Aflatoxin-Albumin Adducts: A Basis for Comparative Carcinogenesis between Animals and Humans

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Abstract
The study objectives were (a) to correlate AFB₁ serum albumin adduct levels with AFB₁-DNA adduct levels in liver in different rodent species to determine whether the former could serve as a marker of hepatic DNA adduct levels irrespective of species, and (b) to relate the levels of both adducts to differences in susceptibility to tumor induction by AFB₁ in the different species. Finally, an attempt was made to compare the dose response for AFB₁-albumin adduct formation in the rodent species with that in human populations exposed environmentally to AFB₁. Three strains of rat (Fischer 344, Wistar, and Sprague-Dawley), and one strain each of guinea pig (Hartley), hamster (Syrian golden), and mouse (C57BL) were treated by gavage with up to 14 daily doses of between 1 and 80 μg AFB₁/kg body weight. Animals were killed 24 h after 1, 3, 7, or 14 days treatment. A dose response in both AFB₁-albumin and AFB₁-DNA adducts was seen for all species and strains with steady-state adduct levels at 14 days. In rat strains at 14 days after treatment with 20 μg/kg, the mean AFB₁-albumin levels were between 24 and 26 pg AFB₁-lysine equivalent/mg albumin, and the mean AFB₁-DNA adduct levels were between 1.5 and 2.5 pmol (8, 9-dihydro-8-(2, 6-diamino-4-oxo-3, 4-dihydro-pyrimid-5-ylforamido)-9-hydroxy) AFB₁/mg DNA. The level of both adducts was in the range of 1.5 to 3 pg AFB₁-lysine equivalent/mg albumin and liver DNA. The levels of AFB₁-albumin adduct also reflect at least qualitatively the relative susceptibility of the different species to AFB₁ carcinogenesis; the rat is sensitive and the hamster and mouse are resistant.

The level of AFB₁-albumin adduct formed as a function of a single dose of AFB₁ in rodents was compared to data from humans exposed environmentally to AFB₁. This comparison yielded a value for the three rat strains of between 0.3 and 0.51 pg AFB₁-lysine equivalent/mg albumin/1 μg AFB₁/kg body weight and a value for the mouse of <0.025. The best estimate for people from The Gambia and southern China was 1.56 pg/mg albumin for the same exposure. These data suggest that humans exposed to AFB₁ form amounts of albumin adducts, and by extrapolation amounts of DNA adducts, closer to those observed in AFB₁-sensitive species and 1–2 orders of magnitude higher levels than the AFB₁-resistant species.

Introduction
AFB₁, has been identified as a human carcinogen based on data from epidemiological studies of HCC in exposed populations, its hepatocarcinogenicity in several animal species, and recent molecular evidence that the presence of a specific mutation in the p53 tumor suppressor gene may result from AFB₁ exposure (1). However, although the evidence that AFB₁ is a carcinogenic risk to humans is convincing, the quantitation of that risk is problematic for a number of reasons. First, there is a striking interspecies variation in susceptibility to AFB₁ carcinogenesis; the Fischer rat is sensitive to dietary levels in the ppb range, whereas the adult mouse is refractory to dietary levels 3 orders of magnitude higher than this (2). The relative susceptibility of humans is not known. Second, although AFB₁ exposure in various parts of the world is widespread in the human food supply, the level of exposure can vary between populations, and between individuals within a population, by several orders of magnitude (3). Third, chronic infection with HBV is a major risk factor for HCC in populations in which AFB₁ exposure is elevated, and there is some epidemiological and experimental evidence that the two factors may interact in the natural history of HCC (4, 5); some evidence points to nutrition (dietary components) as a risk factor in HCC (6).

To improve quantitative risk assessment of AFB₁, a number of requirements can be identified. One is to understand the biological basis of interstrain and interspecies variation in susceptibility to AFB₁ carcinogenesis. Identification of the enzymes involved in AFB₁ activation and detoxification is one important contribution in this respect (7). Another requirement...
is improved quantitative measurement of human exposure at the individual level. Considerable progress has been made in this aspect with the development of measurements of aflatoxin metabolites and macromolecular adducts in human tissues and body fluids (8). AFB1 bound to albumin is correlated with levels of the AFB1-DNA adduct in the liver (9), and this adduct in peripheral blood has been used to examine exposure in several populations, providing a measure of exposure over the past 2–3 months (3, 10, 11). Such measures in different species could improve the basis for cross-species extrapolations of AFB1 carcinogenicity. A third requirement is to determine to what extent AFB1 and HBV interact in the development of HCC and to elucidate the mechanisms behind that interaction (3).

In this experiment we examined liver AFB1-DNA adducts and serum AFB1-albumin adducts in three strains of rats and one strain each of mice, hamsters, and guinea pigs during a 14-day exposure to AFB1, at low doses ranging from 1 to 80 μg AFB1/kg body weight/day. This experiment addressed the question of whether the aflatoxin exposure biomarker, AFB1-albumin adduct, reflects AFB1-DNA adduct in the liver across species and strains and, in turn, whether these adduct levels are related to susceptibility to AFB1 carcinogenesis based on data available from the literature. In addition, aflatoxin-albumin adduct data from human populations were compared to the rodent data to see whether humans resembled sensitive or resistant species in formation of adducts as a function of dose.

Materials and Methods

Animal Treatments. Male animals for all strains and species were used. All animals were kept on a 12-h-light, 12-h-dark cycle and were provided with water and food without restriction. The three strains of rat used were Fischer 344 (F344), Sprague-Dawley, and Wistar obtained from Charles River Japan, Inc. (Hino, Japan), and they weighed between 80 and 140 g at the start of the experiment. The mice were C57BL and were also from Charles River Japan, Inc. (Hino, Japan), and weighed between 16 and 20 g. Hartley guinea pigs were supplied by Charles River Japan, Inc. (Hino, Japan), and weighed 16–20 g. Syrian Golden hamsters were from Japan SLC, Inc. (Hino, Japan), and they weighed between 80 and 140 g at the start of the experiment. The mice were C57BL and were also from Charles River Japan, Inc. (Hino, Japan), and weighed between 16 and 20 g. Hartley guinea pigs were supplied by Charles River Japan, Inc. (Nagano, Japan) and weighed 235–450 g. Syrian Golden hamsters were from Japan SLC, Inc. (Nakaizu, Japan) and weighed between 70 and 130 g at the start of the experiment. Rats, mice, and hamsters were fed on chow diet (Oriental MF diet) from Oriental Yeast Co. (Tokyo, Japan) and guinea pigs on pelleted diet (CG-7) from Japan Clea Co., Ltd. (Tokyo, Japan). All diets were screened for aflatoxins, and no aflatoxins were detected at the 5-ppb level. Animals were divided into groups of four per time point, except for the 14-day time points, at which up to eight animals were available. In some groups, samples were lost during analyses, resulting in smaller group sizes at early time points; this was particularly the case for AFB1-DNA adduct analyses for the mice. Animals were divided into groups so that weight differences between groups were minimized.

Animals were treated with AFB1 (Sigma Chemical Co., St. Louis, MO) dissolved in corn oil by gavage daily for up to 14 days. The doses used are presented in Table 1. Animals were killed 24 h after the final treatment and blood (heparinized) and livers were collected. Plasma and liver tissue was stored at −80°C until analysis. Two groups of control animals were included, either untreated or given corn oil (5 ml/kg) by gavage for 7 days. Because no differences in DNA or albumin adduct levels were noted between the two types of controls, the data were grouped together for purposes of statistical analysis.

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Dose of AFB1 (μg/kg/day)</th>
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<tbody>
<tr>
<td>Rat</td>
<td>Fischer</td>
<td>1, 4, 20</td>
</tr>
<tr>
<td>Syrian Golden</td>
<td>Hartley</td>
<td>20, 80</td>
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<td>Mouse</td>
<td>C57BL</td>
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AFB1-Albumin Analysis. The analysis of AFB1-albumin adducts was by an ELISA method on hydrolyzed albumin as described previously (12), except for some minor changes in albumin extraction as follows: for both the mice, in which the volume of plasma was limited, and the guinea pigs, in which albumin was precipitated at lower concentrations of saturated ammonium sulfate, the initial precipitation of immunoglobulin was with 50% saturated ammonium sulfate instead of 60%. No difference in albumin purity was noted between the two methods as tested by gel electrophoresis of purified proteins, and background inhibitions in ELISA were not altered by this change in albumin extraction procedure (data not shown). Albumin concentration was measured by a protein Coomassie dye binding assay as described (12), using BSA (Sigma Chemical Co., St. Louis, MO) as the protein standard.

AFB1-lysine was used to generate the standard curve in ELISA, and results were expressed as pg AFB1-lysine equivalent/mg albumin. The limit of detection in this series of experiments was 2 pg AFB1-lysine equivalent/mg albumin. All samples were assayed in quadruplicate at least twice, on different days.

AFB1-DNA Adduct Analysis. DNA was analyzed for AFB1-DNA adducts essentially as described before (13). Briefly, DNA was extracted from whole liver by a phenol-chloroform procedure and subjected to alkali treatment to yield AFB1-Fapy, and the concentration was determined by measuring absorbance at 260 nm. A known quantity of this DNA was acid hydrolyzed (0.1 m HCl at 90°C for 45 min). After adjustment to pH 7.4, the aflatoxin residues were purified on Sep-pak C18 cartridges (Waters, Milford, MA) and the eluate was resuspended in PBS (pH 7.4) in a volume equivalent to an original concentration of 2.5 mg DNA/ml for ELISA. Because each ELISA well was tested with 25 μl of sample, this corresponded to aflatoxin residues extracted from the equivalent of 62.5 μg DNA/well. If necessary, dilutions of the sample were made to give an inhibition in ELISA within the limits of the standard curve. The standard curve was generated using AFB1-Fapy (0.01 to 1.0 pmol per 25 μl), which had been prepared as described previously (13) and subjected to the alkali treatment and Sep-pak C18 purification in the same way as the test DNA samples. Samples were assayed in quadruplicate on at least two different days, and results were expressed as pmol AFB1-Fapy equivalent/mg DNA. The detection limit was 0.2 pmol AFB1-Fapy/mg DNA.

Statistical Analysis. The comparison between pairs of group mean adduct levels was made by unpaired Student’s t test or nonparametric Mann-Whitney test, depending on the similarity or otherwise of SDs within the group data. All tests were two-tailed. Testing for differences between more than two
Fig. 1. AFB₁-albumin adducts (a) and AFB₁-DNA adducts (b) in the Fischer rat after various doses of AFB₁ and in the Sprague-Dawley and Wistar rats after 20 µg/kg/day. Boxed area, detection limit of the ELISA assay for AFB₁-albumin (see "Materials and Methods"). Lines, mean; bars, SD. One outlier value of 5.03 pmol AFB₁-Fapy/mg DNA was recorded for F344 rats after 1 day treatment with AFB₁ but was excluded from the analysis presented.

Results

AFB₁-Albumin and AFB₁-DNA Adducts in Rats. A doseresponse relationship was observed for AFB₁-albumin adducts in Fischer rats (Fig. 1a). At the highest dose (20 µg/kg/day) there was an accumulation of adducts up to 7 days with a flattening of the curve between 7 and 14 days. The 4 µg/kg dose demonstrated an increase in adduct levels up to 7 days with a significant decrease at 14 days. With the 1 µg/kg dose, adduct levels were below the detection limit (2 pg AFB₁-lysine equivalent/mg albumin) at all time points other than 7 days. The Sprague-Dawley and Wistar rat
strains treated with 20 μg AFB₃/kg daily showed a similar temporal pattern of adduct levels to the Fischer rat (Fig. 1a), and there were no significant differences between mean adduct levels for the three strains of rat when compared after 1, 3, 7, or 14 days exposure (ANOVA test; $P > 0.1$).

A dose response in hepatic AFB₁-Fapy adduct levels was seen for Fischer rats after 14 days treatment with mean levels of 0.71 ± 0.4 pmol AFB₁-Fapy/mg DNA at 4 μg/kg and 2.44 pmol ± 0.93 pmol/mg DNA at 20 μg/kg. The mean level at the same time point after 1 μg/kg (0.34 ± 0.08 pmol/mg) was not significantly different from the control value, the latter being 0.39 ± 0.35 pmol/mg (data combined for all three strains). The DNA adduct levels in the latter two groups were nevertheless above the detection limit of 0.2 pmol/mg and could originate from a low dietary contamination with aflatoxin (<5 ppb; see “Materials and Methods”). When the mean adduct levels in the three strains were compared by an ANOVA at the various time points, the differences were not statistically significant ($P > 0.1$; Fig. 1b).

### AFB₁-Albumin and AFB₁-DNA Adducts in Guinea Pigs, Hamsters, and Mice

In the guinea pigs, hamsters, and mice, an increase in AFB₁-albumin and AFB₁-DNA adduct levels was also observed with the higher dose (Figs. 2 and 3). The mice showed the lowest level of AFB₁-albumin adduct (see Fig. 3a); with a dose of 20 μg/kg the volumes of plasma obtained did not permit any analysis. The AFB₁-albumin adducts for all three species continued to increase between 7 and 14 days with the 80-μg/kg dose (Fig. 3a), in contrast to the 20-μg/kg dose,
with which evidence of a plateau in adduct levels was obtained (see Fig. 2a). For the DNA adducts at the higher dose of 80 μg/kg (Fig. 3b), similar adduct levels were observed at 7 and 14 days for both the hamsters (1.05 ± 0.53 pmol AFB1-Fapy/mg DNA on day 7 versus 1.39 ± 1.0 pmol on day 14) and the guinea pigs (9.51 ± 3.1 pmol on day 7 versus 12.0 ± 4.9 pmol on day 14). For the mice, data were obtained only for one animal at 7 days, thus precluding comparison. At the lower dose of AFB1, measurements of DNA adducts were made only after 14 days treatment for guinea pigs, hamsters, and mice (Fig. 2b). The mean adduct levels were 1.40 ± 0.61 pmol/mg for guinea pigs, 0.75 ± 0.33 pmol/mg for hamsters, and 0.44 pmol/mg for mice, a value only slightly above the detection limit.

Comparison of Adduct Levels Across Species. A comparison of the AFB1-albumin adduct levels in the rats (data combined for all three strains for clarity of presentation), guinea pigs, and hamsters after 1, 3, 7, and 14 days treatment with 20 μg AFB1/kg (Fig. 2a) demonstrated that the rats and guinea pigs had higher mean adduct levels than hamsters at all time points; differences reached statistical significance except for that between guinea pigs and hamsters at 14 days. The differences between the rat data and guinea pig data were less marked. Comparison of the guinea pigs with the three rat strains individually by ANOVA revealed that there was no significant difference after 1 and 14 days (ANOVA; \( P = 0.17 \) and 0.3, respectively), whereas after 3 and 7 days the differences were just significant (\( P = 0.03 \)). When the data from the three rat strains were combined as in Fig. 2a and compared with the data from the guinea pigs using a nonparametric analysis, the differences were again significant on days 3 and 7 (\( P < 0.01 \)) and
on the borderline of significance at day 1 and 14 ($P = 0.07$ and 0.08, respectively). Further comparison of AFB$_1$-albumin adducts could be made between guinea pigs, hamsters, and mice after treatment with the highest dose of AFB$_1$, 80 µg/kg (Fig. 3a). As with the lower dose of 20 µg/kg AFB$_1$, after 14 days treatment, guinea pigs had the highest adduct levels (mean, 77.6 ± 21.4 pg/mg), significantly higher than those of hamsters (46.0 ± 17.2 pg/mg), which in turn were significantly higher than those of mice (13.4 ± 5.5 pg/mg).

With respect to the liver DNA adducts across species, results are similar to those observed with the AFB$_1$-albumin adducts; adduct levels showed a dose response for all species and mean levels were highest in rats and guinea pigs, lower in hamsters, and lowest in mice (Figs. 2b and 3b).

The mean levels of albumin and DNA adducts at 14 days for all species and strains are presented calculated per µg AFB$_1$/kg body weight (Fig. 4) to permit a more direct comparison of the data across species. Similar interspecies differences are present at each time point (1, 3, 7, and 14 days) of the experiment. This comparison illustrates the fact that for both parameters the adduct levels are highest in rats and guinea pigs, lower in hamsters, and lowest in mice. There is a 7–8-fold range in albumin adduct levels between rats and mice and an 18-fold range in DNA adduct levels.

The ratio of AFB$_1$ binding to peripheral blood albumin and liver DNA across species after 14 days treatment was also examined. Fig. 5 is a linear regression analysis of these two parameters for all individual rats, guinea pigs, hamsters, and mice for which data are available after a dosage of 4, 20, and 80 µg/kg. There is a highly significant correlation between these two parameters ($r = 0.83; P < 0.0001; n = 57$), suggesting that the ratio is relatively constant in rodents. This correlation is weaker but remains highly significant when the four outlying data points from the guinea pigs treated with the high dose of AFB$_1$ are excluded (Fig. 5, inset; $r = 0.58; P < 0.0001$). Data for only two AFB$_1$ doses are available for each
species; therefore, calculation of regression lines for each species individually should be interpreted as preliminary. When such regression analysis was performed for rats, guinea pigs, and hamsters (see legend to Fig. 5), the slopes of the regression lines were similar for the former two species but 2-3-fold greater for hamsters. Although linear regressions were performed on these data, it should be noted that, given the fact only two doses were used, the data are too few to exclude nonlinear relationships for individual species at present. The availability of data at only one dose for mice precluded such a plot for this species.

Discussion

The objective of this experiment was to assess whether the AFB1-albumin adduct reflects AFB1-DNA adduct in the liver across species and strains and, in turn, to determine whether these adduct levels are related to susceptibility to AFB1 carcinogenicity. In addition, we examined whether aflatoxin-albumin adduct data from exposed human populations was comparable to the rodent data to determine whether humans resembled sensitive or resistant species in their dose response. The discussion is arranged around the consideration of these objectives and an evaluation of the possible contribution of the AFB1-albumin adduct in quantitative risk assessment.

A reliable marker of AFB1 exposure at the individual level would be a valuable tool in attempting quantitative risk assessment. AFB1 is metabolized to AFB1 8,9-epoxide, which can bind to DNA, predominantly at the N7 position of guanine (2). This reaction is thought to be important to the carcinogenic process, and this notion is supported by observations linking a p53-specific mutation in human HCC to AFB1 exposure (14-16). Ideally, therefore, for risk assessment, one would want to measure AFB1-N7-guanine adducts in the DNA of the target cell for tumor development, the hepatocyte. Such measurements in human liver have been few, however (17, 18), partially because of the logistical and ethical difficulties in obtaining tissue samples, including samples from appropriate control subjects. An alternative is measurement of urinary AFB1-N7-guanine (19). This noninvasive marker is well correlated with AFB1 exposure at the individual level but has the limitation that adduct excretion is rapid and thus reflects exposure only over the previous 24-48 h. In contrast, the AFB1-albumin adduct gives a measure of exposure over the previous 2-3 months, and there is also a good correlation between aflatoxin intake and adduct level (10, 11, 20). The albumin adduct level is correlated with liver DNA adducts in rats (9) and with urinary excretion of AFB1-N7-guanine in humans (21). An ELISA has been developed that measures predominantly AFB1- rather than aflatoxin G1-induced albumin adducts, and this assay has been used extensively to examine human exposure in a number of populations worldwide (3, 22-24). This adduct, therefore, may provide a reliable basis on which to make cross-species comparisons in adduct formation and in quantitative risk assessment of AFB1 across various animal species and in humans.
Correlation between AFB₁-Albumin and AFB₁-DNA Adducts across Strains and Species. The animals in this study were treated with multiple doses of AFB₁ at levels approaching those occurring in human populations (25) and those used in carcinogenicity studies in experimental animals (2). Both the AFB₁-albumin adducts and the AFB₁-DNA adduct levels measured in the rat liver in the present study are in the range reported previously (9, 26). Previous single- or multiple-dose studies of AFB₁ and liver DNA adduct levels in rats have shown a linear dose response down to 1 ng AFB₁/kg (26). In rats, liver DNA adducts accumulate upon multiple exposure to a maximum level after two to three doses followed in some reports by a decrease, at least partially due to induction of GST (27), and then a steady-state level after about 1 week of dosing (9, 28, 29). By this time most of the adduct is AFB₁-Fapy, which can persist many weeks after cessation of treatment (30).

Thus, after 14 days exposure, a steady state in liver DNA adducts in rats is attained, and our data are consistent with these kinetics (see Figs. 2b and 3b and “Results”). Similarly, the AFB₁-albumin adduct levels are expected to be at steady state at this time point, based on previous studies (9, 29, 31). The AFB₁-albumin data in the current study showed evidence of a plateau at between 7 and 14 days, notably at the lower dose level (Fig. 2a), although an exception was the higher dose in guinea pigs and hamsters, in which there was little or no evidence of a departure from linearity (Fig. 3a). Nevertheless, qualitatively, the interspecies differences in albumin adduct levels were similar, independent of the duration of AFB₁ treatment. Given this observation and the fact that twice as many animals were available for the 14-day time point, we used these latter data for a cross-species comparison.

Liver DNA adduct levels were highest in rats and guinea pig, lower in hamsters, and lowest in mice. The order of AFB₁-albumin adduct levels is the same as for the DNA adducts (Fig. 4). A linear regression analysis of these two parameters including all available data from the different species, strains, and doses of AFB₁ at 14 days shows a significant correlation (Fig. 5), suggesting that the AFB₁-albumin adduct reflects AFB₁-DNA adduct levels in the liver irrespective of species and strain in rodents. A number of earlier studies comparing liver DNA adduct levels between species have been reported. Although they were limited to single doses of AFB₁, and animals were killed a few hours after treatment, the data are consistent with the present work; AFB₁-DNA adduct levels in rats were 1.5-fold higher than in guinea pigs (32), approximately 3-fold higher than in hamsters (33, 34), and between 40- and 600-fold higher than in mice (32, 35-37). The metabolic basis of these differences appears to be mainly associated with the relative ability to detoxify the AFB₁ 8,9-epoxide because, while the activation of AFB₁ to AFB₁ 8,9-epoxide by liver microsomes or liver slices is similar in rats and guinea pigs and is actually up to 3-fold higher in mice and hamsters (34, 37, 38), the conjugation of the AFB₁ 8,9-epoxide to reduced glutathione by cytosolic GST is generally of at least an order of magnitude higher in mice than in guinea pigs and rats, which have similar activity to each other (37, 39-41). Hamsters have 2-3-fold higher activity than rats (38, 42). In addition, various compounds that induce a specific GST isoenzyme in the rat equivalent to that constitutively expressed in the mouse (43) induce resistance to AFB₁-DNA adduct formation and carcinogenicity in rat liver (44, 45). Other differences in detoxification enzymes may, however, be important, including the more recently identified aldehyde reductase acting on AFB₁-dihydrodiol in Fischer rats (46, 47). Finally, mice depleted of hepatic glutathione content had a 30-fold increase in AFB₁-DNA adducts when subsequently treated with a single dose of the carcinogen (41).

Comparison of AFB₁-Albumin Adduct Levels with AFB₁ Carcinogenicity. One limitation in comparing biomarkers of AFB₁ exposure to carcinogenicity data is the scarcity of such data in species and strains other than the male Fischer rat. One approach has been to make a statistical estimate of carcinogenic potency (TD₅₀, dose required to result in a 50% incidence of tumors; Refs. 48, 49). Data from lifetime feeding studies in male rat and mouse strains studied in the present experiments yielded the following TD₅₀ values (µg AFB₁/kg body weight/day): Fischer rat, 1.3; Wistar rat, 5.8; and C57BL mouse, >70 (49). For the hamster, carcinogenicity data are limited to two studies, one using a mixture of aflatoxins (total dose, 5–10 mg) yielded no hepatic tumors or cirrhosis (50). The second study compared male Syrian golden hamsters directly with male Fischer rats (51). In rats treated with 1 mg AFB₁/kg/day (5 days/week for 6 weeks) all animals had developed HCCs by 46 weeks, whereas only one hamster (dose, 2 mg/kg/day) had developed a microscopic HCC when killed at 78 weeks. Comparisons of AFB₁ carcinogenic potency in F344 rats, Wistar rats, and Syrian golden hamsters using a multistage analysis derived values of 2700, 680, and 36 (mg/kg/day)⁻¹, respectively.⁴ We are unaware of data on carcinogenicity in guinea pigs; they are more sensitive than mice and hamsters and similar to rats with respect to the acute hepatotoxic effects of AFB₁. On a purely qualitative scale, therefore, both the AFB₁-albumin and AFB₁-DNA adducts appear to reflect relative carcinogenic susceptibility. More precise data on carcinogenicity, preferably in the same experiment as the adduct measurements are made, would be required to make a more informative quantitative analysis.

Comparison of AFB₁-Albumin Adduct Levels in Animals and Humans. Given the large interspecies variation in susceptibility to AFB₁, it is informative to examine whether the level of AFB₁-albumin adduct formed in humans for a given dietary exposure resembles the sensitive species like the rat and guinea pig or the resistant species like the hamster and mouse. The mean adduct levels in populations from The Gambia and Guanxi, People’s Republic of China, measured by the same ELISA as used for the present animal studies, were 40 and 39 pg AFB₁-lysine equivalent/mg albumin, respectively (3, 52, 53). There are two studies that have examined dietary intake of AFB₁ and AFB₁-albumin adducts in The Gambia and People’s Republic of China. The percentage of the AFB₁ intake that was bound to albumin in the Chinese study was between 1.4 and 2.9% (10, 11), whereas in The Gambia, the apparent percentage was much higher, but this was likely due to an underestimate of exposure (see Ref. 20 for discussion). Taking a range as 1.0% to 3.0%, the mean measured AFB₁-albumin adduct level of 40 pg/mg and 30-fold accumulation of AFB₁-albumin adducts due to the half-life of albumin (54), one can calculate the exposure causing this level of adduct to be between 284 and 853 ng AFB₁/kg body weight/day (see “Appendix”). This exposure value is in agreement with the range of values (3.5–2027 ng/kg/day) reported for these and other high exposure populations in Africa and southeast Asia estimated from levels of aflatoxins in food (25).

On the basis of this calculation, a cross-species comparison is presented in Fig. 6. For this comparison, the data for a

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The highest calculated intake for humans is used in the calculation. For the Fischer rat (4 and 20 µg AFB1/kg) and the guinea pig (20 and 80 µg AFB1/kg), values from two doses could be calculated, and the mean is presented in the figure. For humans, the adduct level represents the mean adduct level in The Gambia and Guanxi, People's Republic of China. All adduct levels are uncorrected for recoveries.

Fig. 6. Cross-species comparison of AFB1-albumin adducts in rodents and humans. For the rodents, the mean adduct level after a single day exposure was used for the calculation. For the Fischer rat (4 and 20 µg AFB1/kg) and the guinea pig (20 and 80 µg AFB1/kg), values from two doses could be calculated, and the mean is presented in the figure. For humans, the adduct level represents the mean adduct level in The Gambia and Guanxi, People's Republic of China. Measured by ELISA (55) adjusted to the adduct level resulting from a single day exposure and expressed as a function of the highest estimate of AFB1 intake. For the Fischer rat (4 and 20 µg AFB1/kg), values calculated for humans is 0.04 µg AFB1/mg albumin/µg AFB1/kg body weight (57). This value calculated for humans is 0.072 pg AFB1-lysine/equivalent mg albumin/µg aflatoxin/m² surface area. The value calculated for humans is 0.041. The value for humans remains, therefore, similar to the value for rats. Alternatively, more sophisticated pharmacokinetic models could be used in the future to better compare these data, as previously discussed (58).

It could be argued that HBV infection influences the dose-response relationship between AFB1 intake and albumin adduct levels in the Gambian and Chinese populations. Although a higher AFB1-albumin adduct level has been observed in HBV-infected children (52, 56), the increase in mean adduct levels compared to noninfected subjects does not significantly alter the overall population mean adduct level, given that only 10% of subjects were HBV-surface-antigen positive. Notwithstanding this, it is important to note that if HBV-infected individuals were considered separately in the cross-species comparison, then their adduct level for a given intake of AFB1 would be still higher.

The human populations in which the AFB1 intake versus AFB1-albumin adduct relationship has been examined are ones in which that intake is high. Examination of the same relationship in low-exposure populations is of importance to test whether the linear dose-response relationships seen in rats at exposures as low as 1 ng AFB1/kg body weight (57) are also observed in humans.

The cross-species comparison presented in Fig. 6 is made on a µg/kg body weight basis. It is recognized that there is considerable discussion as to the most appropriate basis for such comparisons, including the use of body surface area (58), although the limitations of the latter approach have also been discussed (59). If the comparison in Fig. 6 is made on the basis of surface area (surface area in humans, ~1.62 m² for 60 kg and in rats, ~0.2 m² for 100 g), then the adduct range for the three rat strains is 0.055 to 0.072 pg AFB1-lysine/equivalent mg albumin/µg aflatoxin/m² surface area, whereas the value calculated for humans is 0.041. The value for humans remains, therefore, similar to the value for rats. Alternatively, more sophisticated pharmacokinetic models could be used in the future to better compare these data, as previously discussed (58).

An outstanding question from the above exercise is whether the ratio of AFB1-albumin adduct to DNA adduct suggested in rodents (Fig. 5) is the same in humans. Direct evidence for this is not available because of the limitation of measuring DNA adducts in human liver. However, the data are supported by the work of Groopman et al. (19), who report that the percent of AFB1 excreted in the urine as AFB1-N7-guanine is 0.6% in Fischer rats and about 0.2% in Chinese individuals exposed environmentally to AFB1. Given that the amounts of AFB1 intake bound to albumin are also similar between the two species (see above discussion), and assuming that the majority of AFB1-DNA adducts are formed in the liver (2), then the initial ratio between the serum albumin and liver DNA adducts is expected also to be similar in humans and Fischer rats. The assumption that the majority of DNA adducts in human urine originate from the liver may not be valid if recent suggestions concerning the capacity of human intestine to metabolize AFB1 are of relevance in vivo (60).

As discussed above, the basis of the interspecies susceptibility to AFB1 is at least partially explained by the expression of GST isoenzymes, which can efficiently bind AFB1, 8,9-epoxide to reduced glutathione in hamsters and mice and thus reduce the level of binding to DNA. Several studies have reported a low level of such activity in humans (e.g., in a series of liver cytosols from Thai subjects, there was no detectable GST activity toward AFB1, 8,9-epoxide). In comparison with other species, including mice, hamsters, and rats, the human liver had the lowest GST activity toward AFB1, 8,9-epoxide...
(55). These data are again consistent with the cross-species comparisons above, showing higher levels of albumin binding for a given exposure in humans than in hamster and mouse. Routes of detoxification other than GST-mediated ones that could be important in humans (e.g., hydroxylation of AFB1 to aflatoxin Q1, epoxide hydrolase, and the possible role of other enzymes such as aldehyde reductase) require further investigation (46, 47).

Conclusions. The data presented in this paper indicate that the AFB1-albumin adduct in peripheral blood is a reliable marker of AFB1-DNA adducts in the liver in rodents. Both of these parameters are at least qualitatively associated with species susceptibility to AFB1 hepatocarcinogenesis. The cross-species extrapolation to humans suggests that the amount of AFB1-albumin formed for a given exposure more closely approximates that in the sensitive species rather than the that in the resistant species and indicates that the Fischer rat may be a more appropriate model than the mouse for molecular dosimetry studies of AFB1 in, e.g., validating approaches for chemoprevention studies (31).

Carcinogenesis is, however, a multistep process, and the AFB1-albumin adduct is acting as a surrogate marker for only one critical step, the formation of AFB1-DNA adducts in the target cell. The relationship between this marker and the genetic consequences of exposure, as well as the quantitative association with HCC risk in humans, remains to be determined. Carcinogenesis is also multifactorial, and in the case of HCC in humans, HBV infection is a major risk factor. A synergistic interaction has been reported between AFB1 and HBV infection in HCC development in HBV transgenic mice (5), woodchucks (61), and humans (4). This suggests that an understanding of this interaction is vital to any attempts at risk assessment of AFB1, in populations exposed to both factors. The availability of more reliable markers of biologically effective dose of AFB1 should contribute to improving attempts to understand the mechanism of interaction between these two and other risk factors and in contributing to risk assessments aimed at determining the contribution of AFB1 to HCC risk in different countries (62, 63).

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Appendix

Mean adduct level = 40 pg AFB1-lysine equivalent/mg albumin, which represents a 30-fold accumulation over a single day exposure (Ref. 54; i.e., mean adduct level from a single day exposure = 1.33 pg/mg). This value is corrected to 5.33 pg/mg to take into account 25% recovery of adducts by immunosassay (10, 22). Total peripheral blood albumin = 40 (mg albumin/ml plasma) × 40 (ml plasma/kg body weight) × 60 (kg body weight) = 96 g. Total aflatoxin bound to albumin from a single day exposure = 5.33 ng/g × 96 g = 512 ng.

If this amount of aflatoxin represents between 1% and 3% of daily intake (see “Discussion”), then total daily intake (exposure) is between 512/0.03 and 512/0.01 = 17,066 ng and 51,200 ng. Calculating for a body weight of 60 kg gives daily doses of between 284 and 853 ng AFB1/kg body weight.

As mentioned above, the measured level of adduct in humans from a single day exposure is 1.33 pg/mg, and dividing by the higher of the exposure estimates (0.853 pg AFB1/kg body weight) gives a figure of 1.56 pg AFB1-lysine equivalent/mg albumin/μg AFB1/kg.

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