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Determination of Aflatoxin B_1 Biotransformation and Binding to Hepatic Macromolecules in Human Precision Liver Slices

JOHN T. HEINONEN,* ROBYN FISHER,† KLAUS BRENDEL,† AND DAVID L. EATON*,[1]

*Department of Environmental Health, University of Washington, Seattle, Washington 98195; and †Department of Pharmacology and Toxicology, University of Arizona, Tucson, Arizona 85721

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Although epidemiological studies suggest that aflatoxin B_1 (AFB_1) is a human carcinogen, at least in the presence of hepatitis B virus infection, animal studies have demonstrated large differences in species sensitivity to AFB_1, and the sensitivity of humans relative to experimental animals remains unclear. The purpose of this study was to determine the profile of AFB_1 metabolism and the extent of AFB_1 binding to cell macromolecules in human liver slices under experimental conditions that would allow direct comparison to similar endpoints in the rat, a species sensitive to the carcinogenic actions of AFB_1. Liver slices were prepared from three individual human liver samples with a Krumdieck tissue slicer and incubated with 0.5 μM [^{3}H]AFB_1 for 2 hr. Significant interindividual variations were observed in the rates of oxidative metabolite formation and in specific binding to cell macromolecules. The rates of oxidative metabolism of AFB_1 to AFQ_1, AFP_1, and AFM_1 in the three human liver samples were similar to those previously observed in rat liver slices. AFB_1–GSH conjugate formation was not detected in any of the human liver samples, and yet specific binding of AFB_1 to cell macromolecules was considerably lower in the human liver slices relative to that in rat liver slices. AFB_1–DNA binding levels ranged from 3 to 26% of control rat and AFB_1–RNA binding levels ranged from 25 to 49% of control rat. The AFB_1–protein binding level in the one human sample measured was 20% of that observed for control rat. While these results suggest that humans do not form as much AFBO as the rat, they are also consistent with the hypothesis that humans do not possess GST isozyme(s) with high specific activity toward AFBO. Significant individual differences in AFB_1 metabolism and binding between humans suggest the presence of genetic and/or environmental factors that may confer large variability in susceptibility to AFB_1.

Aflatoxin B_1 (AFB_1)[2] is a mycotoxin produced by the molds *Aspergillus flavus* and *parasiticus* (Wogan, 1973). AFB_1 is a potent hepatotoxin and hepatocarcinogen in many animal species and has been implicated as an environmental factor in the etiology of human liver cancer (Groopman *et al.*, 1988; Ross *et al.*, 1992; Eaton *et al.*, 1994; Hall and Wild, 1994). AFB_1 biotransformation occurs principally by the cytochrome P450 (CYP450) mixed function monooxygenase system and involves multiple CYP450 isozymes that show considerable variation in kinetic characteristics and product specificity between species (see Eaton and Gallagher, 1994, for a recent review). The predominant AFB_1 metabolites from CYP450-catalyzed reactions in mammals are aflatoxin M_1 (AFM_1), aflatoxin Q_1 (AFQ_1), aflatoxin P_1 (AFP_1), and aflatoxin B_1-8,9-epoxide (AFBO). AFQ_1, AFM_1, and AFP_1 are substantially less carcinogenic than the parent AFB_1 and are generally considered detoxification products (Campbell and Hayes, 1976; Eaton and Ramsdell, 1991). In contrast, AFBO is a chemically unstable, potent electrophile that readily forms adducts with DNA, RNA, and protein (Lin *et al.*, 1977; Essigmann *et al.*, 1982). Detoxification of AFBO in rodents occurs mainly by conjugation with glutathione (GSH) via glutathione *S*-transferase (GST) (Degen and Newman, 1978; Emerole *et al.*, 1979; Monroe and Eaton, 1987; Ramsdell and Eaton, 1990a,b).

Epidemiological studies of liver cancer incidence in populations where dietary aflatoxin exposures are high have provided much circumstantial evidence for the development of AFB_1-induced liver cancer in humans (Shank *et al.*, 1972; Peers and Linsell, 1973; Van Rensburg *et al.*, 1985). However, in these same populations there also exists a high incidence of hepatitis B virus (HBV) infection which, along with aflatoxin exposure, has been associated with liver cancer (Peers *et al.*, 1987; Yeh *et al.*, 1989). Recent reports

[1] To whom correspondence should be addressed at Department of Environmental Health, XD-41, University of Washington, Seattle, WA 98195. Fax: (206)685-4696.

[2] Abbreviations used: AFB_1, aflatoxin B_1; AFB_1-GSH, 8,9-dihydro-8-(*S*-glutathionyl)-9-hydroxyaflatoxin B_1; AFM_1, aflatoxin M_1; AFBO, aflatoxin B_1-8,9-epoxide; AFP_1, aflatoxin P_1; AFQ_1, aflatoxin Q_1; EQ, ethoxyquin; CYP450, cytochrome P450; DMSO, dimethyl sulfoxide; DOC, dynamic organ culture; GST, glutathione *S*-transferase; HBV, hepatitis B virus; HL, human liver; HLM, human liver microsomes; TAO, troleandomycin.

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from a continuing nested case–control study in China now indicate that the unusually high incidence of liver cancer in this study region may be the result of a synergistic interaction between AFB_1 exposure and HBV infection (Ross *et al.*, 1992; Qian *et al.*, 1994). The most recent report from this study found a relative risk of 3.4 for aflatoxin exposure alone, a relative risk of 7.3 for HBV alone, but a relative risk of about 60 for combined aflatoxin exposure and HBV (Qian *et al.*, 1994). Despite the potential of epidemiologic methods to assess the risk associated with AFB_1 exposure, there are obvious experimental limitations. These limitations underline the importance of developing experimental systems utilizing viable human tissues to study the effects of AFB_1 exposure.

We have recently completed studies of AFB_1 metabolism and binding to cell macromolecules in liver slices isolated from mice, control rats, and rats treated with the dietary antioxidant ethoxyquin (EQ). We found that AFB_1 metabolism and binding levels in control rat liver slices were comparable to those reported previously in *in vivo* rat studies (unpublished observations). In addition, results of experiments using liver slices from EQ-treated rats showed an expected increase in AFB_1–GSH formation with a corresponding decrease in macromolecular adduct formation. Liver slices isolated from mice, a species with high GST activity toward AFBO that is virtually resistant to the carcinogenic effects of AFB_1, had 70-fold higher rates of AFB–GSH conjugation and less than 2% of the AFB–DNA adducts found in control rat liver slices. EQ has been shown in rats to induce two GST isozymes containing a common subunit, designated Yc_2, with notably high activity toward AFBO (Hayes *et al.*, 1991). EQ treatment in rats substantially increases liver cytosolic GST activity toward AFBO (Mandel *et al.*, 1987; Hayes *et al.*, 1991), increases biliary excretion of the AFB_1–GSH conjugate (Kensler *et al.*, 1986), and substantially decreases the level of AFB–DNA adducts and the appearance of hepatic preneoplastic foci in rats administered a carcinogenic dose of AFB_1 (Cabral and Neal, 1983; Kensler *et al.*, 1985). These studies demonstrate that precision-cut liver slices provide an appropriate means to compare the disposition of AFB_1 in intact liver between species, including humans. The human liver slice incubation approach used in this investigation provides AFB_1–DNA binding data for human tissue that would be difficult to obtain otherwise. This technique should more closely account for *in vivo* conditions, such as competing reactions by endogenous substances and the rate of DNA repair in humans, that are difficult to extrapolate from *in vitro* assays and animal models.

MATERIALS AND METHODS

Chemicals. Aflatoxins (B_1, G_1, M_1, P_1, and Q_1), Hepes, Krebs–Henseleit buffer and lauryl sulfate were purchased from Sigma Chemical Co. (St. Louis, MO). DNA grade urea was purchased from Stratagene (La Jolla, CA), and hydroxyapatite was purchased from Calbiochem (San Diego, CA). [^{3}H]AFB (16 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA). HPLC-grade solvents were purchased from J. T. Baker (Philipsburg, NJ).

Human liver slice incubations and analysis. Viable human liver tissue was obtained through the laboratory of Dr. Klaus Brendel at the University of Arizona. Human liver-1 (HL-1) was obtained from a 66-year-old Caucasian male. Intercranial bleeding was the cause of death. HL-2 was obtained from a 30-year-old Caucasian male. The cause of death was listed as cerebrovascular and medical history showed positive toxicology screens for cocaine and benzodiazepine. HL-3 was obtained from a 38-year-old female. Race was listed as unknown and cause of death was listed as closed head injury. Serology tests were negative for all samples.

Human liver slice preparation, incubations, and determination of slice viability by intracellular K^+ were carried out in Dr. Brendel's laboratory. Rat liver slice experiments described under Results were carried out in our laboratory exactly as described for human liver slices. Liver slices were prepared as described by Krumdieck *et al.* (1983) using the Krumdieck tissue slicer and liver slice incubations were performed by dynamic organ culture (DOC) as described by Smith *et al.* (1985a). Briefly, an 8-mm metal biopsy corer was used to prepare tissue cores from the liver samples. Cores were then loaded into the Krumdieck slicer and sliced while submerged in ice-cold Krebs–Henseleit buffer. Optimal slice thickness was previously shown to be 250 μm (Krumdieck *et al.*, 1983). Liver slices were placed in dynamic organ culture vials (2 slices/vial) containing 2 ml of Krebs–Henseleit buffer supplemented with 15 mM Hepes (pH 7.5). Vials were then placed on a rotating vial incubator set at 37°C and 1 rpm. Slices were preincubated for 2 hr and then treated with 20 μl of 50 μM [^{3}H]AFB_1 (1.8 mCi/μmol) in DMSO for a final concentration of 0.5 μM AFB_1 and 1% DMSO. Additional slices were incubated with Krebs–Henseleit buffer and Krebs–Henseleit buffer containing 1% DMSO as media and vehicle controls, respectively. Slice incubations with test chemicals were carried out for 2 hr. Previous studies in rats have shown that AFB_1–DNA binding *in vivo* reaches a maximum at 2 hr following AFB_1 administration (Croy and Wogan, 1981; Essigmann *et al.*, 1982) and the majority of biliary metabolites can be recovered from the bile during the first 2 hr following ip administration of AFB_1 (Holeski *et al.*, 1987). Intracellular K^+ determinations were performed as described by Fisher *et al.* (1990).

Following incubation, human liver slices and media were pooled and then frozen in liquid nitrogen. Samples were shipped on dry ice by next day air to our laboratory for determination of AFB_1 metabolism and binding to cell macromolecules. Briefly, for each liver sample, media from each set of four vials were pooled to obtain a total of three pooled fractions (8 ml/fraction) from [^{3}H]AFB-treated slices and one pooled fraction from DMSO-treated controls. AFB_1 metabolites were extracted from media using BakerBond C^{18} reverse-phase extraction columns (J. T. Baker, Inc., Phillipsburg, NJ.) then quantified by HPLC as described by Monroe and Eaton (1987). Slice DNA, RNA, and protein were isolated from each set of four vials (eight slices total per set) according to the method of Beland *et al.* (1979). Concentrations of purified DNA and RNA were determined spectrophotometrically at 260 nm. Purity was measured as the ratio of absorption at 260 nm to absorption at 280 nm. Reconstituted protein concentrations were determined by the bicinchoninic acid method (BCA) (Pierce Chemical Co., Rockford, IL) using bovine serum albumin as a standard (Smith *et al.*, 1985b). Measurements were made in triplicate on a Molecular Devices Microplate Reader (Menlo Park, CA). [^{3}H]AFB_1-derived radioactivity in DNA, RNA, and protein fractions were quantified in Westchem EcoLite scintillation fluid (Scientific Resource Associates, Inc., Redmond, WA) on a Beckman Model 3800 scintillation counter equipped with automatic quench compensation. The activity of the samples was calculated automatically as dpm (disintegrations per min) using a quench curve constructed with sealed standards. Quantification of [^{3}H]AFB_1-derived radioactivity in all samples was based on dpm values that were at least twofold above background

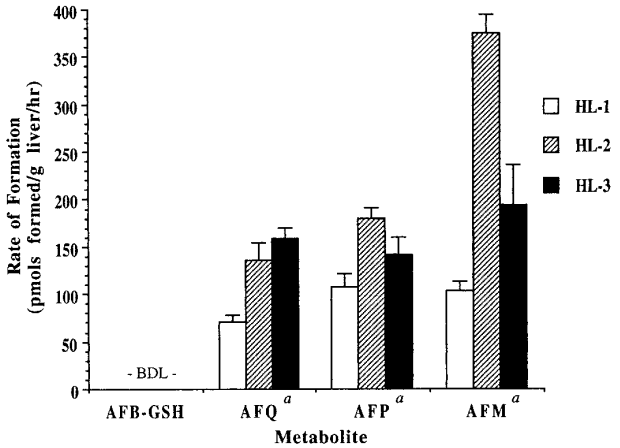

FIG. 1. *In vitro* metabolism of AFB_1 by precision liver slices isolated from three human livers. Incubations were performed as described under Materials and Methods. Slices were preincubated 2 hr in culture media only and then incubated 2 hr in media containing 0.5 μM [^{3}H]AFB_1 and 1% DMSO. Values for human liver samples are given as means ± SE from triplicate analyses. [a]Differences between individuals are significant ($p < 0.05$). Abbreviations used: HL, human liver; BDL, below detection limit

values. Results are expressed as picomole AFB_1 equivalents bound per milligram macromolecule.

Statistical analysis. The StatView statistics program (BrainPower Inc., Calabasas, CA) was used to compare liver samples using analysis of variance (ANOVA). Scheffe's *F* test was used to establish significant differences in AFB_1 metabolism and binding between liver samples. Triplicate analyses for AFB_1 metabolism and binding to cell macromolecules were performed for each liver sample.

RESULTS

Based on intracellular K^+ values, neither DMSO or 0.5 μM AFB_1 had any adverse effect on human liver slice viability (data not shown). Mean intracellular K^+ values for slices incubated in media only (media control) ranged from 49.0 to 59.8 μmol K^+/g liver. Intracellular K^+ values for slices incubated with 1% DMSO (vehicle control) ranged from 95 to 97% of the media control values and slices incubated with 0.5 μM AFB_1 ranged from 87 to 101% of the media control values.

Figure 1 compares the rates of AFB_1–GSH, AFQ_1, AFP_1, and AFM_1 formation in liver slices obtained from three human liver samples. AFB_1–GSH conjugate formation was not detected in any of these samples (detection limits ranged from 46 to 73 pmol/g liver/hr). The lack of AFB_1–GSH formation was attributed to a lack of GST activity rather than a lack of activation because AFB_1 binding data suggested that the human liver slices used in this experiment formed appreciable amounts of AFBO (Fig. 2), and because of *in vitro* experiments which have demonstrated that human liver cytosolic fractions have little or no GST activity toward AFBO (Moss and Neal, 1985; Eaton *et al.*, 1990; Raney *et al.*, 1992a).

The rates of AFQ_1, AFP_1, and AFM_1 formation were found to vary significantly between individuals by one-way analysis of variance (ANOVA) ($p = 0.009$, 0.034, and 0.001 for AFQ_1, AFP_1 and AFM_1, respectively). Interindividual variation in the rate of formation of these metabolites was 2.3-, 1.7-, and 3.6-fold for AFQ_1, AFP_1, and AFM_1, respectively.

Figure 2 compares the specific binding of AFB_1 to cell macromolecules in human liver slices after a 2-hr incubation with 0.5 μM [^{3}H]AFB_1. Again, significant differences existed between individuals ($p = 0.013$ and 0.003 for DNA and RNA binding, respectively). Specific binding to DNA varied as much as ninefold, while specific binding to RNA varied up to twofold. Specific binding to protein in samples HL-2 and HL-3 could not be measured do to technical problems.

Table 1 compares the rates of AFB_1–GSH, AFQ_1, AFP_1, and AFM_1 formation in human liver slices with the rates observed in liver slices isolated from Sprague–Dawley rat. The rates of oxidative metabolism of AFB_1 to AFQ_1, AFP_1, and AFM_1 in the three human liver samples were similar to those previously observed in rat liver slices.

Table 2 compares AFB_1 binding to DNA, RNA, and protein in human liver slices with binding in rat liver slices and with previous binding studies in rat and human hepatocytes. The level of AFB_1–DNA adduct formation in the human samples were all lower than that seen in control rat liver slices. HL-1, HL-2, and HL-3 had AFB_1–DNA binding levels that were 3, 26, and 13% of those for control rat, respectively; and AFB_1–RNA binding levels that were 25, 49, and 40% of those for control rat, respectively. The AFB_1–protein binding level in the one human sample measured was 20%

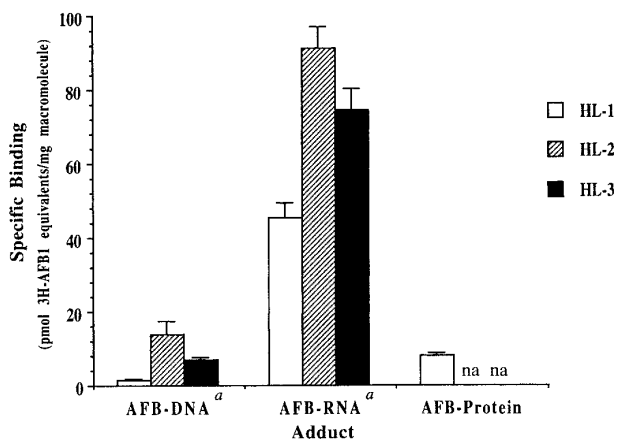

FIG. 2. *In vitro* covalent binding of AFB_1 to hepatic DNA, RNA, and protein in precision liver slices isolated from three human livers. Incubations were performed as described under Materials and Methods. Slices were preincubated 2 hr and then incubated 2 hr with 0.5 μM [^{3}H]AFB_1. Values for human liver samples are given as means ± SE from triplicate analyses. [a]Differences between individuals are significant ($p < 0.05$). Abbreviations used: HL, human liver; na, not available

TABLE 1
Comparison of AFB_1 Metabolism in Liver Slices Isolated from Three Human Livers and from Sprague–Dawley Rat

Sample	Metabolite rate of formation (pmol/g liver/hr)			
	AFB–GSH	AFQ	AFP	AFM
HL-1	<48[a]	70.0 ± 7.1	108 ± 14.2	103 ± 10.3
HL-2	<46[a]	135 ± 19.2	180 ± 11.0	375 ± 19.1
HL-3	<73[a]	158 ± 11.4	142 ± 17.3	194 ± 41.7
SD rat (M)[b]	74.7 ± 12.2	243 ± 19.5	141 ± 30.5	141 ± 19.0

[a] Rate values represent analytical limits of detection (4 pmol AFB–GSH). Data represent means ± SE of triplicate incubations. Incubations were performed as described under Materials and Methods. Slices were preincubated 2 hr in culture medium and then incubated 2 hr in medium containing 0.5 μM [^{3}H]AFB_1 and 1% DMSO.

[b] Data represent means ± SE of triplicate incubations ($n = 6$). Incubations were performed as described under Materials and Methods. Slices were preincubated 2 hr in culture media and then incubated 2 hr in media containing 0.5 μM ^{3}H-AFB_1 and 1% DMSO. Abbreviations used: HL, human liver; M, male; SD, Sprague–Dawley.

of that observed for control rat. For comparison of AFB_1 binding in liver slices with binding in hepatocytes, results from previous studies by Loury *et al.* (1984), Salocks *et al.* (1984), and Cole *et al.* (1988) are included in Table 2.

DISCUSSION

Previous work in our laboratory has shown that when the rate of *in vitro* AFBO formation is expressed as a fraction of total oxidative AFB_1 biotransformation (activation ratio), the proportion of AFBO formed by human and rat liver microsomes is greater at low, nonsaturating, substrate concentrations (16 μM) than at high, saturating, substrate concentrations (128 μM) (Ramsdell and Eaton, 1990b; Ramsdell *et al.*, 1991; Gallagher *et al.*, 1994). These *in vitro* studies suggest that at the relatively low tissue concentrations of AFB_1 that result from dietary exposures, a large proportion of an AFB_1 dose would be converted to AFBO in both of these species. In addition, because humans have negligible GST activity toward AFBO (Moss and Neal, 1985; Eaton *et al.*, 1990; Raney *et al.*, 1992a,b), humans would be expected to be more efficient than rats at generating AFBO–macromolecular adducts. However, in our studies using rat and human liver slices, the three human liver samples had AFB_1–DNA adduct levels that ranged from only 3 to just 26% of control rat levels (Table 2). AFB_1–RNA and AFB_1–protein adduct levels were also substantially lower in human liver slices than in rat liver slices.

In the context of what is known about AFB_1 metabolism and binding to cell macromolecules in rodents, the relatively low level of AFB_1 adduct formation in the human liver slices was unexpected. It can be seen in Table 1 that the overall rates of oxidative metabolism of AFB_1 to AFQ_1, AFP_1, and AFM_1 were found to be comparable between Sprague–Dawley rat and the three human samples. However, the predominant AFBO detoxification product in rodents, AFB_1–GSH, was not detected in any of the human samples. This finding is consistent with previous *in vitro* studies where to date human liver cytosol samples have been shown to have little or no measurable GST activity toward AFBO (Moss and

TABLE 2
Comparison of Present Study with *in Vitro* AFB_1 Binding to DNA, RNA, and Protein in Rat Liver Slices and in Hepatocytes Isolated from Rat and Human Donor Tissue

Reference	AFB exposure		Species		AFB binding (pmol AFB equivalents/mg macromolecule)		
	[AFB] (μM)	Time (hr)			DNA	RNA	Protein
Liver slices							
Present Study	0.5	2	Human	HL-1, C, M	1.6	45.5	7.95
				HL-2, C, M	14.0	91.1	na
				HL-3, F	7.0	74.5	na
	0.5	2	Rat	SD, M	53.6	187	40.4
Hepatocytes							
Loury *et al.* (1984)	0.11	6	Rat	SD, M	150	74	na
Salocks *et al.* (1984)	0.1	6	Rat	SD, M	271	341	31
Cole *et al.* (1988)	0.2	24	Rat	SD, M	203	na	na
				SD, F	38	na	na
			Human	C, M	53	na	na
				C, F	25	na	na
				B, F	69	na	na

Note. Abbreviations used: C, Caucasian; B, black; M, male; F, female; SD, Sprague–Dawley; na, not available.

Neal, 1985; Raney *et al.,* 1992a). The relative lack of human GST activity toward AFBO together with the relatively low adduct levels in human liver slices suggests that humans either do not form as much AFBO as rat or they possess some as yet undescribed mechanism that either protects against adduct formation or provides for rapid repair of AFB_1 adducts to cell macromolecules.

In a previous study by Cole *et al.* (1988), AFB_1–DNA binding was also reported to be lower in humans than in rats (Table 2). In that study, hepatocytes isolated from three human liver samples had AFB_1–DNA adduct levels that ranged from 12 to 34% of control rat levels. Interestingly, AFB–DNA binding levels in these human hepatocytes were on average 6.5-fold higher than in human liver slices. However, when these data are compared on the basis of percentage AFB–DNA binding in rat, the results are remarkably similar despite differences in method, AFB exposure (e.g., AFB concentration and incubation time), and source of human tissue.

One conspicuous difference between our previous work using human liver microsomes (HLM) (Eaton and Gallagher, 1994) and our present study using human liver slices is that humans do not form appreciable amounts of AFP_1 in microsomal assays. The only report of AFP_1 formation in HLMs came from studies of normal and tumorous tissue from liver cancer patients in Thailand (Kirby *et al.,* 1993). Aside from a report by Crespi *et al.* (1990) that shows CYP2A3 is capable of catalyzing AFP_1 formation, little else is known about the enzymes responsible for AFP_1 formation. We have not observed AFP_1 formation in any of the HLM samples we have studied thus far (Ramsdell and Eaton, 1990b; Ramsdell *et al.,* 1991; Gallagher *et al.,* 1994), while in human liver slices we observed a substantial amount of AFP_1 formation (Fig. 1). To determine if this apparent discrepancy is due to the particular human samples used in the respective experiments, or if AFP_1 formation is somehow dependent on the intact nature of the liver slice, warrants further investigation. However, based on the limited *in vivo* human dosimetry data for AFB_1 metabolites, it appears that *in vitro* liver slice data may be relatively more representative of human AFB_1 metabolism *in vivo* when compared to microsomal assays, as AFP_1 has been found to be a major metabolite in urine of people with dietary exposure to AFB_1 (Groopman *et al.,* 1992).

With respect to interindividual variation in AFB_1 metabolism, it is interesting to note that the human liver sample with the substantially higher rate of AFM_1 formation, HL-2 (Fig. 1), also had the highest rate of AFBO formation as measured by specific binding to DNA and RNA (Fig. 2). In fact, there appears to be a positive correlation between AFM_1 formation and AFBO formation among the three human liver samples used in this study. Previous studies in this laboratory suggest that this apparent correlation may not be coincidental. For example, we have previously shown that in a panel of 13 HLM samples, the rate of AFM_1 formation at low substrate concentrations was strongly correlated ($r^2 = 0.97$) with the rate of AFBO formation over a 7-fold range of activity (Eaton *et al.,* 1994). These data, together with the apparent correlation and 3.6-fold variation in the rate of AFM_1 formation in human liver slices, suggest that both AFM_1 and AFBO may be formed by a CYP450 isozyme that is differentially expressed in humans.

More recent work in this laboratory provides strong evidence that both AFM_1 and AFBO are formed by CYP1A2 in humans. Gallagher *et al.* (1994) showed that furafylline, a potent and specific inhibitor of human CYP1A2 (Sesardic *et al.,* 1990; Kunze and Trager, 1993), inhibited AFM_1 formation at both high (128 μM) and low (16 μM) substrate concentrations and strongly inhibited AFBO formation at low substrate concentrations in HLMs (72%, $n = 4$) and in microsomes isolated from human lymphoblastoid cell lines selectively expressing CYP1A2 (>89%). Human CYP3A4 has been shown to form both AFBO and AFQ_1 (Ramsdell *et al.,* 1991; Raney *et al.,* 1992b). However, the ratio of AFQ_1 to AFBO formed by CYP3A4 is approximately 10:1, and CYP3A4 demonstrates nonlinear kinetics such that the contribution of CYP3A4 activity to AFBO formation at very low substrate concentrations appears to be small if significant quantities of CYP1A2 are present (Gallagher *et al.,* 1994, 1995). Interestingly, there appears to be no correlation between AFQ_1 and AFBO formation in the three human liver samples used in the present study. Because liver slice incubations were carried out at relatively low substrate concentrations (0.5 μM AFB_1 in the medium), the lack of correlation between AFQ_1 and AFBO formation and the apparent correlation observed between AFM_1 and AFBO formation in human liver slices is consistent with the hypothesis that CYP1A2 forms AFM_1 and is the principal enzyme involved in AFBO formation at low substrate concentrations.

CYP1A2 expression exhibits considerable genetic variation in human liver (Wrighton *et al.,* 1986; Guengerich and Turvy, 1991). Schweikl *et al.* (1993) recently reported a >40-fold variation in expression of CYP1A2 mRNA among 19 individuals in which CYP1A2 mRNA was reported to be significantly correlated with CYP1A2 protein levels. CYP1A2 is also inducible by polycyclic aromatic hydrocarbons such as those found in cigarette smoke (Sesardic *et al.,* 1988) and charbroiled beef (Conney *et al.,* 1976). Taken together, the differential expression of CYP1A2 and the role of CYP1A2 in AFB_1 activation in human liver suggests that some AFB_1-exposed individuals may be at increased risk of developing AFB_1-induced liver cancer.

In conclusion, no AFB_1–GSH conjugate was identified in human liver slices and yet AFB_1–DNA adducts were considerably lower than those found in rat. In addition, RNA and protein adducts in human slices were also considerably

lower than those found in rat. These results indicate that humans either do not form as much AFBO as rat or possibly possess alternate pathways for AFBO detoxification. A recent human molecular epidemiology study reported a relationship between susceptibility to aflatoxin-related hepatocellular carcinoma (HCC) and the presence of a variant form of human epoxide hydrolase gene (McGlynn *et al.*, 1995). The presence of the GST M1 null allele was not significantly associated with increased risk for HCC, although small sample size hindered the power of this study to detect an association. However, because GST M1 does not appear to have significant catalytic activity toward the carcinogenic AFB-exo-8,9-epoxide (Raney *et al.*, 1992a,b), one would not *a priori* expect that the absence of the GST M1 gene would be associated with increased risk. The results of the present study in human liver slices provide further evidence that constitutively expressed human hepatic GSTs are relatively ineffective at detoxifying AFBO. Based on the relatively large amounts of AFM_1 formed in human liver slices in this study, we expect that significant amounts of AFBO would also have been formed because *in vitro* studies with human liver microsomes demonstrated that AFM_1 is formed almost exclusively by CYP1A2, which also forms AFBO at a rate about three times greater than AFM_1 (Gallagher *et al.*, 1995). Thus the extent of formation of AFBO in these liver slices should have been at least three times that of AFM_1. Because the extent of binding of AFB_1 to hepatic macromolecules was quite low compared to that observed in rat liver slices under identical conditions, these studies suggest that an as yet unidentified protective pathway may exist in human liver. The epidemiological data suggesting that human epoxide hydrolase genotype may be associated with susceptibility to aflatoxin-related hepatocarcinogenesis warrants further study into the possible role of human epoxide hydrolase in the detoxification of AFBO. Significant interindividual differences in AFB_1 metabolism and binding between humans suggest the presence of genetic and/or environmental factors that may make some humans more or less susceptible to AFB_1.

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