Comparison of Human and Rat Hepatocyte Metabolism and Mutagenic Activation of 2-Acetylaminofluorene

Kenneth Rudo, William C. Meyers, Walter Dauterman, and Robert Langenbach

ABSTRACT

A method has been developed to assess the metabolism and mutagenic activation of carcinogens using human and rodent hepatocytes in vitro. A slicing technique which was especially useful for nonperfusable biopsy and resected surgical human liver tissue was used to prepare the hepatocytes. Metabolites of the model carcinogen 2-acetylaminofluorene (AAF) produced by human and rat hepatocytes were similar and consisted primarily of 2-aminofluorene with ring hydroxylated products at the 1-, 3-, 5-, 7-, and 8-positions produced in addition to N-hydroxy-AAF. Sulphate and glucuronide conjugates of ring-hydroxylated metabolites and 2-aminofluorene were detected. Metabolism and cell-mediated Salmonella mutagenicity illustrated interindividual variation with human hepatocytes. Levels of metabolism and mutagenesis were generally higher with human hepatocytes compared to rat hepatocyte results. The increased levels of metabolism and mutagenesis of AAF by human hepatocytes compared to rat hepatocytes probably indicates a different sensitivity to hepatocarcinogenic effects of AAF on humans as compared to rats. Understanding differences and similarities between human and rodent carcinogenesis activation capabilities should be useful in the extrapolation of rodent carcinogenesis data to humans.

INTRODUCTION

In order to understand the carcinogenesis process in addition to testing for agents which cause its occurrence, the factors involved in the development of this disease must be delineated. Animal models have been widely utilized for mechanistic studies and as bioassay systems for potential human carcinogens. However, species differences between humans and animals may lead to problems in the quantitative extrapolation of the effect of specific environmental chemicals in the development of cancer in humans. Differences between human and rat enzymatic pathways can affect the rates of formation as well as the levels of reactive metabolites of environmental chemicals. Metabolism to reactive intermediates represents one of the significant events in the carcinogenic process which can affect the accuracy of animal to human extrapolation (1–9). Therefore, a knowledge of xenobiotic metabolic differences between humans and test animals should contribute to the reliability of the extrapolation process. However, in cases in which human tissues have been utilized in metabolism studies, a high degree of individual variation is evident (10–15) and this must also be considered in the extrapolation process.

Certain aromatic amines, including the model chemical of this class, AAF, have been identified as hepatocarcinogens in a wide variety of animal species (16–19). These studies have also illustrated species differences in the metabolism and mutagenesis of these compounds. The metabolism and mutagenesis of AAF have been studied with animal microsomal, S9, and intact hepatocyte preparations (7, 20, 21) and with human subcellular preparations (22–25). Previous studies comparing subcellular and intact cell metabolism and mutagenicity of aromatic amines in animals have illustrated quantitative and to a lesser extent qualitative differences between the two preparations (26–29). It has been postulated from these experiments that intact hepatocytes may simulate the overall metabolic process (activation and detoxification) as it occurs in the liver better than cell homogenate preparations (26–29).

Therefore, the present study focuses on the differences in AAF metabolism and in its metabolic activation to mutagenic products by hepatocytes from humans and the rat. A modified slicing technique (30) for hepatocyte preparation from rat and human liver allowed the use of nonperfusable tissue samples for experiments. The Salmonella typhimurium assay has proven to be a sensitive measure of aromatic amine mutagenesis (27, 31) and is used in this study to measure cell-mediated mutagenesis of AAF. Levels of metabolism, specific metabolite formation, and extent of mutagenicity were measured to determine interindividual differences in humans and the extent of species variation. The metabolism and mutagenicity data are discussed in relation to the rodent to human extrapolation process used in the prediction of carcinogenic risk.

MATERIALS AND METHODS

Chemicals. [14C]AAF (specific activity, 57.0 μCi/μmol) was purchased from Amersham (Arlington Heights, IL). It was found to be >99% radiochemically pure by HPLC. Nonradiolabeled AAF, AF, 1-OH-AAF, 3-OH-AAF, 5-OH-AAF, 7-OH-AAF, 8-OH-AAF, 9-OH-AAF, and N-OH-AAF were purchased from the National Cancer Institute (Bethesda, MD). Desferal mesylate was obtained from Ciba Pharmaceutical Co. (Summit, NJ). β-Glucuronidase (Type B-10, 100,000 units/mg), ethylene glycol bis(β-aminoethyl ether)N,N′-tetra-acetic acid, arosulfatase (33 units/mg, Type VIII), calcium chloride, bovine serum albumin, and D-saccharic-1,4-lactone were purchased from Sigma Chemical Co. (St. Louis, MO). Dulbecco’s PBS (without calcium and magnesium), Eagle’s Minimum Essential Medium (without L-glutamine), collagenase, gentamicin, and HBSS (without calcium and magnesium) were obtained from Gibco-BRL (Grand Island, NY). HPLC spectral grade ethylacetate, methanol, acetone, diethyl ether, ethanol, and isopropanol were purchased from Burdick and Jackson Laboratories, Inc. (Muskegon, MI). Avasol-2 and Protosol were obtained from Dupont/New England Nuclear (Boston, MA). Trichloroacetic acid and dimethyl sulfoxide were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ). All treatments, extractions, evaporation, and HPLC procedures were carried out under yellow light.

Isolation of Human and Rat Hepatocytes. Human liver tissue was obtained from nine surgical specimens at Duke University Medical Center (Durham, NC). Table 1 lists the ages, sex, cause of liver tissue removal, and viability of liver cells from each individual. Tumor tissue was separated from normal tissue by the pathology department at Duke University Hospital. Tissue specimens for this study were transported...
Hepatocyte metabolism and mutagenic activation of AAF

Table I Liver donor patient data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Disease/surgical procedure</th>
<th>% of Cell viability</th>
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<td>30</td>
<td>Male</td>
<td>Organ donor (motorcycle accident)</td>
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<tr>
<td>II</td>
<td>43</td>
<td>Male</td>
<td>Rectal carcinoma, liver metastasis; subtotal left hepatectomy</td>
<td>66.9</td>
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<tr>
<td>III</td>
<td>30</td>
<td>Male</td>
<td>Fibromellar hepatocellular cancer, liver metastasis; subtotal left resection</td>
<td>81.6</td>
</tr>
<tr>
<td>IV</td>
<td>61</td>
<td>Male</td>
<td>Hemangiomia; partial left hepatomecy</td>
<td>70.7</td>
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<tr>
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<td>79.1</td>
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<td>VI</td>
<td>31</td>
<td>Female</td>
<td>Retropitoneal and liver cancer; partial right hepatomecy</td>
<td>79.0</td>
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<td>34</td>
<td>Female</td>
<td>Adenocarcinoma of the colon, liver metastasis; ductotomy</td>
<td>92.1</td>
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<tr>
<td>VIII</td>
<td>38</td>
<td>Female</td>
<td>Right hepatic mass; subtotal right lateral resection</td>
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<tr>
<td>IX</td>
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Table II Patient data

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<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
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<td>IX</td>
<td>45</td>
<td>Female</td>
<td>Organ donor</td>
<td>80.0</td>
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</table>

Water-soluble AAF metabolites were identified after β-glucuronidase or aryl sulfatase treatment of the aqueous phase. To ensure the removal of all organic-soluble metabolites, a final reextraction of the aqueous phase with diethyl ether was done before the pH was adjusted to 5.0 with 0.5 N acetic acid. One-half of each aqueous sample (2.5 ml) was treated with β-glucuronidase (1000 units) while the other half was treated with aryl sulfatase (250 units) plus 19.2 mg of β-saccharin-1,4-lactone (30). The samples were mixed thoroughly and were incubated at 37°C for 24 h. After incubation, the pH of the aryl sulfatase samples was readjusted to 7.5 with 1 N NaOH and all samples were extracted as described above with the organic layer being removed following centrifugation. The samples were then analyzed by HPLC.

The amount of AAF bound to cellular macromolecules was determined as follows. Bovine serum albumin (10 mg) was added to the combined enzymatically hydrolyzed aqueous phase containing cell debris after the final organic extraction. Both bovine serum albumin and cell debris were precipitated by the addition of 1 ml 20% trichloroacetic acid to 5 ml of aqueous phase. Each tube was mixed with 1 ml Protosol overnight in a 37°C water bath and neutralized with 1 ml 0.5 N HCl. Aqosal-2 (10 ml) cocktail was added and each sample counted in the liquid scintillation counter.

High Pressure Liquid Chromatography of AAF Metabolites. The analysis of AAF metabolites and HPLC conditions is a modification of methods previously described by Smith and Thorgerisson (21) and modified by Langenbach et al. (30). Metabolite identification was accomplished by co-chromatography with authentic standards. Samples were chromatographed on a DuPont Model 8800 chromatograph equipped with a UV (254 nm) absorbance detector (DuPont Instruments, Wilmington, DE). A flow rate of 1.5 ml/min using a Zorbax C8 column, 4.6 mm (inside diameter) x 15 cm (DuPont Instruments, Wilmington, DE) was used. Solvent A consisted of a mixture of 0.01 M acetic acid and 0.01% desferal mesylate and solvent B was isopropanol with 0.01% desferal mesylate. The desferal mesylate prevented chemisorption of N-hydroxy-AAF to the column (21). The gradient system used and the separation of standard and radiolabeled metabolites are shown in Fig. 1. The top profile is the HPLC chromatogram of each standard metabolite. The lower profile represents the radioactivity collected corresponding to each separate metabolite produced by rat or human hepatocytes. The AAF metabolites identified were the 1- and 3-, 5/9-, 7-, and 8-hydroxy AAF, N-OH-AAF, and AF. Attempts to separate the 5- and 9-hydroxy-AAF as previously reported (21) were unsuccessful and therefore these metabolites are combined in subsequent tables. The 8-OH-AAF has been previously reported as a metabolite of AAF in the rat (33), but the present study is the first HPLC detection of this metabolite. The 8-OH-AAF and 5/9-OH-AAF were clearly separated as seen in the radiochromatogram. Eluted fractions were collected directly into scintillation vials with variable collection times: 1 min/fraction for the first 2 min, 6 sec/fraction from 2 to 6 min to resolve closely eluting early metabolites, and 30 sec/fraction until the end of the chromatographic procedure. The fractions were counted in a Packard Tri Carb 2000 CA Liquid Scintillation Counter (Packard Instrument Co., Downer's Grove, IL) in Aqosal-2 (DuPont/New England Nuclear, Boston, MA). Medium controls with [3H]AAF but not containing cells were run in each experiment. Recovery of radioactivity from the column was greater than 90%.

S. typhimurium Assay. The S. typhimurium assay was performed according to methods reported by Ames et al. (31) as modified by Fix et al. (34) for using isolated hepatocytes. The following were added to 2 ml of top agar at 45°C: 100 μl of a 16-h nutrient broth culture of S. typhimurium TA98; the test chemical dissolved in dimethyl-sulfoxide (50 μl) and HBSS containing the isolated hepatocytes (1 ml). The volumes varied with cell concentration and amount of chemical per
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Therefore, all hepatocytes including those from rat liver were prepared by the slicing technique in subsequent studies.

The dose responses and cell number responses for human and rat hepatocyte-mediated Salmonella mutagenesis are shown in Figs. 2 and 3. Each Roman Numeral in the figures represents a separate human donor (see Table 1) with results from Individuals III–VII illustrated because only these cases yielded a sufficient number of cells necessary to do complete dose and cell number response experiments. In addition, mutagenesis data for Individuals I, II, and IX were obtained using 10 μg/AF/plate at a cell concentration of 1 x 10^6 cells/plate. Hepatocytes from these individuals induced 651, 226, and 481 revertants.

Fig. 1. Profiles of HPLC chromatogram (—) and corresponding radioactivity chromatogram (---) of AAF metabolites. Separation via HPLC was achieved under the following condition; Segment 1, 28% isopropanol with 0.01% desferal mesylate:72% 0.01 M acetic acid with 0.01% desferal mesylate for 20 min; Segment 2, 100% isopropanol with 0.01% desferal mesylate for 10 min.

Table 2. Viability and Salmonella mutagenesis comparison of AF and AAF by hepatocytes prepared by the slicing and perfusion technique.

Both techniques were performed on liver from each individual in the table.

<table>
<thead>
<tr>
<th></th>
<th>Individual I</th>
<th>Individual III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sliced</td>
<td>Perfused</td>
</tr>
<tr>
<td>AF concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 μg/plate</td>
<td>18</td>
<td>21</td>
</tr>
<tr>
<td>1 μg/plate</td>
<td>63</td>
<td>97</td>
</tr>
<tr>
<td>10 μg/plate</td>
<td>203</td>
<td>182</td>
</tr>
<tr>
<td>25 μg/plate</td>
<td>298</td>
<td>250</td>
</tr>
<tr>
<td>AAF (25 μg/plate)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Viability (%)</td>
<td>92</td>
<td>90</td>
</tr>
</tbody>
</table>

^ TA 98 revertant counts based on 2 plates/point at a cell concentration of 0.5 x 10^6 hepatocytes/plate.

\(^2\) Dimethyl sulfoxide control for background revertant measurements.

\(^3\) ND, not done.

\(^4\) Viability determined by trypan blue exclusion procedure.

Plate. The agar mix was vortexed gently and poured on Vogel-Bonner Medium E plates and incubated inverted at 37°C. The mixture of test chemicals, hepatocytes, and bacteria was preincubated for 30 min at 37°C. Each experiment was carried out with duplicate plates per treatment point. A negative control with dimethyl sulfoxide instead of AF or AAF was run as well as a rat liver S9 positive control (using 2-aminoanthracene) to monitor the quality of each assay. Colonies were counted 48 h later.

RESULTS

Cell-mediated Salmonella Mutagenesis. Because preliminary studies indicated that many human liver samples received were not sufficiently perfusable to yield adequate numbers of viable hepatocytes, the possibility of using the slicing technique on these samples was investigated. The data in Table 2 indicate that hepatocytes obtained by the slicing technique (30) and the perfusion technique (32) from liver samples from the same individual were comparable in their viabilities and in their abilities to activate AAF and/or AF to Salmonella mutagens.

Fig. 2. Effect of AF concentration and human and rat hepatocyte-mediated mutagenesis of Salmonella TA98. Results are from individual human cases and the average of three rat experiments using 1 x 10^6 hepatocytes/plate. The revertant values are the average of 2 plates/concentration. The variation between the 2 averaged revertant values is ±15% of the stated value. Zero axis values, dimethyl sulfoxide controls.

Fig. 3. Effect of human and rat hepatocyte number on Salmonella TA98 revertants. a, revertant values for AF at 25 μg/plate; b, revertant values for AAF at 25 μg/plate. Results are from individual human cases and the average of three rat experiments. The revertant values are the average of 2 plates/cell number. The variation between the 2 averaged revertant values is ±15% of the stated value. Zero axis values, dimethyl sulfoxide controls.
HEPATOCYTE METABOLISM AND MUTAGENIC ACTIVATION OF AAF

perturbs/plate, respectively. As shown in Fig. 2, levels of *Salmonella* revertants increased with AF concentration for both human and rat hepatocytes. Each human case exhibited a higher mutagenic response at 10 and 25 µg/plate than was observed with rat hepatocytes, although at the 1 µg/plate dose the differences were smaller. In Fig. 3, a and b, the cell number responses for AF and AAF, respectively, indicate that at all cell concentrations, the mutagenic response was higher with human hepatocytes than with rat hepatocytes. In Figs. 2 and 3, the variation in the ability to activate the aromatic amine by the different human samples is apparent. However, rat hepatocytes prepared from different animals exhibited a relatively consistent level of mutagenic activity. For example, in studies at 10 µg/plate AF at a cell concentration of 1 x 10^6 cells/plate, the range for eight human samples was about 6-fold (198–1225 revertants/plate) with the variation by rat hepatocytes being less than 1.5 fold.

Metabolism of AAF. To determine concentration and times for AAF metabolism studies, dose-response studies with rat hepatocytes and a time course experiment with human and rat hepatocytes were conducted. In Table 3, the overall percentage of metabolism and percentage of metabolism to water-soluble products by rat hepatocytes were measured at four AAF concentrations. With increasing AAF levels the percentage of dose metabolized decreased. In addition, the ratio of percentage of water-soluble:percentage of organic-soluble metabolism decreased with increasing AAF concentration.

Fig. 4 illustrates the time-dependent rat and human hepatocyte metabolism of AAF separated into different metabolite fractions. Rat and human hepatocytes showed an increase in the amount of organic-soluble metabolite formation with time; however, hepatocytes from Humans (HL) IV and V demonstrated peaks in ether extractable metabolites at 1 and 2 h, respectively (Fig. 4a). Levels of human hepatocyte organic-soluble metabolite formation from these three cases were higher at all time points than in the rat. Both rat and human hepatocytes also showed a time-dependent increase for water-soluble metabolites and for covalently bound products (Fig. 4, b and c). Despite lower metabolism, rat hepatocytes had equal or higher levels of covalent binding of AAF than the three human cases. Total AF production increased with time although human hepatocytes deacetylated AAF to a greater extent than rat hepatocytes (Fig. 4d). C-OH-AAF (summation of all carbon hydroxylated metabolites) formation exhibited variability among human hepatocytes with peaks at 1 h for Individual IV, 2 h for Individual V, and an increase over the 4-h time period for Individual VI and by rat hepatocytes. N-OH-AAF formation varied in the three different human hepatocyte preparations, was lower in the rat hepatocytes than in Individual V and VI, but was similar to the hepatocytes from Individual IV. Based on the results from the dose-response (Table 3) and time-course experiments (Fig. 4), human and rat hepatocyte metabolism

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>% of total AAF metabolized</th>
<th>% of water-soluble metabolism</th>
</tr>
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<tbody>
<tr>
<td>0.44</td>
<td>26.7</td>
<td>20.2</td>
</tr>
<tr>
<td>4.4</td>
<td>19.6</td>
<td>8.1</td>
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<tr>
<td>44</td>
<td>9.9</td>
<td>3.2</td>
</tr>
<tr>
<td>440</td>
<td>3.3</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*The percentage of total AAF metabolized and percentage of water-soluble metabolism were based on the initial amount of [14C]AAF added. Total percentage of AAF metabolized includes the percentage of organic-soluble and the percentage of water-soluble metabolism.

with 4 µM AAF was measured at 4 h. In Table 4, total metabolism by hepatocytes from each individual human case and the average of 3 experiments for the rat are shown. Hepatocytes from Individual I had the highest overall metabolism (49.4%) of AAF and highest metabolism to water-soluble products (45.4%) with Individual IV exhibiting the lowest metabolism (19.8% and 7.3%, respectively). The range of conjugative metabolism by human hepatocytes was greater (7.3–45.4%) than the range of overall metabolism (19.8–49.4%). Except for Individual IV, human hepatocyte metabolism of AAF to organic and water-soluble products was greater than rat hepatocyte metabolism.

The ranges of specific metabolite levels formed by hepatocytes from the eight human cases and the rat are shown in Table 5. The major organic-soluble metabolites formed by both human and rat hepatocytes were AF, 7-OH-AAF, and the
HEPATOCELLULAR METABOLISM AND MUTAGENIC ACTIVATION OF AAF

unknown metabolite(s). The primary glucuronide- and sulfate-conjugated product formed by human and rat hepatocytes was the 7-OH-AAF although sulfate and glucuronide conjugates were formed with 8-OH-AAF, 5/9-OH-AAF, 1/3-OH-AAF, N-OH-AAF, and AF. Levels of organic-soluble and water-soluble N-OH-AAF formation were low compared to other metabolites formed by both human and rat hepatocytes. AF formation was highest in Individuals V, VI, and VII and lowest in Individuals I and VIII (data not shown). It is interesting that while total metabolism or water-soluble metabolism with human hepatocytes varied 2.5- and 6-fold (Table 4), respectively, the range of individual metabolites such as AF (organic-soluble) varied 35-fold while glucuronide and sulfate conjugates of 7-OH-AAF varied 30- and 36-fold, respectively, in eight human cases (Table 5).

DISCUSSION

In the present study, human hepatocytes were compared to rat hepatocytes for their ability to metabolize AAF and AF and to gain insight into interindividual variations in the human population for AAF metabolism. The slicing technique used to prepare hepatocytes in the present study is valuable because it utilizes samples from biopsy or resected tissue that are too small or are otherwise not perfusable. In six of nine cases in this study, the tissues obtained were not perfusable. The slicing technique therefore allows studies to be done on a more frequent basis with human hepatocytes and yields hepatocytes with a viability and mutagenic activity comparable to hepatocytes prepared via the perfusion technique (Table 2).

Intact hepatocytes are useful in metabolism and genotoxicity studies because of their advantages when compared to isolated subcellular preparations (20, 27, 34, 35). Intact hepatocytes catalyze Phase I and Phase II reactions at rates and in the sequence that they occur in vivo (28), as well as preserving their metabolizing capabilities for a longer period of time than subcellular fractions (36). Differences in levels of mutagenic activity are also apparent between subcellular fractions and intact hepatocytes (27, 34). Levels of AF and AAF mutagenic activity previously measured (20, 23, 30, 35, 37-39 and data not shown) are higher in S9 preparations than intact hepatocytes from humans and rats possibly due to a lack of detoxifying pathways that are present in intact hepatocytes but not in subcellular preparations. Previous investigation of AAF metabolism by human liver microsomes (23, 40) did not detect AF, although deacetylation activity has been measured in microsomal fractions from guinea pigs, dogs, hamsters, mice, and rats (41) and with intact hepatocytes in the present study. Other studies have illustrated extensive species differences in the metabolism and mutagenesis of many environmental compounds (7-10, 30, 39, 42-45) and these results also may be useful in the extrapolation of animal data to represent hazard levels in humans (45).

In the present study, rat and human hepatocytes metabolized AAF in a qualitatively similar fashion. The major metabolite produced by rat and human hepatocytes was AF. Evidence of the importance of AF as an AAF metabolite is provided by our findings that levels of AF mutagenesis were substantially higher than with AAF and that AF was the major metabolite formed while N-OH-AAF, which is a mutagenically active metabolite isolated primarily in microsomal preparations (21, 23, 40, 46) was a minor metabolite in both human and rat hepatocytes. It should be noted, however, that in hepatic microsomal preparations N-OH-AAF was more mutagenically active than AF (47). Because AF may be further metabolized (48, 49) and N-OH-AAF can be metabolically changed primarily via deacetylation to a reactive form (18, 38, 50), the relative contribution of these two compounds to the mutagenic process cannot be clearly established.

The C-hydroxylation of AAF by human and rat hepatocytes represents a step in the detoxification process. In addition to the normal C-hydroxylated products the tentative identification of 8-OH-AAF was reported in rat hepatocytes in this study and previously (30) and in human hepatocytes for the first time. The 8-OH-AAF had also been detected in rat urine (33). The unknown peak in the HPLC chromatogram and 7-OH-AAF were the major organic-soluble C-hydroxylated metabolites with the 5/9-OH-AAF, 3-OH-AAF, 1-OH-AAF, and 8-OH-AAF appearing in roughly equivalent levels in hepatocytes from human individuals. The 7-OH-AAF was the primary glucuronide and sulfate conjugate formed by human and rat hepatocytes. It has been previously reported that C-hydroxylated glucuronides are the only detectable water soluble metabolites formed in rat hepatocytes (7). In the present study both conjugates were detected using longer enzymatic times than in previous sulfatase and β-glucuronidase hydrolysis experiments (51). AF-conjugated products were also identified (Table 5) based on the extractable radioactivity after sulfatase and β-glucuronidase treatment, but the structural nature of the conjugates could not be positively identified.

Quantitative differences in rat and human hepatocyte metabolism were evident when comparing levels of overall organic-soluble and water-soluble metabolite formation with human hepatocyte metabolism higher than that by rat hepatocytes. This was also true for water-soluble metabolite formation in 7 of 8 human cases in which the specific metabolite levels, espe-
ially AF, 7-OH-AAF, and 5/9-OH-AAF, were appreciably higher in humans than rats. However, there was a higher conversion of total water-soluble metabolites by rat hepatocytes to glucuronide and sulfate conjugates (>85%) than by human hepatocytes (40–67%), although humans exhibited a higher overall water-soluble metabolite level. Although it was not investigated in the present study, a possible reason for this discrepancy is that more glutathione and amino acid conjugates of AAF metabolites are produced by human hepatocytes than are produced by rat hepatocytes. The higher level of such conjugates produced by human hepatocytes may indicate a species difference between humans and rats in the sensitivity and susceptibility to the hepatocarcinogenicity of aromatic amines. Rat, guinea pig, and hamster differences in hepatocarcinogenic susceptibility have been reported (18, 19) and Holme and co-workers (7) have illustrated differences in the ability of these species to detoxify AAF.

Another factor to be considered when extrapolating animal data to humans is the wide degree of interindividual variation in humans. The extent of interindividual variation in humans is well documented with the emphasis divided on factors related to environment or heredity (9, 52, 53). Genetic factors, nutrition, drug intake, cigarette smoking, and exposure to pollutants are but a few of the factors that modify the way human enzymatic systems and in particular the cytochrome P-450 system metabolize foreign and endogenous substances (52, 54–56). The metabolic polymorphisms and substrate specificities of certain P-450 isozymes in the hydroxylation of compounds such as debrisoquine and sparteine (4, 5, 9, 37), populations divided into slow and fast acetylators (38, 57), and findings that have identified multiple P-450s all attest to a widespread variation in the present study shows the practicality and utility of investigating the metabolism and activation of carcinogens with human tissues. Such data when used in combination with rodent bioassay data and other results from frequently used short-term assays can be useful for risk assessment of environmental chemicals to humans.

**REFERENCES**


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