IN VITRO METABOLISM OF ARYLAMINE HAIR DYES IN HUMAN HEPTIC MICROSOMES AND HUMAN HEPATOCYTES

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ABSTRACT

Metabolism is a key consideration in the safety assessment of primary amines used in oxidative hair dyes, especially for the assessment of potential occupational occupational carcinogenicity. Since hydroxylation formation by CYP450 is regarded as a key step in the activation of amines, it is important to understand the metabolism of these compounds in human hepatic microsomes and to compare microsomal metabolism with in vitro metabolism of the same compounds in human hepatocytes. Previous studies have shown that p-phenylendiamine and 2,5-diaminotoluene, two widely used oxidative hair dye ingredients, do not form mono-oxygenated metabolites in cryopreserved human hepatocytes, toxic microsomes or recombinant CYPs. Only the mono- and dihydroxylated metabolites were observed in human hepatocytes. The purpose of the present work was to screen additional arylamine hair dyes for oxidative metabolism in human hepatic microsomes and to compare microsomal metabolism studies with any compounds exhibiting evidence of oxidative metabolism. Five primary amines that are widely used in oxidative hair dyes were screened and showed no evidence of oxidative metabolism. One compound, 4-amino-2-hydroxytoluene, was concluded to be not biologically reactive. There was no evidence of constant binding of 1-C3-Hydroxylated AHT to human microsomal protein, suggesting that the mono-hydroxylated metabolite formed by CYP1A2 was not biologically reactive. One of these metabolites was confirmed to be hydroxylated on the methyl group and the other is likely to be ring hydroxylated. Further in vitro studies in human, rat, and mouse hepatocytes indicated that phase II reactions (sulfation, acetylation, glucuronidation) prevent the intact hepatocyte and that mono- and di-oxygenated metabolites are not detected. Our results indicate that none of the hair dyes tested showed evidence of hepatic metabolism potentially directly reactive oxidative metabolites. As such, these metabolites are of particular interest from the point of view of human hair coloring.

INTRODUCTION

In a case-control epidemiology study conducted in Los Angeles an association was found between the use of permantin (i.e., oxidative) hair dyes and bladder cancer risk (1). In a subsequent publication from the same study the increase in bladder cancer risk was reported to be associated with the use of arylamine-based hair dyes (2). These investigations concluded that modification of the risk by NAT2 genotype/phenoype implicates arylamines used in permanent hair dyes as playing a role in bladder carcinogenesis. More recent epidemiology studies have not shown an association between the use of oxidative hair dyes and bladder cancer risk estimate (3). Nevertheless, the Los Angeles study had raised questions about permanent hair dyes (which contain arylamines as ingredients) because some arylamines (e.g., benzidine, 4-amino-methylpropiol, and 4-aminophenol, are known to be human bladder carcinogens, and 4-aminophenol, and diaminodiphenyl ethers are involved in the metabolism of these carcinogens. At the time the Los Angeles study was published there were few data in the literature on the metabolism of hair dye amines. Previous work, p-phenylendiamine and 2,5-diaminotoluene have shown evidence for formation of hydroxylated metabolites by human hepatic microsomes or by specific human CYP enzymes (4). The experiments presented here tested the metabolism of four newly identified amines and in the previously published work (5,6) those are used at the highest volume in permanent hair dyes.

METHODS

Pooled Human Liver Microsomes

Pooled human liver microsomes were obtained from Gentest, Woburn, MA. The microsomes were pooled from microsomal preparations from 26 female and 20 male subjects. Characterization of the enzymatic activity of the pooled microsomes was described by the supplier.

Microsomal Incubations

Test chemicals (10 and 100 µM) and the positive control (100 µM 3-amino-1-methyl-1-2-naphthylamine) were incubated with pooled liver microsomes (1 mg protein) in the presence of an NADPH generating system. The reactions were initiated by the addition of microsomes, incubated at 37°C for one hour, and terminated by the addition of an equal volume of acetone. The samples were filtered through a 0.22 µm pore size filter membrane and the supernatant was flash-frozen and stored at -70°C until analysis.

Cryopreserved Human Hepatocytes

Cryopreserved human hepatocytes were obtained from In Vitro Technologies. Suspended hepatocytes (1x10⁶/ml) were incubated with sodium (10 and 100 µM) for up to four hours. 500 µl aliquots were removed at selected time points and the reaction stopped by the addition of an equal volume of acetone. Samples were filtered through a 0.22 µm pore size filter membrane and the supernatant was flash-frozen and stored at -70°C until analysis.

Sample Analysis

Automated HPLC analysis was carried out using reverse phase HPLC with MS detection. For MS detection, a Waters Quatran MS was used in spotting mode (positive or negative) for selected ion detection of the parent ion and potential metabolites. The conditions of the HPLC were optimized to achieve a high degree of peak separation. For redetection detection, a Beckman-Radial CH105 detector was used. The limit of detection was approximately 5% of the peak height, which was determined with TLC. The mass spectrometer was operated in the quadrant mode and was equipped with a quadrupole filter. The nitrogen was used as both the ionization gas and collision gas. The compounds were analyzed under positive ionization and negative ionization. Analytes were identified based on accurate mass measurements obtained from selected ion monitoring for the parent ion and respective metabolites. The response was determined for each compound and the corresponding metabolites. The mass spectrometer was operated in the quadrant mode and was equipped with an electrospray ionization source. The nitrogen was used as both the ionization gas and collision gas. The compounds were analyzed under positive ionization and negative ionization. Analytes were identified based on accurate mass measurements obtained from selected ion monitoring for the parent ion and respective metabolites. The response was determined for each compound and the corresponding metabolites. Treatment of the data was done using Masslynx 4.1 software. For MS detection, a Waters Quatran MS was used in spotting mode (positive or negative) for selected ion detection of the parent ion and potential metabolites. The conditions of the HPLC were optimized to achieve a high degree of peak separation. For redetection detection, a Beckman-Radial CH105 detector was used. The limit of detection was approximately 5% of the peak height, which was determined with TLC. The mass spectrometer was operated in the quadrant mode and was equipped with a quadrupole filter. The nitrogen was used as both the ionization gas and collision gas. The compounds were analyzed under positive ionization and negative ionization. Analytes were identified based on accurate mass measurements obtained from selected ion monitoring for the parent ion and respective metabolites. The response was determined for each compound and the corresponding metabolites. Treatment of the data was done using Masslynx 4.1 software.

CONCLUSIONS

There is no evidence that the hair dye arylamines investigated in this study undergo hepatic oxidative metabolism. In epidemiological studies that investigated the relationship between occupational exposure to hair dye arylamines and human cancer risk, no correlation could be found between occupational exposure and bladder cancer risk. However, it has been shown that occupational exposure to hair dye arylamines is associated with bladder cancer risk (1). These findings are supported by the results of the present study, which demonstrated that no oxidative metabolism was observed in human hepatic microsomes and human hepatocytes. The results of the present study suggest that oxidative metabolism of hair dye arylamines is not a significant contributor to the risk of bladder cancer associated with occupational exposure to these compounds.