Comparison of the polymorphic regions of the cytochrome P450 CYP2E1 gene of humans and patas and cynomolgus monkeys

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Cytochrome P450 2E1 (CYP2E1) metabolizes low molecular weight toxicants. CYP2E1 gene polymorphisms have been linked to risk of various cancers and liver disease in humans. Since the patas monkey is a promising model for study of cancer-related alcohol/nitrosamine interactions, we examined CYP2E1 in this monkey for characteristics of two regions that are polymorphic in humans, an Rsa I site in the 5′ promoter region and a Dra I site in intron 6. Another monkey species often used in biomedical research, the cynomolgus monkey, was also examined. Human DNA primers used to amplify a 413 bp segment around the Rsa I site also amplified a segment of similar size (409 bp) from DNA of 25 patas monkeys, whereas a product of ~800 bp was amplified from DNA of eight cynomolgus monkeys. Rsa I did not cut the amplified DNA product from either monkey species. Sequencing revealed that the patas Rsa I site was identical to that in humans with the c2c2 CYP2E1 genotype, GTAT. The equivalent cynomolgus sequence, CTAC, has not been observed in humans. Thus, the patas monkey appears to be a useful model for CYP2E1 c2c2 humans, and this genotype, present in 2–25% of humans, may be more primitive than c1c1. For the Dra I site, the human primers amplified DNA products similar in size to those from humans, from all patas and cynomolgus monkey DNA samples; none were cut by Dra I. Thus, both monkey species appeared to be generally similar to humans of CYP2E1 CC Dra I genotype, which is the rarer form of the gene.

Abbreviations: CYP2E1, cytochrome P450 2E1; NDMA, N-nitrosodimethylamine; PCR, polymerase chain reaction.

Cytchrome P450 2E1 (CYP2E1) metabolizes important low molecular weight environmental toxicants and carcinogens, including solvents and N-nitrosodimethylamine (NDMA) (1). Several gene polymorphisms have been identified in humans, which correlate in complex ways with risk of cancer at several anatomic sites (2). The incidence of these polymorphisms appears to vary with race, and the alternate forms of the gene may influence the degree of gene expression (2). In particular, a site in the promoter region of the 2E1 gene recognized by the Rsa I restriction enzyme has a variant allelic form that is more common in Orientals (up to 20% of individuals) than in Caucasians or Blacks. The more common allele is designated c1, and the more unusual variation is c2. Evidence related to the physiological significance of this variation is limited, but suggests that the c2 allele is more highly expressed, possibly because of an effect on a binding site for hepatic nuclear factor 1 (HNF1) (3–9).

Another CYP2E1 polymorphism, at a Dra I restriction site in intron 6, has also been linked to cancer risk in some studies (10–12). A relationship of this polymorphism to gene expression has been postulated (13,14) but has not yet been directly demonstrated.

We have utilized a non-human primate model, the patas monkey, to study the interactions of ethanol with NDMA, in pursuit of the mechanism of enhancement of some tobacco-associated cancers by alcoholic beverages. In patas monkeys, ethanol potently inhibited the in vivo clearance of NDMA, and caused large (up to 20-fold) increases in levels of promutagenic methyl DNA adducts, derived from NDMA, in many tissues (15). These results were interpreted as a consequence of competitive inhibition by ethanol of hepatic first-pass clearance of NDMA by CYP2E1. The findings appeared to explain in part the 10–20-fold increase in head-and-neck cancers in humans when smoking is combined with drinking (16). On the biochemical level, CYP2E1 was detected in patas liver by western immunoblot analysis and observed to be similar in size to that of humans; it was increased in amount after isopropanol treatment (17).

In view of the polymorphic differences in CYP2E1 gene structure among humans, related in part to race, we wished to determine whether the region of the patas CYP2E1 gene homologous to the Rsa I site in humans is similar to the human c1 or c2 variant. Further, to discover whether this gene region might be highly conserved among primates, we also included DNA from cynomolgus monkeys, which are commonly used models in biomedical research. CYP2E1 has been also detected in cynomolgus liver by immunoblotting and enzymology (18).

Patas (Erythrocebus patas) and cynomolgus (Macaca fascicularis) monkeys were maintained at BioQual (Shady Grove, MD), in a facility fully accredited by the American Association for Accreditation of Laboratory Animal Care, as described previously (19). Genomic DNA was isolated from whole blood of 25 patas and eight cynomolgus monkeys as described previously (20). Polymerase chain reaction (PCR)–restriction fragment length polymorphism was utilized to analyze the polymorphic Rsa I site in the 5′-regulatory region and Dra I site in intron 6 of CYP2E1. The primers used, derived from the human sequence (GenBank accession number J02843) were 5′-CCA-GTC-GAG-TCT-ACA-TTG-TCA-3′ (upper) and 5′-TTC-ATT-CG-TCT-AAC-TGG-3′ (lower). A PCR kit from Perkin–Elmer Applied Biosystems (Foster City, CA) was used. The reaction volume was 50 µl and contained 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 125 µM dNTPs, 0.2 µM each of the primers, 100 ng DNA and 2 U Taq polymerase. A modified hot start reaction with
CYP2E1 gene has not been completely sequenced, but it is clear that most of this additional fragment is located downstream of the RsaI site (Figure 2). For the region that has been sequenced, homology with the human gene is 89.3% and with the patas gene 93.1%. Homology with the human sequence within the HNF1 binding site was again 83%, but the differences were not the same as those found for the patas CYP2E1 in this region. The RsaI site was again altered so that cutting did not occur, but in this case the first base was changed to C, a difference not yet described for humans.

It appears that the CYP2E1 gene promoter region at the RsaI site and immediate vicinity was more similar to the human structure for patas than for cynomolgus monkeys. The presence in the cynomolgus CYP2E1 gene of an extra ~370 bp downstream from the RsaI site suggests substantial modification of gene structure. In humans, c2 variant has been associated with higher CYP2E1 expression in comparison with c1 genotype (5,6). Further comparison of CYP2E1 expression between patas and cynomolgus monkeys may be crucial to determine the role of the CYP2E1 promoter region to the level of expression.

Although nothing can be concluded from the study of just two monkey species, it is of interest that patas monkeys live on the savannas of Africa, where the ancestors of man dwelt for hundreds of thousands of years. By contrast, cynomolgus monkeys, and most other non-human primates utilized in biomedical research, are arboreal, forest-living animals. It is
possible that humans and patas monkeys have greater similarity in gene structure because of common evolutionary pressures. Another speculation is that the c2 allele is the more ancient, and has been selected against during human evolution, especially in the Caucasian and Black races, where its frequency is 5% or less.

The similarity of the patas CYP2E1 gene promoter region to that in c2c2 humans suggests that results obtained with patas monkeys may be relevant to the risk characteristics associated with the c2 allele. Substantial DNA alkylation from NDMA was detected in numerous patas tissues, including nasal cavity, oral cavity, esophagus, stomach and large intestine (15). Nitrosamines have been suspected in the etiology of cancers in all of these organs, and other diet-derived toxicants, such as the newly-discovered mutagen 2-chloro-4-methylthiobutanoic acid in salted, pickled fish (22), may also be substrates for CYP2E1. Possession of the c2 allele has been linked with risk of nasopharyngeal carcinoma (20) and of gastric cancer (23) in Chinese. Alcohol-related liver cancer and other diseases have also been associated with the c2 allele, in both Oriental and Caucasian populations (24–28), although other studies have implicated the c1 allele (29,30) and yet others have found no associations (31–33). This inconsistency may be a result of rare frequency of c2 allele in the general population. The patas monkey may be useful for modeling CYP2E1 c2-related disease risk in these organs.

DNA from both monkey species was examined for gene structure in the region of the Drai polymorphic site. A human CYP2E1 CD heterozygote from our previous study (20) was used as a positive control and presented the 874 and 121 bp fragments from the C allele and the 572, 302 and 121 bp fragments from Drai digestion of the D allele (Figure 3). DNA from all of the monkeys tested, both patas and cynomolgus, yielded a product with the human primers that was similar in size to that from the human DNA. All showed only two fragments after Drai digestion, and were thus genotypically similar to CYP2E1 CC humans, at least in product length and lack of Drai cutting, suggesting that the general structure of this region, though intronic, is conserved, and that the CC form is the more ancient. The C allele has been associated with increased risk of lung cancer in Japanese (10,13) and with breast cancer in smoking, pre-menopausal Caucasians (12). On the other hand, the DD genotype was associated with increased risk of lung cancer in Mexican-American and African-American male smokers (34). Non-human primates may provide a model for functioning of this region of the gene.

References

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Fig. 3. Separation by gel electrophoresis of PCR products, using human primers for the DraI site of CYP2E1, followed by DraI digestion. Numbers indicate individual monkeys. PCR products from a CD human DNA sample are on the far right. The 121 bp fragment in some of the monkey DNA samples is faint in the photograph, but was evident in the original gel.

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