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Original article

Correlation between *UGT1A1* polymorphisms and raltegravir plasma trough concentrations in Japanese HIV-1-infected patients

Hiroki Yagura [a], Dai Watanabe [b, *], Misa Ashida [b], Hiroyuki Kushida [a], Kazuyuki Hirota [b], Motoko Ikuma [b], Yoshihiko Ogawa [b], Keishiro Yajima [b], Daisuke Kasai [b], Yasuharu Nishida [b], Tomoko Uehira [b], Munehiro Yoshino [c], Takuma Shirasaka [b]

[a] Department of Pharmacy, National Hospital Organization Osaka National Hospital, Japan
[b] AIDS Medical Center, National Hospital Organization Osaka National Hospital, Japan
[c] Department of Pharmacy, National Hospital Organization Himeji Medical Center, Japan

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ABSTRACT

Raltegravir (RAL), an HIV integrase inhibitor, is metabolized mainly by UDP-glucuronosyltransferase 1A1 (UGT1A1). Polymorphisms in *UGT1A1* may cause alterations in the pharmacodynamics of RAL, which is taken twice daily with no dietary restrictions. We compared the effect of two polymorphic alleles in this gene, *UGT1A1*6* and *UGT1A1*28* on plasma RAL concentrations in Japanese HIV-1-infected patients. Of 114 Japanese HIV-1-infected patients who received RAL, the frequencies of *UGT1A1*6* and *UGT1A1*28* were 18% and 13%, respectively. The percentage of homozygotes for *UGT1A1*6* and *UGT1A1*28* was 6% and 4%, respectively, the percentage of compound heterozygotes for *UGT1A1*6* and *UGT1A1*28* was 2%, and that of heterozygotes for *UGT1A1*6* and *UGT1A1*28* was 22% and 17%, respectively. RAL plasma trough concentrations were compared for each polymorphism. Significantly higher levels of RAL were observed with patients who were homozygous for *UGT1A1*6* (median: 1.0 μg/mL) than for the normal allele (median: 0.11 μg/mL; $p = 0.021$). Multivariate logistic regression analysis showed that the presence of one or two alleles of *UGT1A1*6* or two alleles of *UGT1A1*28* were independent factors associated with high RAL plasma trough concentrations (≥0.17 μg/mL). These results indicated that *UGT1A1*6* and *UGT1A1*28* are both factors influencing the RAL plasma trough concentrations in Japanese HIV-1-infected patients.

1. Introduction

Raltegravir (RAL) is a potent HIV integrase inhibitor, and its efficacy and safety during long-term administration have been demonstrated in clinical trials [1,2]. Major guidelines recommend RAL as a key drug for initial treatment of HIV-1 infection [3,4]. RAL has fewer drug interactions than other anti-HIV drugs, because it has neither inhibitory nor inductive effects on cytochrome P450 (CYP), a drug-metabolizing enzyme, and is metabolized mainly by UDP-glucuronosyltransferase 1A1 (UGT1A1) [5].

UGT1A1 is mainly expressed in the liver, and is involved in bilirubin glucuronidation and drug metabolism. Several *UGT1A1* polymorphisms have been correlated with *UGT1A1* expression levels. Six TA repeats are present in the TATA box of the *UGT1A1* promoter. The *UGT1A1*28* allele involves an additional TA repeat, resulting in decreased *UGT1A1* mRNA and protein expression [6]. Allele frequencies of *UGT1A1*28* vary with race and are relatively high in Caucasian and African-American populations [7].

Another *UGT1A1* polymorphism, *UGT1A1*6* (211G > A), a single nucleotide polymorphism in exon 1, also decreases UGT1A1 enzymatic activity. Allele frequencies of *UGT1A1*6* also vary with race, and are low in Western populations but high in Asian populations [8].

Effects of *UGT1A1*6* and *UGT1A1*28* on RAL plasma trough concentrations have only been described in a few reports [9,10]. The *UGT1A1*6* polymorphism has been less well studied, because it is

* Corresponding author. AIDS Medical Center, National Hospital Organization Osaka National Hospital, 2-1-14 Hoenzaka, Chuo-ku, Osaka 540-0006, Japan. Tel.: +81 6 6942 1331; fax: +81 6 6946 3652.
E-mail address: dai@onh.go.jp (D. Watanabe).

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less common in Western populations. Here, we examined the association between the *UGT1A1*6* and *UGT1A1*28* polymorphisms and RAL plasma trough concentrations in Japanese HIV-1-infected patients. Because these polymorphisms decrease UGT1A1 enzymatic or gene activity, we predicted that their presence would decrease RAL metabolism, resulting in elevated RAL plasma trough concentrations.

2. Patients & methods

2.1. Patients

This study was reviewed and approved by the Institutional Review Board of National Hospital Organization Osaka National Hospital (approval number: 0838). Japanese HIV-1-infected patients aged ≥20 years and receiving RAL therapy at the National Hospital Organization Osaka National Hospital were included. Samples were collected after obtaining written informed consent. Patients were interviewed regarding adherence to RAL treatment, and those showing poor adherence were excluded. The exclusion criteria were as follows: co-administration with a potent inhibitor of UGT1A1 (atazanavir), potent inducers of UGT1A1 (rifampicin, carbamazepine, phenytoin, and phenobarbital), and other drugs known to influence RAL plasma concentration (aluminum- and/or magnesium-containing antacids, proton pump inhibitors, and histamine H2-receptor antagonists).

2.2. UGT1A1 genotyping

Saliva samples were collected using a cotton swab, absorbed to filter paper (FTA Micro Card, GE Healthcare, Little Chalfont, UK), and allowed to dry. A piece of the filter paper was removed and used as a template for PCR (Ampdirect Plus, Shimadzu, Kyoto, Japan). The promoter region and exon 1 of *UGT1A1* were amplified by nested PCR to determine the presence or absence of gene polymorphisms by direct sequencing. Sequences of the primers used for PCR and sequencing are shown in Table 1.

2.3. RAL plasma trough concentrations

Blood samples were collected at 11–13 h after oral administration of RAL under repetitive administration for 14 days or more. Samples were centrifuged at 3000 rpm for 10 min to separate 2 mL of plasma. The plasma was stored at −80 °C until required for analysis. Plasma RAL concentration was measured by reverse phase-high performance liquid chromatography, as previously reported [11].

2.4. Statistical analysis

The Wilcoxon rank sum test was used for multiple comparisons, and significant results were further analyzed using the Steel–Dwass post-hoc test. Factors influencing RAL plasma trough concentrations were examined by multivariate logistic regression analysis. Patients were classified into two groups based on the median RAL plasma trough concentration (0.17 μg/mL). The following were used as variables for analysis with the forced-entry method: age (≥40 y), body weight (<60 kg), estimated glomerular filtration rate (<80 mL min^{-1} 1.73 m^{-2}), as calculated by a modified version of the Modification of Diet in Renal Disease formula of the Japanese Society of Nephrology [12], detectable HIV-1-RNA levels (≥20 copies/mL) after 24 weeks of RAL treatment, and allele frequencies of *UGT1A1*6* and *UGT1A1*28*. A hazard ratio of 5% was considered significant. Statistical analyses were conducted using JMP (version 10.0.0, SAS Institute).

3. Results

3.1. Frequencies of UGT1A1 polymorphisms in Japanese HIV-1-infected patients

The frequencies of alleles of the *UGT1A1* polymorphisms in 114 HIV-1-infected patients who participated in this study are shown in Table 2. Six percent of cases (n = 7) were homozygous for *UGT1A1*6*, 2% of cases (n = 2) were compound heterozygous for *UGT1A1*6* and *UGT1A1*28*, and 22% of cases (n = 25) were heterozygous for *UGT1A1*6*. Gene frequencies for *UGT1A1*6* and *UGT1A1*28* were 18% and 13%, respectively. The general characteristics of the HIV-1-infected patients with the different *UGT1A1* polymorphisms are shown in Table 2. The percentage of participants with undetectable HIV-1-RNA levels (<20 copies/mL) at the time of sampling for *UGT1A1* genotyping was significantly different (p = 0.0432). However, 20 patients (18%) had RAL treatment with a duration of 30 days or less at the time of sampling. To clarify the association between the antiviral effect and *UGT1A1* polymorphisms, the percentage of participants with undetectable HIV-1-RNA levels was compared at 24 weeks after the initiation of RAL treatment. No resistant mutation to RAL was found. Two patients stopped RAL treatment within 24 weeks due to liver dysfunction or at the patient's request. These patients were both heterozygous for *UGT1A1*6*, and RAL plasma trough concentrations were 0.171 μg/mL and 0.184 μg/mL, respectively. These patients were excluded from the analysis after 24 weeks of RAL treatment. At 24 weeks of RAL treatment, there was no significant difference among the different *UGT1A1* polymorphism groups. Use of co-administrated antiretroviral agents did not differ significantly.

3.2. Correlation between UGT1A1 polymorphisms and RAL plasma trough concentrations

RAL plasma trough concentrations were classified according to gene polymorphism and compared between the groups (Fig. 1; Wilcoxon rank sum test, p = 0.0046). RAL plasma trough concentrations in patients homozygous for *UGT1A1*6* (median: 1.0 μg/mL) were significantly higher than the normal allele (−/−, median: 0.11 μg/mL; p = 0.021). RAL plasma trough concentrations of patients homozygous for *UGT1A1*28* (median: 0.28 μg/mL),

Table 1
Primers for amplification and sequencing of *UGT1A1* polymorphisms.

	Direction	*UGT1A1* polymorphism	Sequence
1st amplification	Forward	*6 and *28	5′-ttcactacatagtcgtccttcttcc-3′
1st amplification	Reverse	*6 and *28	5′-aattgattactatccactggagcac-3′
2nd amplification and sequencing	Forward	*28	5′-tgagtatgaaattccagccagtt-3′
2nd amplification and sequencing	Reverse	*28	5′-ccactgggatcaacagtatcttc-3′
2nd amplification and sequencing	Forward	*6	5′-atataagtaggagagggcgaacct-3′
2nd amplification and sequencing	Reverse	*6	5′-agacaaaagcatagcagagtcctt-3′