To the Editor:
Cytochrome P450 2B6 (CYP2B6) metabolizes several clinically important drugs, such as cyclophosphamide (1). Eight variants, CYP2B6*2 through *9 have been reported to date, and assays combining PCR and restriction fragment length polymorphism (RFLP) analysis have been developed for genotyping of CYP2B6*1 through *7 (2, 3). We previously performed PCR-RFLP assays for genotyping of CYP2B6*1 through *7 in 147 Japanese volunteers and found an individual heterozygous for the G516T, A785G, and C1459T mutations in the CYP2B6 gene. The individual was classified

Fig. 1. Protocol for haplotype determination to distinguish CYP2B6*1/*7 and *5/*6 using PCR and RFLP methods. Exons containing mutations encoding amino acid changes are shown as filled boxes; exons encoding the wild-type amino acid sequence are shown as open boxes. Open arrows connected by lines show PCR products that were amplified by the primers.

An individual heterozygous for G516T, A785G, and C1459T mutations in CYP2B6 gene

Step 1: long-PCR amplification of exon 4-9

Step 2; allele specific PCR amplification of CYP2B6*1 and *5

Step 3; Alw I-RFLP analysis for detecting CYP2B6*5-related C1459T mutation

1067-bp fragment

1286-bp fragment

digestion

non-digestion
as CYP2B6*1/*7 or *5/*6, but we could not determine the CYP2B6 genotype of this individual because the PCR-RFLP assays failed to distinguish the two genotypes. An individual classified as CYP2B6*1/*7 or *5/*6 has also been reported by other authors (4). It is necessary to distinguish CYP2B6*1/*7 and *5/*6 because CYP2B6*5/*6 and *7 have been associated with changes in the production and function of CYP2B6, respectively (2, 4). In haplotype determination, a cloning method is usually performed, but this is time-consuming. We therefore developed a method for rapid haplotype determination based on PCR to distinguish CYP2B6*1/*7 and *5/*6.

The local Institutional Review Board approved this study, and informed consent for participation in this study was obtained from all volunteers.

Primers CYP2B6-A516G (5’-ACC CCA CCT TCC TCT AGC AG-3’) and CYP2B6-9R-II (5’-GGA TGA GAC CTC-3’) were designed on the basis of the published sequence (5). A GeneAmp® PCR System 9700 (Applied Biosystems) was used for PCR amplifications. In step 1 (Fig. 1), the following mixture was prepared for the long PCR: 15.75 μL of water, 2.0 μL of 10× NEBuffer 4, 1.0 μL of AlwI (5 U/μL; New England Biolabs), and 10 μL of the allele-specific PCR product. The AlwI RFLP mixture was incubated at 37 °C for 2 h. The resulting fragments were identified on a 2% agarose gel stained with ethidium bromide.

In the AlwI RFLP analysis, the allele-specific PCR products derived from CYP2B6*1 and *5 yielded 1067- and 1286-bp fragments, respectively. Our results showed that the 1286-bp fragment was from the individual heterozygous for the G516T, A785G, and C1459T mutations of the CYP2B6 gene, which indicated that the CYP2B6 genotype of this individual was CYP2B6*5/*6.

In conclusion, we have established a method for haplotype determination based on PCR that enables CYP2B6*1/*7 and *5/*6 to be distinguished. This method, which is suitable for routine assay of CYP2B6 genotype, takes ~10 h and is faster than a cloning method.

References

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Interference by Gelatin-Based Plasma Substitutes in Capillary Zone Electrophoresis

To the Editor:
Analysis of serum proteins by capillary zone electrophoresis (CZE) is used to detect dysproteinemias and monoclonal proteins [reviewed in (1)]. Most interferences in this method are caused by interference with ultraviolet detection [reviewed in (2)]. For example, radio-opaque contrast agents and, to a lesser extent, some antibiotics absorb at 200–214 nm and produce distinct peaks on the electropherogram. A gelatin-based plasma substitute has also been reported to produce an increase in the γ-region in a polyclonal-like way (3). We describe the systematic evaluation of commercially available synthetic colloidal plasma substitutes for their potential to interfere with serum protein analysis by CZE.

We used the Paragon 2000® (Beckman Coulter) and the Capillarlys® (Sebia) CZE systems. The former uses 214 nm and the latter 200 nm as detection wavelength. The Hydragel® (Sebia) agarose gel electrophoresis (AGE) system was used for comparison.

Synthetic colloidal plasma substitutes can be divided into products based on dextran, starch, or gelatin (4). We studied a dextran-based