Role of human CYP2A enzymes on metabolism of nicotine and environmental chemicals

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Abstract

Human cytochrome (CYP) 2A subfamily consists of two functional isoforms, CYP2A6 and CYP2A13. The purpose of my study was to investigate the clinical significance of the CYP2A enzymes. The effects of genetic polymorphisms of *CYP2A6* on nicotine metabolism and the role of CYP2A13 in the metabolism of various environmental chemicals were evaluated. CYP2A6 catalyzes the conversion of nicotine to cotinine. This study clarified that there was no ethnic difference in the nicotine metabolism potency between European-Americans and African-Americans. Therefore, the higher lung cancer risk in black smokers than that in white smokers was not owing to the difference of the CYP2A6 enzyme activity. Novel polymorphic alleles, *CYP2A6*17*, *CYP2A6*18*, *CYP2A6*19*, *CYP2A6*20* and *CYP2A6*1X2B*, were identified. The *CYP2A6*17* and *CYP2A6*20* found in African-Americans and *CYP2A6*19* found in Koreans were causal for the decreased nicotine metabolism potency, whereas the *CYP2A6*1X2B* found in African-Americans was causal for the increased nicotine metabolism. It was demonstrated that large interindividual variability was mostly explained by the *CYP2A6* genetic polymorphisms. Although CYP2A13 was reported to catalyze the activations of nitrosamines, the knowledge of the CYP2A13 substrates was limited. This study found that CYP2A13 metabolizes 4-aminobiphenyl, naphthalene, styrene, and toluene with higher efficiencies than other CYP isoforms. CYP2A13 might be one of the important enzymes for the activation or detoxification of environmental chemicals. The knowledge from this study would increase our understanding of clinical and toxicological significance of human CYP2A enzymes.

Dissertation abstract

Cytochrome P450 (CYP) enzymes comprising a superfamily of heme-containing monooxygenases are involved in the metabolism of drugs, environmental pollutants, dietary chemicals, and endogenous compounds. Human CYP2A subfamily consists of three isoforms, CYP2A6, CYP2A7 and CYP2A13. CYP2A6 and CYP2A13 are functional, but CYP2A7 is not. CYP2A6 is mainly expressed in human liver, whereas CYP2A13 is predominantly expressed in human respiratory tract and bladder. The purpose of my study was to investigate the clinical significance of CYP2A enzymes. The effects of genetic polymorphisms of *CYP2A6* on nicotine metabolism and the role of CYP2A13 in the metabolism of various environmental chemicals were evaluated.

Relationship between *CYP2A6* genetic polymorphisms and nicotine metabolism

In humans, 70-80% of the absorbed nicotine is metabolized to cotinine by CYP2A6. CYP2A6 is also responsible for the activation of tobacco specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Smokers adapt their smoking behavior to maintain nicotine levels in the brain. Therefore, it is considered that the variability of CYP2A6 enzyme activity is associated with the smoking status and lung cancer risk. The present study found that the nicotine metabolism potency in African-Americans was similar to that in European-Americans with a phenotyping method using a nicotine gum[1]. Many research groups had reported that the plasma cotinine levels in black smokers were higher than those in white smokers. Furthermore, lung cancer risk in black smokers seems to be higher than that in white and Japanese smokers. The results in this study could exclude the ethnic difference in CYP2A6 enzyme activity between European-Americans and African-Americans.

The present study found large interindividual variability in nicotine metabolism in European-Americans and African-Americans. The relationship between the *CYP2A6* genetic polymorphisms and interindividual variability in nicotine metabolism was analyzed. In the process of the *CYP2A6* genotyping, novel polymorphic alleles, *CYP2A6*17*, *CYP2A6*18*, *CYP2A6*19*, *CYP2A6*20* and *CYP2A6*1X2B*, were identified. The *CYP2A6*17* allele was specifically found in African-Americans at 10.5% of allele frequency. Recombinant CYP2A6.17 showed the decreased enzyme activity in coumarin 7-hydroxylation and nicotine *C*-oxidation. Furthermore, the in vivo cotinine/nicotine ratio in heterozygotes or homozygotes of *CYP2A6*17* was significantly lower than that in homozygotes of *CYP2A6*1*. These results demonstrated that *CYP2A6*17* allele is causal for the decreased enzyme activity[2]. The *CYP2A6*18* allele was found in European-Americans and Koreans at 2.1% and 0.5% of allele frequencies, respectively, whereas the *CYP2A6*19* allele was found in Koreans at 1.0% of

allele frequency. Recombinant CYP2A6.18 showed the decreased enzyme activity in coumarin 7-hydroxylation and 5-fluorouracil formation from tegafur, but not in nicotine *C*-oxidation, whereas recombinant CYP2A6.19 showed the decreased enzyme activity in all reactions. The in vivo study also suggested that the *CYP2A6*19* was causal for the decreased nicotine metabolism potency[3]. The *CYP2A6*20* allele was also specifically found in African-Americans at 1.7% of allele frequency. In vitro study clarified that this allele produces a truncated protein due to a lack of two deoxyadenosines, resulting in no enzyme activity. The effect of the *CYP2A6*20* allele on in vivo nicotine metabolism could not be directly evaluated due to the absence of homozygotes of this allele, but heterozygotes of this allele showed low cotinine/nicotine ratio in plasma. Thus, it was assumed that *CYP2A6*20* is causal for the decreased nicotine metabolism[4]. The *CYP2A6*1X2B*, a novel duplication type was specifically found in African-Americans at 1.7% of allele frequency. This duplication is considered to be created by unequal crossover with *CYP2A7* gene at 5.2 to 5.6 kb downstream of the stop codon and a reciprocal product is the *CYP2A6*4B* allele. The subjects with this duplication showed 1.4-fold higher cotinine/nicotine ratio in plasma than homozygotes of *CYP2A6* wild type although the difference was not statistically significant. The *CYP2A6*1X2B* allele likely increases the nicotine metabolism potency[5].

Finally, this study demonstrated that large interindividual variability in nicotine metabolism was mostly explained by the *CYP2A6* genetic polymorphisms. In European-Americans, a few variant alleles were found with low frequency. Thereby the relationship between the *CYP2A6* genetic polymorphisms and interindividual variability in nicotine metabolism was not dramatic. However, in African-Americans, it was demonstrated that the *CYP2A6*2*, *CYP2A6*4*, *CYP2A6*9*, *CYP2A6*17*, and *CYP2A6*20* alleles cause the decreased nicotine metabolism. In Japanese and Koreans, the *CYP2A6*4*, *CYP2A6*7*, *CYP2A6*9*, *CYP2A6*10* alleles were variants causing low enzyme activity in Japanese and Koreans[6, 7]. The combined frequencies of the alleles lacking or reducing enzyme activity were 9.1% and 21.9% in European-Americans and African-Americans, respectively, whereas 43.4% and 50.5% in Koreans and Japanese respectively. It was demonstrated that the nicotine metabolism potency in Japanese was significantly lower than those in other populations[8]. The high frequencies of variant alleles might be one of the reasons for the low nicotine metabolism in Japanese. The findings in my thesis could provide the useful information for the interindividual variability of smoking status and lung cancer risk.

Role of CYP2A13 in the metabolism of various environmental chemicals

CYP2A13, which is predominantly expressed in respiratory tract and bladder, was found to catalyze the metabolism of *N*-nitrosodiethylamine, 2,6-dichlorobenzonitrile, and NNK, but the knowledge of the CYP2A13 substrates was limited. This study found that CYP2A13

metabolizes various environmental chemicals, 4-aminobiphenyl, naphthalene, styrene, and toluene with higher efficiencies than major CYP isoforms catalyzing them[9, 10]. CYP2A13 showed overlapping substrate specificity with CYP1A2 and CYP2E1, although the amino acid homology between CYP2A13 and CYP1A2 or CYP2E1 is low[10, 11]. However, CYP2A6 showed no or substantially decreased activity in above reactions, although the amino acid identity with CYP2A13 is as high as 96.5%. The substrates of CYP2A13 are not necessarily compatible with those of CYP2A6. Overall, CYP2A13 might contribute to the activation or detoxification of various environmental chemicals in human respiratory tract and bladder with the different substrate specificity from that of CYP2A6.

Conclusion

CYP2A6 polymorphic alleles including novel alleles identified in this study were associated with the interindividual variability in nicotine metabolism. CYP2A13 could efficiently catalyze the activation or detoxification of various environmental chemicals with the different substrate specificity from CYP2A6. Finally, the knowledge from this study would increase our understanding of clinical and toxicological significance of human CYP2A enzymes.

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学位論文審査結果の要旨

Cytochrome P450 (CYP) は多くの薬物や外来異物ならびに内因性化合物の代謝を触媒している酵素である。本研究では、ヒト CYP2A6 と CYP2A13 の生体内における臨床意義を明らかにすることを目的とした。CYP2A6 はニコチンをコチニンに代謝する。喫煙者において黒人は白人よりも血中コチニン濃度が高いことが報告されているため、CYP2A6 代謝能に人種差が存在する可能性が示唆された。そこでニコチンガムを用いたフェノタイピング法により白人187名と黒人176名について代謝能を解析したところニコチン代謝能に人種差はないことを明らかにした。しかし、ニコチン代謝能に大きな個体差が認められ、その原因として *CYP2A6* 遺伝子多型を解析し、酵素活性を低下させる新規変異型 *CYP2A6*17*、*CYP2A6*18*、*CYP2A6*19*、酵素活性を消失させる *CYP2A6*20* と遺伝子重複型 *CYP2A6*1X2B* を発見した。一方、CYP2A13 はタバコ特異的ニトロソアミンである NNK の代謝的活性化を触媒することが知られているが、他にどのような化合物を代謝するのか情報が少ない。呼吸器官に高く発現していることから環境化学物質の代謝について解析した結果、4-アミノビフェニル、ナフタレン、スチレン、トルエンをこれまで代謝することが知られていた分子種よりも効率的に代謝することを明らかにした。本研究成果は、CYP2A の臨床意義の解明に大きく貢献し、博士（薬学）に値すると判定した。