuals with the 521CC genotype precluded pair-wise comparisons between this and the other two genotypes at the same locus. Thus, for pair-wise comparison between genotypes we combined homozygous and heterozygous carriers of the variant 521C allele. This showed that trough concentrations of lopinavir in the plasma of 521C carriers were significantly higher relative to the wild-type genotype (Mann–Whitney test, *P* = 0.03). No association was observed between the trough concentrations of lopinavir and ritonavir in plasma and demographic variables such as age, weight or ‘race/colour’ of the enrolled individuals.

Discussion

Collectively, the present observations are consistent with the original report of Shalcross *et al.* [7], in that patients

Figure 1

Scatter plot of the distribution of trough concentrations of lopinavir in blood plasma among 99 HIV-infected subjects, according to *SLCO1B1* 521T→C genotypes. The lines denote the median values for each genotype

with the 521T→C polymorphism displayed higher lopinavir exposure than those with wild-type genotype. We could not verify the previously reported higher plasma concentration of lopinavir in CC individuals compared with either CT or TT [7], because of our small numbers of the CC genotype.

The OATP/SLCO family represents an important class of drug uptake transporters that mediate the sodium-independent transport of a diverse range of amphipathic organic compounds, including the PIs [6]. In humans, OATP1B1 is predominantly expressed at the sinusoidal membrane of hepatocytes, and the 521T→C polymorphism has been consistently associated with reduced transport activity *in vivo* [11, 12], leading to reduced uptake by the hepatocytes and higher plasma levels of different classes of therapeutic drugs, such as hypocholesterolaemic statins and meglitinide analogue hypoglycaemics [12–14]. A similar mechanism may account for the increased trough concentration of lopinavir in the plasma of *SLCO1B1* 521C, observed in our cohort and previously reported by Shallcross *et al.* [7]. The clinical usefulness of this information is uncertain at present, in view of both the extensive overlap of the trough concentrations of lopinavir in the plasma across the three 521T→C genotypes and the low prevalence (<5%) of the homozygous variant genotype (521CC), observed in our study as well as by Shallcross *et al.* [7]. We concur with the latter authors that further studies to confirm the importance of *SLCO1B1* polymorphisms in lopinavir pharmacokinetics are warranted.

Competing interests

None to declare.

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