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Complete protection against aflatoxin B₁-induced liver cancer with a triterpenoid: DNA adduct dosimetry, molecular signature and genotoxicity threshold

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Abstract

In experimental animals and humans, aflatoxin B₁ (AFB₁) is a potent hepatic toxin and carcinogen. The synthetic oleanane triterpenoid 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im), a powerful activator of Keap1-Nrf2 signaling, protects against AFB₁-induced toxicity and preneoplastic lesion formation (GST-P positive foci). This study assessed and mechanistically characterized the chemoprotective efficacy of CDDO-Im against AFB₁-induced hepatocellular carcinoma (HCC). A lifetime cancer bioassay was undertaken in F344 rats dosed with AFB₁ (200µg/kg rat/day) for 4 weeks and receiving either vehicle or CDDO-Im (three times weekly), one week prior to and throughout the exposure period. Weekly, 24-hour urine samples were collected for analysis of AFB₁ metabolites. In a subset of rats, livers were analyzed for GST-P foci. The comparative response of a toxicogenomic RNA expression signature for AFB₁ was examined. CDDO-Im completely protected (0/20) against AFB₁-induced liver cancer compared to a 96% incidence (22/23) observed in the AFB₁ group. With CDDO-Im treatment, integrated level of urinary AFB₁-N⁷-guanine was significantly reduced (66%) and aflatoxin-N-acetylcysteine, a detoxication product, was consistently elevated (300%) after the first AFB₁ dose. In AFB₁-treated rats, the hepatic burden of GST-P positive foci increased substantially (0% to 13.8%) over the 4

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weeks, but was largely absent with CDDO-Im intervention. The toxicogenomic RNA expression signature characteristic of AFB₁ was absent in the AFB₁ + CDDO-Im treated rats. The remarkable efficacy of CDDO-Im as an anticarcinogen is established even in the face of a significant aflatoxin adduct burden. Consequently, the absence of cancer requires a concept of a threshold for DNA damage for cancer development.

Keywords

aflatoxin; chemoprevention; gene expression; DNA adducts; triterpenoid

Introduction

Hepatocellular carcinoma (HCC), the most common form of primary liver cancer, is the third leading cause of cancer mortality worldwide (1). It has been estimated that between 4.6 and 28.2% of all HCC cases worldwide may be attributed to aflatoxin exposure (2). Thus, a considerable research effort has focused on prevention strategies to reduce the impact of aflatoxin-induced HCC, including post-harvest mitigation of food contamination by the mold *Aspergillus flavus* that forms aflatoxin (3) and dietary change to foodstuffs less prone to fungal contaminations (4). The use of chemical or dietary interventions to block, retard, or reverse carcinogenesis, a strategy termed chemoprevention, represents another promising approach for the reduction of HCC. Initial cancer prevention bioassays in aflatoxin-treated rats utilizing the antischistosomal drug oltipraz demonstrated reductions in the incidence of HCC from 20% to 0% when low doses of aflatoxin B₁ (AFB₁) were used (5) and from 83% to 48% when a higher total dose of AFB₁ and a longer duration of dosing was used (6). In both cases, significant, but incomplete reductions in levels of hepatic aflatoxin-derived DNA adducts were observed in parallel cohorts of animals. Oltipraz was subsequently used in randomized, placebo controlled Phase II chemoprevention trials in an aflatoxin-endemic region of China, where pharmacodynamic action indicative of enhanced detoxication of aflatoxin was reported (7; 8).

More recently, the synthetic oleanane triterpenoid 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im) has been shown to inhibit aflatoxin-induced tumorigenesis in the rat as evidenced by a significant reduction in hepatic focal burden of glutathione S-transferase placental form (GST-P positive foci) preneoplastic lesions (9). Notably, CDDO-Im was nearly 100-times more potent than oltipraz in this short-term *in vivo* model. Mechanistic studies illustrated that CDDO-Im is an exceptionally potent activator of Keap1-Nrf2 signaling (10, 11), which leads to enhanced conjugation of the 8,9-epoxide of AFB₁ with glutathione through the actions of glutathione S-transferases (GSTs) and consequent diminution of DNA adducts formed from this ultimate carcinogenic electrophile. Based on this unparalleled potency of CDDO-Im, we employed a sub-chronic aflatoxin dosing regimen in a lifetime bioassay in F344 rats to assess protective efficacy against hepatocarcinogenesis. Serial urine collections during dosing afforded the opportunity to assess the impact of intervention on AFB₁ genotoxicity and detoxication. The model was also used to assess whether a short-term toxicogenomic signature of aflatoxin hepatocarcinogenicity could predict the extent of risk reduction by the intervention.

Materials and Methods

Chemicals

Aflatoxin B₁ was purchased from Sigma-Aldrich (St. Louis, MO). CDDO-Im was synthesized as previously described (12).

Animals

Male F344/NHsd rats were purchased from Harlan (Indianapolis, IN) and housed under controlled conditions of temperature, humidity, and lighting. Animals were fed AIN-76A purified diet (Teklad, Madison, WI) without the addition of the dietary antioxidant ethoxyquin. Food and water were available *ad libitum* and fresh diet was provided to animals at least twice per week. Rats were weighed daily during the dosing period and twice weekly thereafter. All experiments were approved by The Johns Hopkins University Animal Care and Use Committee.

Treatment protocol for protection against hepatocarcinogenesis

Rats were acclimated to diet and housing conditions for 1 week. Figure 1 outlines the treatment schedule. At 5 weeks of age and approximately 85 g body weight, the rats were randomly assigned to the two treatment groups: AFB₁ + vehicle (henceforth, called AFB₁) or AFB₁ + CDDO-Im. The rats were gavaged with either 30 μ mol (16.2 mg)/kg rat with CDDO-Im or a vehicle of 10% dimethyl sulfoxide, 10% Cremophor-EL, and phosphate buffered saline, three times per week for 5 successive weeks. This vehicle has been used previously with CDDO-Im (9, 11). Beginning at week 6 and 2 hours before CDDO-Im treatment, all rats received 200 μ g AFB₁ dissolved in dimethyl sulfoxide/kg body weight via oral gavage daily for 4 weeks. Following the first dose of AFB₁ and at weekly intervals during AFB₁ treatment, all rats were housed in glass metabolic cages and urine was collected on ice for a period of 24 hours.

Analysis of cancer

All rats were euthanized and necropsied when clinical observations indicated that the rat was in pain or would likely not survive longer than 12 hours. The latter criteria were substantial (>15%) and rapid loss of body weight, failure to groom, and/or inability to ambulate. Standardized sections of normal hepatic tissue and all abnormal tissues including all hepatic tumors were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin. Hepatic histopathological analyses were performed according to the criteria of Eustis et al. (13).

Analysis of aflatoxin metabolites in urine

Immediately following the urine collection, samples were centrifuged at $150 \times g$ and adjusted to an acidic pH using 0.5 M ascorbic acid. Urines were analyzed for levels of aflatoxin-N⁷-guanine and aflatoxin-N-acetylcysteine by isotope dilution mass spectrometry (14). Levels were normalized to creatinine content as measured using a spectrophotometric creatinine kit (Eagle Diagnostics, Inc., De Soto, TX).

Treatment for hepatic aflatoxin-DNA adducts, hepatic foci, and gene signature analysis

In order to analyze liver tissue following AFB₁ exposure with or without CDDO-Im treatment, a subset of rats were treated as in Figure 1 and serially sacrificed 24 hours following the last AFB₁ dose at the end of each week (i.e., weeks 7, 8, 9, and 10 of age). Multiple 2-mm-thick sections were cut from the left lateral, median, and right lobes of the liver and fixed in 4°C acetone, stained for expression of GST-P positive foci and analyzed by light microscopy. The observed focal data of number of foci per unit tissue area and their focal transectional areas were subjected to morphometric transformation resulting in the volume percent of liver occupied by GST-P positive foci (15, 16). Within minutes of sacrifice, the remaining liver was flash frozen in liquid nitrogen and stored at -80°C. DNA was isolated (17) and analyzed for levels of aflatoxin-DNA adducts by isotope dilution mass spectrometry. Total DNA content was determined spectrophotometrically using diphenylamine. Total RNA was isolated from frozen liver tissue (18). The levels of RNA for all genes were analyzed by qRT-PCR. Each gene was normalized to that of β -actin and the relative value for the control samples was set at one. The genes analyzed were based on a transcriptome signature shown to be predictive of aflatoxin hepatocarcinogenesis (19 – 21).

Statistical analyses

Body weights and levels of biomarkers were compared between groups using the Student *t*-test. Kaplan-Meier curves were compared by the log rank test.

Results

Growth of rats and AFB₁ toxicity

Figure 2 shows the growth inhibitory effects of AFB₁ and amelioration of this persistent toxicity by concurrent treatment with CDDO-Im. Within one week, the weight gain of the AFB₁-treated group was significantly diminished ($p < 0.001$) compared to the AFB₁ + CDDO-Im group (Fig. 2, insert). While this impact on growth somewhat subsided with termination of daily AFB₁ exposure at week 10, the diminished body weight of unprotected, early in life AFB₁ treatment persisted throughout their lifetime.

Lifetime cancer bioassay

Profound differences in HCC incidence were seen between treatment groups. In the AFB₁ group, the first HCC appeared in a rat that died at 44 weeks of age. The incidence of HCC in the AFB₁ group was 96% (22 of 23 rats) with the majority of the rats presenting with multiple HCCs that often appeared to coalesce into one larger tumor mass obliterating the normal gross hepatic architecture. The one animal in the AFB₁ group that was not diagnosed with HCC had 5 large preneoplastic foci with an average focal transactional diameter of 1.3 mm when it died, relatively young, at 35 weeks of age. The histopathological picture was radically different in the AFB₁ + CDDO-Im group as no HCCs were found. The most advanced AFB₁-related lesions were putative preneoplastic foci detected in only 3 of the 20 rats. These 3 rats died at 93, 95, and 111 weeks of age and the foci were less than half the size of foci seen in the AFB₁-treated rat that died at 35 weeks of age.

The median age of death in the AFB₁ group was 74 weeks compared to the AFB₁ + CDDO-Im group median of 90 weeks ($p < 0.01$). The overwhelmingly positive impact of CDDO-Im on prevention of HCC and ultimately survival against a large exposure to AFB₁ is shown in Fig 3. Aged control F344 rats largely die of mononuclear cell leukemia (22) or marked bilateral chronic progressive nephropathy (23). In the AFB₁ + CDDO-Im group, the incidence of nephropathy was 75% (15/20) and leukemia was 50% (10/20) with the majority having both pathologies. In the AFB₁ group, the prevalence of these lesions was much lower: 17% (4/23) had nephropathy and 30% (7/23) had leukemia. Because the rats treated with CDDO-Im lived longer and free of HCC, they then became susceptible to the common chronic diseases of age in this rat strain. The burden of HCC was almost certainly the major contributing factor to the clinical decline and death of these AFB₁ rats rather than these comorbidities.

AFB₁ DNA adducts and mercapturic acid

Urinary biomarkers of aflatoxin were measured in the lifetime bioassay rats during the 28-day AFB₁-treatment period to determine relationships with protection by CDDO-Im. All animals had biomarker levels well above the analytical limit of determination. Figure 4A depicts the urinary excretion of aflatoxin-N⁷-guanine, a biomarker of the biological effective dose of AFB₁. Also shown is the average administered daily dose of AFB₁. The AFB₁ + CDDO-Im rats excreted significantly less aflatoxin-N⁷-guanine than the AFB₁ rats (overall, 34% of the AFB₁ group) despite receiving a cumulative 20% higher dose of AFB₁. At the end of the last week of the dosing, the AFB₁ rats had more than 7 times as much aflatoxin-N⁷-guanine per day in urine as did the protected group.

A major route of detoxication of AFB₁ is through conjugation of the aflatoxin-8,9-epoxide with glutathione by GSTs and ultimately, excretion in the urine as a mercapturic acid. Elimination of aflatoxin-N-acetylcysteine in the AFB₁ + CDDO-Im rats was initially 3-fold higher than in the AFB₁ group and remained at this elevated rate for the duration of the dosing period (Figure 4B). An adaptive response in the AFB₁ rats was an increase in aflatoxin-N-acetylcysteine formation and elimination beginning in the second week of dosing and thereafter increasing to levels greater than in the AFB₁ + CDDO-Im rats.

In a separate cohort of animals treated identically, livers were collected 24 hours post- AFB_1 dose at weekly intervals over 4 weeks and were analyzed for the hepatic burden of aflatoxin-DNA adducts. The major and stable aflatoxin-N⁷-guanine-derived adduct in liver is 8,9-dihydro-8-(2,6-diamino-4-oxo-3,4-dihydropyrimidin-5-yl formamido)-9-hydroxyaflatoxin B₁ (FAPyr). As shown in Figure 5, hepatic FAPyr levels remained 2- to 3-fold higher in the AFB₁ group than in the AFB₁ + CDDO-Im group. Although initially the primary adduct formed is aflatoxin-N⁷-guanine, by 24 hours aflatoxin-N⁷-guanine has either undergone repair, depurinated, or ring-opened to form FAPyr. Overall, aflatoxin-N⁷-guanine adducts were 53% lower in the AFB₁ + CDDO-Im livers compared to those treated with AFB₁ and for FAPyr, the reduction was 69%.

Putative preneoplastic lesions

The hepatic burdens of GST-P positive foci were analyzed from histopathological sections obtained from the serially sacrificed animals of the second cohort. GST-P positive foci were not present following the first dose of AFB₁, but were histologically obvious at 8 days and increased over the course of the 28-day experiment in the AFB₁ group. As shown in Table 1 in the AFB₁ group, the GST-P positive focal volume percent was 0.01, 0.25, 3.22, and 13.81 at 8, 15, 22, and 28 days, respectively. In the AFB₁ + CDDO-Im group, foci were totally absent at 8 and 15 days, and at 22 and 28 days the focal volume percent was 0.02 and 0.01, respectively. Only one animal in each of the day 22 and 28 groups had any foci; whereas, all AFB₁ treated rats had multiple foci from day 15 onward. Clearly, the CDDO-Im intervention largely prevented the formation of these putative preneoplastic, microscopic foci commonly seen after aflatoxin exposure.

Validation of a AFB₁ gene signature

Merrick et al. (20) defined a discrete 14 gene signature derived from transcript profiling that predicted hepatocarcinogenic responses to subchronic AFB₁ exposure (19). These genes, *Wwox*, *Fhit*, *Adam8*, *C8orf46* homolog, *Mybl2*, *Abcb1b*, *Cdh13*, *Ddit4l*, *Akr7a2*, *Akr7a3*, *Abcc3*, *Cxcl1*, *Gsta5*, and *Grin2c*, represent cell cycle progression, DNA damage response and xenosensor, and detoxication pathways. However, in a separate experiment, we observed that treatment of rats with a single dose of 30 µmol CDDO-Im/kg body weight by itself led to the induction of transcripts for *Mybl2*, *Ddit4l*, *Abcc3*, *Gsta5*, and *Akr7a1* (*Akr7a2* and *Akr7a3* are actually human genes) and a decrease in *Cxcl1* independent of AFB₁ (data not shown). After censoring for these genes, we examined the effect of the AFB₁ + CDDO-Im intervention on the expression of the remaining 7 AFB₁ signature genes in livers after 28 days of AFB₁ exposure (Figure 6). The significant up or down regulation of these 7 signature genes in AFB₁-treated rats compared to vehicle was entirely consistent with the literature report (20). Moreover, the intervention with CDDO-Im almost completely abrogated the AFB₁-induced changes in RNA expression. Five (*Wwox*, *Fhit*, *Adam8*, *Cdk13*, and *Grin2c*) of the 7 genes were expressed in the protected group at levels no different than measured in the livers of control animals not receiving AFB₁ and expression of the other two, *Abcb1b* and *C8orf46*, declined 67% and 75%, respectively, towards values of untreated animals.

Discussion

The inclusion of risk-reduction cohorts in animal bioassays allows not only the evaluation of novel preventive agents, but provides a unique perspective to assess possible genotoxicity thresholds and to validate predictive molecular signatures of carcinogenesis. This study demonstrates the remarkable efficacy and extreme potency of a synthetic oleanane triterpenoid as an inhibitor of carcinogenesis, in this instance, induced by a known human carcinogen. The complete ablation of HCC development coupled with extended survivorship by CDDO-Im in a model in which aflatoxin induces a 96% incidence of HCC is unparalleled, irrespective of the dose or chemical class of chemopreventive agent used (24). Decades of mechanistic studies on aflatoxin hepatocarcinogenesis in rats provide a clear perspective on the roles of carcinogen metabolism, DNA damage, and hepatotoxicity on this

pathogenesis (7). Previous studies by our group have indicated that the protection provided by CDDO-Im in this model is achieved largely through interaction with signaling pathways mediated by the transcription factor Nrf2 (9, 11). Hepatic expression of Nrf2 target genes known to be involved in aflatoxin detoxication, namely, aldo-keto reductase 7A1 and GSTs, are elevated by CDDO-Im. The present results in which hepatic and urinary levels of aflatoxin-DNA damage products are substantively, but incompletely reduced by CDDO-Im treatment during the period of AFB₁ dosing are consistent with this view. As seen here and also reported previously in another aflatoxin-chemoprevention rat model, reduction in hepatic aflatoxin-DNA adduct burden underestimates the efficacy of chemopreventive interventions (25). In this context, it is likely that CDDO-Im, which is known to be a multifunctional agent with anti-inflammatory, antiproliferative, apoptotic as well as cytoprotective activities, is affecting multiple targets and pathways (26, 27).

Interestingly, in the 3rd and 4th weeks of carcinogen exposure, the dynamics of AFB₁ metabolism and elimination change dramatically. Urinary levels of aflatoxin-N⁷-guanine and aflatoxin N-acetylcysteine increase substantially. Also at this time, the percentage of hepatocytes expressing the presumptive preneoplastic phenotype of GST P-positivity dramatically rises from a fraction of a percent to over 10%. These foci typically harbor an increased capacity to detoxify carcinogens (28) and likely account for the increased excretion of aflatoxin-N-acetylcysteine to levels even higher than those induced by CDDO-Im. Indeed, evidence of resistance to AFB₁ cytotoxicity in AFB₁-induced preneoplastic lesions has been associated with increased glutathione levels and GST activity (29). The marked elevation in excretion of aflatoxin-N⁷-guanine may reflect the increased apoptosis observed in the residual hepatocytes of the AFB₁-treated animals at these later points in the dosing regimen (30). Aflatoxin-induced DNA damage is a key mechanistic step in the induction of HCC. The major DNA adducts produced by aflatoxin are aflatoxin-N⁷-guanine and its stable DNA oxidation product aflatoxin-FAPyr (31). Studies of the mutational potency of these two DNA damage products have revealed that the FAPyr adduct is about ten-times more mutagenic than the aflatoxin-N⁷-guanine adduct which in turn is a more toxic lesion (32, 33). Analysis of the DNA adducts in our investigation reveals that at 24 hours post-dosing, the FAPyr lesion already predominates. In the AFB₁ group, the FAPyr adduct burden is about 1 lesion per 250,000 nucleotides (~40,000 adducts/cell) compared to 1 lesion per 650,000 nucleotides (~15,000 adducts/cell) in the AFB₁ + CDDO-Im group.

There is a substantial steady-state burden of aflatoxin DNA adducts in the livers of the AFB₁ + CDDO-Im treated animals that are not producing toxicities of any consequence to these animals. It is possible that these adducts are either sequestered in non-parenchymal cells in the liver or reside in non-transcribed regions of the hepatocyte genome (34). As a consequence of the experimental design, exposure to aflatoxin is actually higher in the AFB₁ + CDDO-Im group compared to AFB₁ because of dosing on a per body weight basis. In current quantitative cancer risk assessment, it is commonly assumed that genotoxic agents exhibit linear dose-response curves for the formation of covalent adducts, and thereby no 'safe level' or threshold dose exists. Recent literature (35) has challenged this default "no-threshold" assumption, demonstrating direct acting alkylating agents such as ethyl methanesulfonate and methyl- and ethylnitrosourea do not follow a linear pattern at low dose levels. Mechanistic studies show these low dose exposures may be influenced by

homeostatic mechanisms, such as repair by DNA methyltransferases (36, 37). Aflatoxin acts as a classic genotoxin characteristically binding to the N⁷ atom of guanine (38). Previous studies conducted in the F344 rat have shown AFB₁ observes a linear dose-response curve in regards to macromolecular adduct formation over a wide range (39 - 43) extending to doses as low as 0.16 ng/kg (44). Consequently, it has been concluded the 'no threshold' paradigm exists. By combining tumor incidence data from long-term rat and trout studies, Bechtel (45) extended this observation to support that hepatic cancer risk is linearly proportional to hepatic aflatoxin DNA adduct concentration. However, our data lead to a different conclusion, pointing to a view that substantial aflatoxin-DNA damage is not sufficient for development of HCC. Moreover, the absence of cancer in this experimental setting supports the concept of a threshold for biological mode of action that links DNA damage to the development of HCC. Kaden et al. (46) demonstrated an apparent saturation of mutation induced by AFB₁ despite a linear increase in the amount of aflatoxin-DNA adducts formed in human lymphoblast cells. They hypothesized the presence of an inducible error-free DNA repair system at higher levels of adduct burden. Clearly, while DNA adducts may lead to mutations, an adduct is not the equivalent of a mutation. Smela et al. (33) have reported that aflatoxin-N⁷-guanine requires 30 lesions per induced mutation; whereas, FAPyr is 10-fold more mutagenic and 35% of FAPyr adducts result in mutation.

Additional evidence that CDDO-Im is altering the carcinogenic mode of action at steps beyond adduct formation come from measurements of a predictive genomic signature of carcinogenicity. Current rodent bioassays are time consuming and very expensive, and are incapable of evaluating the high numbers of existing and new chemicals awaiting testing. Molecular expression analyses are being undertaken to predict the carcinogenicity of untested chemicals using relatively inexpensive, high-throughput platforms (47). Such gene expression-based predictive models have been shown to be effective tools for identifying hepatocarcinogens (19). Transcript profiles following subacute and chronic dosing regimens with a range of rat hepatocarcinogens including AFB₁ were used to derive predictive computational models for their classification prior to tumor development. Merrick et al. (20, 21) confirmed this AFB₁ gene signature in a 90-day feeding study (1 ppm AFB₁) using microarray, RNA-Seq and qPCR methods. These genes included the disintegrin metalloprotease *Adam8*, the drug transporter *Abc1b1*, the DNA damage sensitive *C8orf46* homologue as well as genes that are repressed after genotoxic challenge (*Wwox* and *Fhit*) (19). Results from our 28-day, high dose AFB₁ study completely affirm this predictive signature. More notable, this signature disappeared in the context of our risk ablation intervention with CDDO-Im. Thus, these gene expression changes appear to accurately reflect the underlying biology that drives the liver towards HCC.

Collectively, this lifetime bioassay with the human carcinogen AFB₁, coupled with an intervention with the synthetic oleanane triterpenoid CDDO-Im, (i) establishes the remarkable efficacy of CDDO-Im as an anticarcinogen, (ii) defines operationally the presence of a threshold in this carcinogenesis model, and (iii) provides an additional, unique element for the validation of a predictive molecular signature of hepatocarcinogens.

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Fig. 1. Schematic of dosing protocol. Time of treatment with (*) 30 μmol CDDO-Im, *p.o.*, (▼) 200 μg AFB₁/kg body weight, *p.o.*, and (U) collection of 24 hr urine sample.

Fig. 2. Growth rates of rats treated with (●) AFB₁ or (○) AFB₁ + CDDO-Im. *Insert*, mean body weight around the period of dosing (bar). An asterisk indicates significant difference by treatment group, $p < 0.05$. Standard errors were smaller than symbols unless indicated.

Fig. 3.
Kaplan Meier curves for proportion of rats free of hepatocellular carcinoma after receiving
(●) AFB₁ or (○) AFB₁ + CDDO-Im.

Fig. 4.

Urinary and hepatic levels of aflatoxin-DNA adducts and mercapturic acid. (A) Urinary excretion of aflatoxin-N7-guanine during dosing phase in rats maintained on lifetime bioassay (bars). Circles indicate the mean dose of AFB₁ administered as calculated at weekly intervals during the dosing period. (■, ●) AFB₁ or (□, ○) AFB₁ + CDDO-Im. (B) Urinary excretion of aflatoxin-N-acetylcysteine (mercapturic acid) during dosing phase in rats maintained on lifetime bioassay. (■) AFB₁ or (□) AFB₁ + CDDO-Im. Values are mean ± SE (n=10).

Fig. 5.

Hepatic levels of aflatoxin-N⁷-guanine (N7) receiving () AFB₁ or (□) AFB₁ + CDDO-Im and 8,9-dihydro-8-(2,6-diamino-4-oxo-3,4-dihydropyrimid-5-yl formamido)-9-hydroxyaflatoxin B₁ (FAPyr) receiving (■) AFB₁ or (▨) AFB₁ + CDDO-Im in DNA isolated 24 hr after the most recent dose of AFB₁ over a 1 to 4 week dosing period. Values are mean ± SE (n=3-7).

Fig. 6.

Effect of aflatoxin alone and with CDDO-Im intervention on levels of transcript signatures of aflatoxin-induced hepatocarcinogenesis at day 28 of dosing protocol. RFC, relative fold change. Values are mean $\pm$ SE (n=3). * Differs from concurrent untreated rats (no AFB₁), $p < 0.05$. † Differs from AFB₁, $p < 0.05$. *Lower right*: RFC in AFB₁ group plotted versus AFB₁ + CDDO-Im group. Proximity of the gene name to 1 on the Y-axis indicates near return of gene expression levels to control (no AFB₁ treatment) levels.

Table 1

Hepatic burden of GST P-positive foci

Treatment	Days of AFB ₁	Number of rats	Focal Volume %, Mean (range)
None	0*	3	0
AFBi	8	3	0.01 (0, 0.04)
	15	3	0.25 (0.10, 0.34)
	22	3	3.22 (1.89, 5.86)
	28	6	13.81 (5.25, 23.11)
AFB ₁ +CDDO-Im	8	3	0
	15	3	0
	22	3	0.02 (0, 0.064)
	28	4	0.01 (0, 0.02)

* Sacrificed on day 28