Development of a Simple Method for Detection of the HLA-A*31:01 Allele

Kazuki UCHIYAMA 1, Fumika KUBOTA 1, Noritaka ARIYOSHI 1,2,*, Jun MATSUMOTO 1, Itsuko ISHIH 1,2 and Mitsukazu KITADA 1,2

1Department of Clinical Pharmacology, Faculty of Pharmaceutical Sciences, Chiba University, Chiba, Japan
2Division of Pharmacy, University Hospital, Chiba University School of Medicine, Chiba, Japan

Note

Summary: It is known that rare but severe cutaneous adverse drug reactions (cADRs), such as Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DIHS), are induced by carbamazepine (CBZ). Recent studies have shown an association between HLA-A*31:01 and CBZ-induced severe cADRs in Japanese and Caucasian populations. In this study, we developed a simple method to detect the HLA-A*31:01 allele by nested allele-specific primer-polymerase chain reaction combined with restriction fragment length polymorphism analysis. Accuracy of the developed method was evaluated by direct sequencing analysis of PCR products amplified from DNA samples with known HLA-A genotypes and by consigning diagnosis of DNA samples with unknown HLA-A genotypes to a company providing clinical laboratory testing. The method developed in this study is simple, rapid, and of low cost compared to outsourcing tests and may be useful for in-house testing of the HLA-A*31:01 allele.

Keywords: HLA genotyping; carbamazepine; idiosyncratic adverse drug reaction; HLA-A*31:01; Stevens-Johnson syndrome; CBZ

Introduction

Cutaneous adverse drug reactions (cADRs) are known to be induced by a number of drugs including anticonvulsants, antibiotics and non-steroidal anti-inflammatory drugs. Carbamazepine (CBZ) is a widely prescribed drug for the treatment of epilepsy as well as trigeminal neuralgia and bipolar disorder. However, CBZ is known to be one of drugs that cause idiosyncratic cADRs, which are characterized as dose-independent, unpredictable and sometimes life-threatening acute inflammatory reactions of skin and mucous membranes. Stevens-Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DIHS) are known as severe idiosyncratic cADRs. SJS is associated with a mortality rate of 5%, and TEN is a more extensive skin detachment with mortality rates of 25 to 35%. Although detailed mechanisms underlying the development of these severe idiosyncratic cADRs are not yet clear, recent studies have demonstrated that some specific types of human leukocyte antigen (HLA) are associated with the onset of SJS/TEN. First, it was demonstrated that HLA-B*15:02 was strongly associated with SJS/TEN induced by CBZ in the Han-Chinese population. Subsequently, this association was confirmed in people from Hong Kong, Malaysia, Thailand, and India and in descendants of immigrants from Southeast Asia. In fact, HLA-B*15:02 genotyping prior to CBZ administration could be applied for prediction and prevention of SJS/TEN. However, the prevalence of the HLA-B*15:02 allele is only 0.1% in Japanese compared to that in Southeast Asians (8.6%), suggesting that another HLA allele is responsible for SJS/TEN induced by CBZ in the Japanese population. More recently, a genome-wide association study (GWAS) revealed an association between the HLA-A*31:01 allele and CBZ-induced SJS/TEN in Japanese and also in Caucasians. Thus, HLA-A genotyping prior to CBZ administration is expected to be useful for preventing CBZ-induced SJS/TEN. However, HLA genotyping provided by laboratory testing service companies is expensive because it is not yet covered by medical insurance in Japan. Moreover, precise HLA genotyping conducted frequently in the field of transplantation is a labor- and time-consuming assay.

Here we propose a simple method that focuses on clarifying the existence of a specific HLA allele of interest. This new concept of HLA detection allows quick and low-cost in-house testing for the HLA-A*31:01 allele, which may be responsible in part for CBZ-induced SJS/TEN in Japanese and Caucasians.

Materials and Methods

Human genomic DNA samples: Genomic DNA was isolated from whole blood donated by unrelated Japanese healthy volunteers. This study was approved by the Life-Ethics Committee of the Department of Clinical Pharmacology, Faculty of Pharmaceutical Sciences, Chiba University.

Received December 2, 2012; Accepted February 3, 2013

J-STAGE Advance Published Date: February 12, 2013, doi:10.2133/dmpk.DMPK-12-NT-136

*To whom correspondence should be addressed: Noritaka ARIYOSHI, Ph.D., Division of Pharmacy, University Hospital, Chiba University School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8677, Japan. Tel. +81-43-226-2208, Fax. +81-43-226-2208, E-mail: ariyoshi@ho.chiba-u.ac.jp
the Graduate School of Medicine, Chiba University (approval No. 5–24), and written, informed consent was obtained from all volunteers.

Detection of the HLA-A*31:01 allele by the nested allele-specific PCR-RFLP method: The primer set (forward: HLA-AF 5'-GAGGTTGGCGRGGTCTCAAGCCA-3' and reverse: HLA-AR 5'-GGGYYATATTCTAGGTGTGTCCTAATTGT-3') for the first PCR was designed to amplify specifically from the HLA-A locus. The first PCR was performed in a reaction mixture of 25 μL containing 50 ng of genomic DNA, 0.625 units of Prime STAR GXL (Takara Bio, Otsu, Japan), 1.0 mM MgCl₂, and 0.2 mM dNTPs with 0.2 μM of each primer. PCR conditions were 94°C for 3 min, followed by 25 cycles of 98°C for 10 s, and 68°C for 30 s. PCR products were visualized by ethidium bromide under UV irradiation with 1.5% (w/v) agarose gel electrophoresis.

The first PCR products were then diluted 20 times with sterilized milliQ water and used as a template for the second nested allele-specific PCR. Primer set A (forward: A3101-F1 5'-CCA-CCTCCATGAGGTATTTCA-3' and reverse: A3101-R 5'-CTCGGC-TCTGGTTGTTAGTAG-3') was designed to amplify specifically from HLA-A*31:01 or HLA-A*33:03, the latter of which is highly homologous to the HLA-A*31:01 allele. Primer set B (forward: A3101-F2 5'-YCACTTCCATGAGGTATTTCA-3' and reverse: A3101-R) was designed to amplify from HLA-A alleles other than HLA-A*31:01 and HLA-A*33:03. The second PCR was carried out in a reaction mixture of 25 μL containing 1 μL of diluted first PCR product, 1.0 units of EX-Taq Hot Start version (Takara Bio), 1.5 mM MgCl₂, and 0.2 mM dNTPs with 0.2 μM of each primer. The second PCR conditions were 94°C for 3 min, followed by 30 cycles of 95°C for 20 s, 63°C for 10 s, and 72°C for 10 s. Amplification of DNA fragments was confirmed by 2.5% (w/v) agarose gel electrophoresis.

An aliquot (5 μL) of the second PCR products were then digested with 0.4 units of Stu I (New England Biolabs, Beverly, MA) in the presence of 1× Buffer 4 (New England Biolabs) at 37°C for 3 h. Restriction fragment length polymorphism (RFLP) analyses were performed by 2.5% (w/v) agarose gel electrophoresis.

Direct sequencing analysis: DNA sequences were determined using a BigDye Terminator Cycle Sequencing Kit ver.1.1 (Applied Biosystems, Foster City, CA) and an ABI Prism 310A Genetic Analyzer (Applied Biosystems) according to the manufacturer’s instructions.

HLA genotyping: HLA-A genotypes were analyzed by the HLA Laboratory (Kyoto, Japan).

Results and Discussion

Figure 1 shows a brief strategy of detection of the HLA-A*31:01 allele. The first PCR was conducted to separate HLA-A genes from other HLA genes and selectively amplify regions from exon 2 to exon 3, where the sequences are highly variable, in the HLA-A gene. In order to isolate the HLA-A*31:01 allele from other HLA-A alleles, nested allele-specific PCR was performed by using A3101-F1 and A3101-R primers. However, PCR with these primers amplifies the product from not only the HLA-A*31:01 allele but also the HLA-A*33:03 allele, because both alleles possess adenine at the corresponding position for the 3’-end of the A3101-F1 primer.

On the other hand, another allele-specific PCR by using A3101-F2 and A3101-R primers amplifies products from major HLA-A alleles other than HLA-A*31:01 and HLA-A*33:03. As shown in Supplementary Figure 1, by being used with A3101-R, A3101-F1 amplifies a gene from HLA-A*31:01 and A*33:03 but not from the other 12 alleles, whose allele frequencies are over 0.4% (ranging from 0.40 to 35.94%) in the Japanese population (n = 21,705, http://www.hla.or.jp/haplo/haplo_search.php?type=arlil&loci=A&lang=ja). On the other hand, another allele-specific PCR (A3101-F2) gives amplicons from the 12 HLA-A alleles found commonly in the Japanese population but not from HLA-A*31:01 or A*33:03. Consequently, the second allele-specific PCR gives one of three patterns (I, II or III) as shown in Figures 1 and 2b. To distinguish between the HLA-A*31:01 and HLA-A*33:03 alleles, restriction digestion with Stu I followed by RFLP analyses was conducted when pattern I or II was observed. For instance, if pattern I was observed, there are three possibilities, namely, as shown in Figure 1, the individual is homozygous for the HLA-A*31:01 (I-i), homozygous for the HLA-A*33:03 allele (I-ii) or compound heterozygous for the HLA-A*31:01 and HLA-A*33:03 alleles (I-iii). Pattern II, on the other hand, indicates that the individual has either one HLA-A*31:01 (II-i) or one HLA-A*33:03 allele (II-ii). The remaining pattern III indicates that no HLA-A*31:01 exists in the individual.

Six genomic DNA samples isolated from Japanese with known HLA-A genotypes were examined (Fig. 2). After the first PCR, a single band with the size of 929 bp derived from the HLA-A gene was amplified from all samples (Fig. 2a). As shown in Figure 2b, three samples showed pattern I, two samples showed pattern II, and one sample showed pattern III. No band was found in the left lane for the sample with HLA-A*11:01/*24:02, as expected. RFLP analyses of the second PCR products amplified from 5 samples support the validity of the developed method.

In addition to the RFLP analysis, direct sequencing analysis was carried out to further confirm accuracy of the developed method. Supplementary Figure 2 shows sequences of the second
PCR products: left lanes of samples No. 1 (*31:01 homo) and No.3 (*31:01 hetero), right lane of sample No. 6 (no *31:01) in Figure 2b. The sequences obtained from the remaining three samples (No. 2, 4 and 5 in Fig. 2b) were completely in agreement with the expected sequences (data not shown).

Finally, we applied this method to detect the HLA-A*31:01 allele in other samples, for which HLA genotypes were unidentified. Supplementary Figure 3 and Table 1a show the results obtained from our analysis. These results were subsequently compared with those from the company providing clinical laboratory testing (Supplementary Table 1b). This comparison further confirmed that the method developed in the present study can accurately detect the HLA-A*31:01 allele.

The new method is superior to outsourcing examination in terms of time and cost. In this study, for example, it took 5 days to obtain results of HLA-A genotypes for 10 samples from the outsourcing company, whereas it took only 5 h to learn how many individuals possessed the HLA-A*31:01 allele. Moreover, the cost for consumables and reagents for the present method was $2.27 per sample, whereas total cost for outsourcing test by the company used was $136 per sample. More importantly, anyone can perform the assay without extensive training, because the techniques we adopted in this method are extremely basic. Recently, Aoki et al.17) reported a new genotyping method for the HLA-A*31:01 allele. The method used an InvaderPlus® technique, which allows PCR followed by an Invader reaction in a single tube, leading to reduced handling and incubation time for Invader reaction. However, this method requires unique reagents including a fluorescence resonance energy transfer (FRET) cassette and Cleavase® enzyme, which are not commercially available. On the other hand, all reagents needed for the present method are inexpensive and commercially available. Furthermore, the required instruments are a thermal cycler and an apparatus for electrophoresis that is remarkably cheaper than a real-time PCR system, which is necessary for the InvaderPlus® assay. Conversely, compared with the InvaderPlus® assay, the disadvantage of the present method is the longer turn-around time, which includes the process of transferring PCR product to another tube for dilution or restriction digestion. However, because the number of samples to be diagnosed at the same time is usually one or two in the clinical setting, substantial...
handling time is short and the risk of taking the wrong sample is virtually low. Another limitation of the present method may be application to other ethnic populations. Since HLA-A*31:01 is one of the promising biomarkers for CBZ-induced severe cADRs not only in Japanese but also Caucasians, usefulness of the present method in Caucasians must await a future study.

In conclusion, we have developed a simple and rapid method for detection of the HLA-A*31:01 allele, which is thought to be responsible in part for CBZ-induced severe cADRs. The method is expected to be useful for a prospective clinical trial to determine the significance of HLA typing prior to CBZ treatment and/or could be useful for in-house pre-screening for patients who are scheduled to receive CBZ administration.

References