Correlation between Acetylator Phenotypes and Genotypes of Polymorphic Arylamine N-Acetyltransferase in Human Liver*

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Southern blot analysis was performed with genomic DNAs from 86 human subjects using the 32P-labeled cDNA for polymorphic arylamine N-acetyltransferase (EC 2.3.1.5) in human liver recently cloned in our laboratory. Three types of N-acetyltransferase gene were identified. Gene 1 contains a 5.5-kilobase (kb) KpnI fragment with a BamHI site; gene 2 contains a 5.5-kb KpnI fragment without a BamHI site; and gene 3 contains a 5.0-kb KpnI fragment with a BamHI site. The combination of these three genes generated five genotypes. Acetylator phenotypes were determined in 29 healthy volunteers by isoniazid loading tests, and they were classified as rapid (10 subjects), intermediate (16 subjects), or slow (3 subjects) acetylators. Rapid acetylators were homozygotes of gene 1. Intermediate acetylators were heterozygotes of either genes 1 and 2 or genes 1 and 3. There were two exceptional cases who were classified as intermediate acetylators but were homozygotes of gene 1. Slow acetylators were either heterozygote of genes 2 and 3 or homozygotes of gene 3. These results indicate that gene 1 corresponds to high N-acetyltransferase activity, while gene 2 and gene 3 give rise to low N-acetyltransferase activity.

N-Acetylation polymorphism has been implicated in the toxicity of amine-containing drugs and in the susceptibility to bladder cancer, systemic lupus erythematosus, and other diseases (1-6). Studies using animal models and human liver specimens have shown that acetylator phenotypes are determined by a low or high arylamine N-acetyltransferase activity in the liver. A slow acetylator phenotype was shown to be inherited by an autosomal recessive gene in man, rabbit, and other mammals (7-13). The molecular and genetic bases of N-acetyltransferase polymorphism are partially understood (14, 15). Recently the cDNAs (16) and genomic DNA (17) for polymorphic N-acetyltransferase in human liver have been cloned and sequenced. One of our laboratories has isolated two types of cDNA coding for the polymorphic N-acetyltransferase in human liver. The cDNAs differed in one nucleotide in the coding region near the C terminus, resulting in a substitution of an amino acid residue and the generation of a BamHI site. A study of the expression of the cDNAs in Chinese hamster ovary cells has indicated that the two cDNAs are correlated with low and high N-acetyltransferase activity (16). On the other hand, genomic Southern blot analysis has demonstrated that there was a restriction fragment length polymorphism (RFLP)' in the KpnI digests of polymorphic N-acetyltransferase gene. In this report, we have analyzed the polymorphic N-acetyltransferase gene by Southern blot hybridization in Japanese subjects and have shown that there is a close correlation between acetylator phenotypes and the genotypes of polymorphic N-acetyltransferase gene in human liver.

MATERIALS AND METHODS

Genomic Southern Blot Analysis—Genomic DNA was prepared from leucocytes or autopsy liver specimens as described (18). The DNAs (10 μg) were digested with 30-40 units of KpnI, KpnI plus BamHI, EcoRI, or EcoRI plus BamHI at 37 °C for 5 h, separated on a 0.8% agarose gel at 25 V for 16 h, and transferred to GeneScreen Plus filters. The filters were prehybridized at 42 °C in 50% formamide, 1 μg/mL NaCl, 1% dextran sulfate, 1% SDS, and 150 μg/mL denatured salmon sperm DNA for 3 h. The filters were hybridized in the above solution containing 3 × 10^6 cpm/mL of either the 32P-labeled specific cDNA or whole cdNA probe. The cdNAs were 32P-labeled using the multi-prime labeling system (Amersham, United Kingdom) to a specific activity of 5 × 10^9 cpm/μg. After 24 h at 42 °C, the filters were washed in 2 × SSC (1 × SSC = 0.15 μg/mL NaCl, 0.015 μM sodium citrate, pH 7.4) containing 1% SDS at 42 °C for 1 h by changing the solution twice and exposed to X-ray film at -80 °C for 24-36 h.

Determination of Acetylator Phenotypes—Acetylator phenotypes were determined on 29 healthy volunteers (16 men and 13 women) who lived in Beppu city and neighboring areas. Their ages were between 20 and 56 (mean 37) years. All subjects gave informed consent to participate in this study. The subjects fasted for at least 12 h before and 3 h after the drug administration. At 9 a.m. after blank blood had been drawn, a single dose of isoniazid (INH) (10 mg/kg of body weight) as powder was orally administered with 150 mL of water. Blood was obtained at 1, 2, and 3 h after the INH administration. Heparinized blood samples were immediately centrifuged, and the plasma were separated. Plasma levels of INH and acetylisoniazid (acetyl-INH) were measured by the high performance liquid chromatography procedure as described by Hutchings et al. (19).

RESULTS AND DISCUSSION

To investigate the correlation between two types of cdNAs recently identified in our laboratory and the RFLP observed in the KpnI digest of human polymorphic N-acetyltransferase gene, genomic DNAs obtained from leucocytes of healthy volunteers were digested with KpnI, KpnI plus BamHI, EcoRI, or EcoRI plus BamHI (Fig. 1b). For hybridization, the BamHI-EcoRI fragment of the cdNA at the 3' terminus was used as a specific probe for the polymorphic N-acetyltransferase (Fig. 1a), while the whole cdNA detected not only the polymorphic N-acetyltransferase gene but also other homologous genes including a monomorphic N-acetyltransferase gene (16). This analysis revealed five patterns of DNA fragments.

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1 The abbreviations used are: RFLP, restriction fragment length polymorphism; INH, isoniazid; SDS, sodium dodecyl sulfate; kb, kilobase(s).
FIG. 1. a, structure of cDNAs for polymorphic N-acetyltransferase from human liver (16) and cDNA probes used for Southern blot hybridization. Dotted and open boxes indicate coding and noncoding regions, respectively. b, Southern blot analysis of genomic DNA. Filters were hybridized either with the specific cDNA (left column) or with the whole cDNA probe (right column) as described under "Materials and Methods." Wild type λ phage DNA digested with HindIII was used for size markers, shown on both sides in kilobases.

(Fig. 1b). Subjects 1 and 2 showed a 5.5-kb fragment in KpnI digests which was shortened upon digestion with KpnI plus BamHI. Since the 5.5-kb fragment could be detected with the specific probe, it corresponded to the 3'-flanking region of the cDNA. Digestion of genomic DNA with EcoRI produced a 1.9-kb fragment in all subjects, which was detected with both the specific probe and the whole cDNA probe. Upon digestion with EcoRI plus BamHI, the 1.9-kb fragment in subjects 1 and 2 was shortened to 1.6 kb and could not be detected with the specific probe but was detected with the whole cDNA probe, indicating that it contained a BamHI site. This result indicates that subjects 1 and 2 possessed two alleles of gene 1, as shown in Fig. 2a. In subjects 3 and 4, the 5.5-kb fragment in KpnI digests separated into two bands upon digestion with KpnI plus BamHI, indicating that the fragment of one allele contained a BamHI site while the fragment of the other allele had no BamHI site. In support of this conclusion, digestion of DNA with EcoRI plus BamHI revealed two bands; a 1.9-kb band was detected with the specific probe, and another 1.6-kb band was detected only with the whole cDNA probe. Thus both subjects 3 and 4 contained genes 1 and 2. In subjects 5 and 6, both 5.5- and 6.0-kb fragments were detected in KpnI digests, and both were shortened by digestion with KpnI plus BamHI, indicating that both fragments contained a BamHI site. Upon digestion with EcoRI plus BamHI, the 1.9-kb fragment was shortened and could be detected only with the whole cDNA probe. Thus both subjects 5 and 6 contained genes 1 and 3. Subject 7 showed both 5.5- and 5.0-
might be homozygous rapid acetylators but exhibit an apparently intermediate acetylator phenotype due to asymptomatic dysfunctions of liver or gut. It has been documented that drug

This analysis has revealed that there are at least three types of N-acetyltransferase gene. Gene 1 contains a 5.5-kb KpnI fragment with a BamHI site; gene 2 contains a 5.5-kb KpnI fragment without a BamHI site; and gene 3 contains a 5.0-kb KpnI fragment with a BamHI site (Fig. 2a). Genomic Southern blot analysis was performed with the genomic DNAs from leucocytes of 52 healthy volunteers and from 34 livers obtained at autopsy. They were divided into five genotypes (Fig. 2b). Genotype I was predominant and represented 47% of the subjects examined, while genotypes IV and V were rare. We have not yet detected the homozygote of gene 2.

To match the genotypes to acetylator phenotypes, oral INH loading tests were performed on 29 healthy volunteers. Acetylator status was determined from analysis of the plasma INH level at 3 h, INH half-life, and the ratio of acetyl-INH to INH at 3 h. These three indices showed good correlation, and the 29 subjects were classified as rapid, intermediate, or slow acetylators. The subjects who showed a concentration ratio of acetyl-INH to INH below 0.4 were classified as slow acetylators, while those who showed the ratio above 4.4 were classified as rapid acetylators. The details of the results will be published elsewhere. Ten subjects were rapid acetylators, 16 were intermediate, and 3 were slow. These observations showed a good agreement with a previous report concerning populations of acetylator phenotypes in Japanese (20). The acetylator phenotypes as expressed by the ratio of acetyl-INH to INH at 3 h were correlated with the genotypes of the polymorphic N-acetyltransferase (Fig. 3). The 10 rapid acetylators belonged to genotype I. Intermediate acetylators were divided into three genotypes: 7 out of the 16 subjects belonged to genotype I, 2 subjects to genotype II, and 12 subjects to genotype III. One of the three slow acetylators belonged to genotype IV and the other two to genotype V. This result indicates that gene 1 is correlated with high N-acetyltransferase activity, while gene 2 and gene 3 give rise to low N-acetyltransferase activity. Genotype V, which contained two alleles of gene 3, and genotype IV, which contained genes 2 and 3, both produced a slow acetylator phenotype. Genotype III, containing genes 1 and 3, and genotype II, comprised of genes 1 and 2, both showed an intermediate acetylator phenotype. These rules, however, did not apply to two subjects who were classified as intermediate acetylators but showed genotype I. We performed genomic Southern blot analysis using an additional 12 restriction enzymes but could not detect any RFLP between the two subjects and the rapid acetylators. The following possibilities are conceivable to explain the two exceptional cases. First, there might be another mutation in the N-acetyltransferase gene, which results in low N-acetyltransferase activity but which cannot be detected by the present RFLP analysis. Second, the two subjects might be homozygous rapid acetylators but exhibit an apparently intermediate acetylator phenotype due to asymptomatic dysfunctions of liver or gut. It has been documented that drug

**FIG. 3. Correlation between acetylator phenotypes and genotypes of polymorphic N-acetyltransferase in human liver.** Genomic DNAs were analyzed as described under "Materials and Methods," and genotypes were determined. The numbers in the figure indicate the subjects whose genomic DNA analyses are shown in Fig. 1b. The correlation between the acetylator phenotypes, as expressed in the ratio of acetyl-INH to INH at 3 h, and the genotypes of the polymorphic N-acetyltransferase is demonstrated.

Tests sometimes do not discriminate the homozygous rapid acetylator from the heterozygous intermediate acetylator (7). Further analysis of N-acetyltransferase gene in the two subjects should provide an answer to this question.

Recently we have shown that the cDNA containing a BamHI site in the coding region expresses a high N-acetyltransferase activity, while the cDNA which lacks a BamHI site expresses a low N-acetyltransferase activity when introduced into Chinese hamster ovary cells. RNA blot and immunoblot analyses have indicated that the low N-acetyltransferase activity produced by the latter cDNA is due to a low N-acetyltransferase protein content (16). It could be that a single amino acid substitution renders the enzyme protein unstable and results in its rapid degradation. Thus gene 1 would code for the cDNA with a BamHI site, while gene 2 codes for the cDNA without a BamHI site. The present analysis has also indicated that the 5.0-kb KpnI fragment is correlated with low N-acetyltransferase activity. A preliminary study with autopsy livers has indicated that the human livers of genotype V contained a low level of mRNA for the polymorphic N-acetyltransferase. It could be that the mutation associated with gene 3 results in a defect in the transcription of the N-acetyltransferase gene. Analysis of gene 3 is in progress in one of our laboratories to identify another mutation associated with low N-acetyltransferase activity. The present study has shown that in 27 of 29 Japanese subjects there was a close correlation between the acetylator phenotypes and the genotypes of the polymorphic N-acetyltransferase in the liver. Thus Southern blot analysis of genomic DNA can predict acetylator phenotypes in most cases, although an additional mutation in a small fraction of the population has not been excluded. The information and methods reported here should prove of use in the identification of acetylator phenotypes and in the investigation of correlations.
between the acetylator genotypes and the susceptibility to certain diseases (5–7).

REFERENCES