Sulforaphane-Mediated Reduction of Aflatoxin B$_1$-N$^7$-Guanine in Rat Liver DNA: Impacts of Strain and Sex

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Abstract

Aflatoxin B₁ (AFB₁) is a DNA-binding toxin that contributes to the burden of liver cancer in tropical areas. AFB₁-DNA adducts are powerful biomarkers that discern individual and population risk from exposure to this carcinogen. The discovery of concordance between the metabolic pathways of the male Fischer rat and humans allowed data from rats to guide the development of chemoprevention strategies employed in clinical trials in high-risk regions. In this study, the variables of strain and sex are studied in the rat model, as a step toward understanding how ethnic differences and sex influence DNA adduct formation and the induction of enzymes by chemoprotective agents. Sulforaphane (SF), which induces Phase II enzymes including glutathione S-transferases (GSTs), was evaluated for its ability to induce GST activity and reduce the AFB₁-DNA adducts in livers of both sexes of two rat strains that differ in susceptibility to AFB₁ hepatocarcinogenesis. A dose-dependent relationship was found for SF for both induction of GST and reduction in of AFB₁-N⁷-guanine in both Fischer (sensitive to AFB₁) and Sprague-Dawley rats (relatively resistant). Sprague-Dawley rats exhibited the greatest increase in GST levels and the largest reduction in AFB₁-N⁷-guanine in liver DNA. Males and females of each strain were also compared to determine if the ability of SF to induce GST and reduce AFB₁-N⁷-guanine correlated with gender differences in sensitivity to AFB₁ carcinogenesis. No gender-specific responses to SF were observed. These results support the view that SF induction of liver GST activity may play a role in its chemoprotective activity.
INTRODUCTION

Aflatoxin B1 (AFB1) is a known carcinogen, along with hepatitis B, that contributes to the burden of human liver cancer (Kensler et al., 2010). Aflatoxin-DNA adducts are proven biomarkers that enable estimation of individual and population risk resulting from exposure to this carcinogen (Groopman et al., 2002; Groopman et al., 2008). For example, it has been shown that significant similarities exist between the relevant metabolic pathways of humans and the male Fischer rat, which is the principal model used over the past four decades (Groopman et al., 1992b). Insights obtained from these studies have provided important guidance for chemoprevention strategies employed in clinical trials in high-risk regions (Groopman et al., 2008).

Despite a wealth of experimental data, major gaps remain in our understanding of the mechanisms by which chemopreventive compounds act to decrease cancer risk. Knowledge of these mechanisms in experimental animals is important for the design of future translational research efforts. Of particular importance is the lack of data concerning the influences of strain and sex on levels of key xenobiotic metabolizing enzymes and their inducibility by chemoprotective agents. This fundamental work is required to refine our understanding of relationships between humans and experimental animal models and is of particular relevance to the design of intervention studies in genetically diverse human populations. Previous studies have shown that treatment of rats with dithiolethiones, such as oltipraz, inhibits the formation of AFB1-DNA adducts and preneoplastic lesions in the liver of animals dosed with AFB1 (Roebuck et al., 2003). In the present investigation, we evaluated the chemopreventive activity of sulforaphane, the predominant isothiocyanate found in broccoli (Fahey et al., 1997), which has been used in clinical trials and shown to reduce aflatoxin-DNA adduct biomarkers in exposed populations (Kensler et al., 2005). These data emphasize the importance of further elucidation of sulforaphane effects in experimental animal models to strengthen a mechanistic understanding of the
potential of this agent to decrease cancer risk. With that objective, we compared the effects of sulforaphane in the Sprague Dawley rat, which is relatively resistant to aflatoxin-induced liver carcinogenesis, and the Fischer rat, which is comparatively sensitive (Busby and Wogan, 1984). We also compared responses of males and females in both strains to assess the potential role of sex as a modulator of response to chemopreventive agents.

Our experiments were designed to compare induction of hepatic glutathione S-transferases (GSTs) \textit{in vivo} by sulforaphane and the resultant impact on the formation of AFB$_1$-N$^7$-guanine adducts in both sexes and both strains of rats. It has been established that aflatoxin-induced liver tumors develop more slowly in female than in male rats of both strains (Busby and Wogan, 1984). However, little information exists to indicate the predictive importance of the genotoxic DNA damage caused by AFB$_1$ and post-initiation events that produce hepatocellular carcinoma. These initial studies in the rat were designed as part of a larger effort to define key biochemical markers related to age, gender and strain that are sentinels for sensitivity and resistance to carcinogens. Identification of biomarkers that can be correlated with reduced cancer risk will aid in the efficient development of chemopreventive compounds and will permit assessment of their efficacy in clinical studies.
MATERIALS AND METHODS

Chemicals. R,S-Sulforaphane (SF) and 3H-1,2-dithiole-3-thione (D3T) were purchased from LKT Laboratories (St. Paul, MN). Cremophor and aflatoxin B₃ (AFB₃) were from Sigma Chemical (St. Louis, MO). Unless otherwise noted, all other chemicals and reagents were of ACS grade or better.

Animals. Sprague Dawley and Fischer CDF male and female rats (21 days old; Charles River, Wilmington, MA) were fed AIN76A diet (TestDiet, Richmond, IN) for one week prior to the start of the experiment. They were housed in facilities maintained at standard relative humidity and temperature, and 12h:12h light:dark conditions, with food and water available ad libitum. All procedures involving animals followed NIH guidelines and were approved by the Massachusetts Institute of Technology Committee on Animal Care.

Treatment Protocol. Treatment groups consisted of 200 animals randomly assigned into 10 sub-groups, each containing five animals of each strain and sex. At the beginning of the study all animals were 30 days of age. Treatment regimens included dosing with SF at either 0.7 or 1.4 mmol/kg dissolved in corn oil. For D3T, animals received 0.3 mmol/kg dissolved in 80% PBS, 10% Cremophor, 10% DMSO (D3T vehicle). Beginning at 30 days of age, rats received a total of three doses by gavage every other day. Twenty four h after the third dose, half the animals in each group were killed, livers removed and flash frozen in liquid N₂ for storage prior to analysis of GST activity. The remaining animals in each group were injected ip with 25 μg AFB₁ in DMSO. Aflatoxin-exposed animals were killed 4 h later and livers removed and flash frozen as above for AFB₁-DNA adduct analysis. This experimental protocol is schematically illustrated in Figure 1. Agents such as AFB₁ that can be metabolized to form strong electrophiles have been shown to affect and modulate GST activity. Previous studies have shown that AFB₁ can either induce (Harris et al., 1998) or inhibit (Fatemi et al.,
2006) GST activity depending on, age, dose and duration of treatment. Thus, to determine the impact of AFB\textsubscript{1} on GST levels in our experimental rat groups, 25 µg AFB\textsubscript{1} was administered 24 hours following the last treatment of SF, D3T or vehicle. Hepatic GST levels were then assayed 4 h later when the DNA adduct levels were determined.

**GST activity analysis.** Total GST activity was measured in 100,000 x g liver cytosol preparations using 1-chloro-2,4-dinitrobenzene as a substrate according to a previously described method (Habig et al., 1974).

**Isolation of liver DNA and hydrolysis to yield AFB\textsubscript{1}-DNA adducts.** DNA was isolated from livers of AFB\textsubscript{1}-treated rats and quantified by the diphenylamine reaction as previously described (Groopman et al., 1980; Kensler et al., 1986). For adduct analysis AFB\textsubscript{1}-DNA adducts were released by hydrolysis in 1.0 N HCl at 99°C for 15 min (Groopman et al., 1981).

**DNA adduct analysis.** Ultra-high performance liquid chromatography (UPLC) was used to separate DNA adducts prior to quantification using isotope-dilution mass spectrometry as previously reported (Egner et al., 2006). An Acuity C18 1.7 µm 1.0 x 150 mm UPLC column was used with the initial mobile phase of 14% methanol, 1% acetonitrile, 0.1% formic acid and 85% water. An 8-minute linear gradient was employed with the final mobile phase condition of 37% methanol, 2% acetonitrile, 0.1% formic acid and 61% water. The flow rate was 120 µl per minute. The hydrolyzed solution was diluted into the initial UPLC mobile phase, then injected for MS/MS analysis of AFB\textsubscript{1}-DNA adduct levels.

**Statistical analysis.**

Data are presented as $\bar{x} \pm SD$. Differences between various groups were determined using a two sample $t$-test, with statistical significance set at $p<0.05$.  

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RESULTS

Sex and strain effects on sulforaphane induction of GST activity

The objective of our study was to assess the influence of sex and strain on the ability of sulforaphane to affect the metabolism of AFB₁ and formation of DNA adducts in rat liver. Since prior information was available concerning SF induction of GSTs in vivo in rat liver, initial studies were designed to evaluate the extent to which sex and strain differences affected the modulation of these enzymes. To provide a baseline for comparison, 3H-1,2-dithiole-3-thione (D3T) was used as a positive control, since D3T had previously been shown to be a potent inducer of GST activity and greatly reduce AFB₁-DNA adduct formation in the Fischer rat (Kensler et al., 1992).

SF was well-tolerated by all animals, although a slight, statistically insignificant reduction in body weight gain occurred following the final dose in animals receiving the highest level of SF (1.4 mmol/kg). Hepatic GST activity levels were measured 24 h after the final dose of SF or D3T. Vehicle control animals of both strains and sexes showed similar levels of GST activity (Table 1A and Figure 2). In the vehicle control groups, female rats of both strains had slightly, but not significantly, lower levels of GST activity compared to males. Figure 2 also reveals that treatment with graded levels of SF resulted in an increase in hepatic GST activity that ranged from 1.4- to 2.6-fold compared with vehicle treated animals across all groups. A modest increase in GST activity was found at the lowest dose of SF (0.7 mmol/kg), with no statistically significant differences between treatment groups for this regimen. SF caused greater induction of GST activity in Sprague-Dawley rats of both sexes, and combining all of the strain data showed that the highest dose of SF (1.4 mmol/kg) caused a greater elevation of GST activity in Sprague-Dawley rats (2.2-fold; up to 555 ± 92 nmol/mg/min) compared to Fischer rats (1.6-fold; up to 408 ± 50 nmol/mg/min). Consistent with our prior findings (Kensler et al., 1992), treatment
with D3T (0.3 mmol/kg) resulted in a markedly greater increase in GST activity in both sexes and strains (2.5- to 3.7-fold) compared with treatment with SF (Figure 2). In this case, GST levels were higher on average in the Fischer strain, but the strain difference did not reach statistical significance because of wide interindividual variation in GST activity among Sprague-Dawley males.

The data in Table 1B show that there were no consistent strain- or sex-related effects of AFB₁ treatment on GST activity levels in vehicle-, D3T- or SF-treated groups. Further, no change in GST activity, or at most a modest increase (1.2- to 1.4-fold) occurred as a result of AFB₁ treatment. In only one aflatoxin-treated group, namely the Sprague Dawley corn oil-treated males, was inhibition (22%) of GST activity following AFB₁ treatment detected. Therefore, the AFB-DNA adduct data described below assumes that GST modulation of DNA adduct levels was attributable to SF and D3T alone.

Impacts of increased levels of liver GST activity in SF and D3T-treated rats on AFB₁-N⁷-guanine DNA adduct levels.

For this purpose, one dose consisting of 25 μg AFB₁ was administered 24 hours after the final dose of SF or D3T. This aflatoxin dose was used because it is the level employed in the multi-dose regimen that is highly effective in liver tumor induction in male Fischer rats (Egner et al., 1995). Four hours after AFB₁ administration, liver DNA was isolated for AFB₁-N⁷-guanine adduct measurement by quantitative isotope dilution mass spectrometry. This technology represents a significant advance over many previous studies in which radioactivity was used to assess levels of DNA adduct burden. Using MS/MS analysis of the AFB₁-N⁷-guanine adduct, the protonated parent ion (m/z 480.1) was selected and the collision-induced fragment ion (m/z 389.1) was monitored to quantify levels of adduct in the sample (Egner et al., 2006). Figures 3A and B depict the calculated levels of AFB₁-N⁷-guanine adducts in liver tissue from vehicle control-, D3T- and SF-treated rats. The highest adduct levels were found in vehicle-
treated animals of both strains and sexes. The differences in the initial adduct burden in the control
groups between genders and strains were attributed to variations in body weights of the animals at time
of dosing. Thus, 35-day-old male Fischer rats weighed 86 ± 6 g, while at the same age male Sprague
Dawley rats weighed 137 ± 14 g. Therefore, Fischer rats, on average, received a 1.6-fold higher dose of
AFB$_1$ on a body weight basis. Likewise, females of both strains weighed on average significantly less
than males resulting in a greater body weight dose of AFB$_1$ to female animals and correspondingly
higher AFB$_1$ adduct levels.

Comparison of the AFB$_1$-N$^7$-guanine adduct in liver DNA within treatment groups of each rat
strain reveals that both dose levels of SF were effective in reducing hepatic AFB$_1$-N$^7$-guanine levels
(Figures 3A and 4B). In rats that received the 0.7 mmol/kg dose, average adduct levels were lower than
those found in vehicle-treated controls; the differences attained statistical significance in the Sprague
Dawley strain that had a 45-50% reduction in amount of AFB$_1$-N$^7$-guanine in liver DNA ($p<0.05$). The
average level of AFB$_1$-N$^7$-guanine was also reduced in Fischer rats but the magnitude of reduction did
not reach statistical significance in males or females at this lower level of SF administration. In animals
that received the 1.4 mmol/kg dose of SF, the Sprague-Dawley strain also had the greatest reduction in
AFB$_1$-N$^7$-guanine levels. As shown in Figure 3A, AFB$_1$-N$^7$-guanine levels were reduced by 70-75% in
both male and female Sprague-Dawley rats ($p<0.05$). The Fischer strain (Figure 3B) had smaller
reductions in adduct levels with a 30% decrease in males ($p=0.07$) and 40% in females ($p<0.05$) (Table
2). Overall, D3T was most effective in reducing AFB$_1$-N$^7$-guanine levels in the liver. In all groups
treated with D3T, the amounts of AFB$_1$-N$^7$-guanine in liver DNA were reduced by 85 to 90% of those in
the corresponding vehicle-treated control groups regardless of strain or gender (see data in Figure 3 and
Table 2). In summary, these data comprise the first demonstration of the ability of SF to cause a
reduction in aflatoxin DNA adduct damage in vivo in Sprague Dawley and Fischer rats of both sexes.
Our findings provide further justification for exploring the chemoprotective properties of this naturally occurring isothiocyanate.
DISCUSSION

Aflatoxin is a known human carcinogen that significantly contributes to the burden of hepatocellular carcinoma in many parts of the world (Kensler et al.). This mycotoxin is also a potent experimental carcinogen in many different animal models, especially the rat. Age, sex, strain and diet have been demonstrated to be important factors that affect the susceptibility of animals to AFB$_1$-induced cancers. Among natural dietary components, dithiolethiones and isothiocyanates have been shown to possess anticarcinogenic properties in experimentally induced animal tumor models including AFB$_1$-induced tumors in the rat (Zhang et al., 1994; Fahey et al., 2002; Roebuck et al., 2003). Some of the chemoprotective effects of these compounds have been attributed to their abilities to inhibit carcinogen activation and/or to induce metabolic pathways that detoxify electrophilic metabolites that damage DNA and other cellular molecules (Fahey and Talalay, 1999; Langouet et al., 2000; Gross-Steinmeyer et al., 2010). Our study compares the effects of two anticancer compounds, the naturally occurring SF, and the synthetic compound, D3T, on the formation of genotoxic aflatoxin liver DNA adducts. The study compared adducts in the Fischer rat, recognized as a sensitive strain to aflatoxin carcinogenesis, with the less sensitive Sprague Dawley strain. Our comparative studies were designed to reveal possible sex and strain differences in response to the natural and synthetic chemopreventive agents.

Early studies of aflatoxin carcinogenesis in both male and female Fischer rats revealed a 100% incidence of hepatocellular carcinoma in both sexes when aflatoxin was fed in the diet for periods of up to one year (Wogan and Newberne, 1967). Of significance in this study was the observation that while both sexes were highly sensitive to aflatoxin-induced liver cancer, female rats developed tumors later in life than males (Wogan and Newberne, 1967). Studies that explored strain differences between the Fischer and Sprague Dawley rat found that the Sprague Dawley was about 50% less sensitive to liver cancer following aflatoxin exposure (Rogers et al., 1971). Few subsequent studies, however, have
compared aflatoxin metabolism and DNA damage in sensitive and resistant rats. One \textit{in vitro} study did not find significant differences in microsomal AFB$_1$ metabolites among several rat strains, but prior treatment of rats with testosterone was reported to increase the rate of AFB$_1$ metabolism (Gurtoo and Motycka, 1976), suggesting that male rats could activate AFB$_1$ more efficiently than females.

Formation of DNA adducts by AFB$_1$ requires metabolic activation by Cyp1A2 or Cyp3A4/5 to form the electrophilic AFB$_1$-8,9-oxide. The highly reactive AFB$_1$-8,9-oxide can be detoxified by GSTs. Both sulforaphane and D3T have been found to be potent inducers of hepatic GST activity, which has been established as a predictor for chemopreventive efficacy in aflatoxin carcinogenicity studies (Dinkova-Kostova \textit{et al.}, 2005). In the present study we found that treatment of Fischer or Sprague Dawley rats with SF or D3T resulted in significant increases in total GST activity and concomitant and somewhat proportional reductions in the amounts of AFB$_1$-N$^7$-guanine formed in liver DNA. Treatment with either SF or D3T resulted in statistically significant reductions in aflatoxin-DNA adduct formation in animals of both strains and sexes. Consistent with prior data, synthetic chemopreventive agent, D3T, proved to be extremely effective in lowering DNA damage by nearly 90% (Groopman \textit{et al.}, 1992a). Collectively these findings suggest that initial formation of aflatoxin-DNA adducts can be a good predictor of the relative differences between tumor incidence and time-to-tumor seen across the strains and genders in rats.

As an experimental carcinogen that is active in many different animal models, aflatoxin has been useful for elucidating pathways of metabolic activation and DNA adduct formation (Kensler \textit{et al.}, 2003). Additionally, the rat has proven to be an excellent experimental model for assessing impacts of human exposures because it parallels the human pathways of aflatoxin metabolism as well as the formation and genotoxic DNA adducts. This parallelism between the experimental model and humans exists for very few chemical carcinogens, and studies of aflatoxin have provided a valuable template for
many other environmental agents of concern. Given the public health impact of hepatocellular carcinoma in many populations around the world, investigators have worked to develop both primary and chemopreventive strategies to blunt the biological consequences of aflatoxin exposure in these high risk settings. Once again experimental models, particularly the rat model, have been very valuable for the development of intervention strategies. Complementary epidemiologic data from studies of hepatocellular carcinoma have shown that men experience between three and eight times higher incidence of this cancer than women. Further studies are necessary to explore post-initiation events and the resultant biology of promotion and progression to identify the underlying mechanisms for the strain and sex differences. These studies will be very important in our long-term translational efforts to mitigate liver cancer by aflatoxin in high-risk human population.

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**Tables**

**Table 1A. Effect of sulforaphane or D3T on liver GST activity**

| Treatment Group      | SD Male   | SD Female  | Fischer Male | Fischer Female |
|----------------------|-----------|------------|--------------|----------------|
| Corn Oil             | 288.3 ± 41.2 | 212.3 ± 51.0 | 268.4 ± 27.6 | 261.8 ± 97.3   |
| SF (0.7 mmol/kg)     | 409.0 ± 67.8 (1.4) | 321.1 ± 39.0 (1.5) | 366.9 ± 37.1a (1.4) | 372.2 ± 28.3a (1.4) |
| SF (1.4 mmol/kg)     | 557.5 ± 90.2a (1.9) | 553.2 ± 104.7 (2.6) | 433.6 ± 98.2b (1.6) | 397.1 ± 29.2 (1.5) |
| D3T Vehicle          | 284.1 ± 38.3 | 220.7 ± 44.2a | 305.0 ± 71.0 | 227.0 ± 14.4   |
| D3T (0.3 mmol/kg)    | 709.7 ± 379.8 (2.5) | 722.1 ± 94.2 (3.3) | 1012.2 ± 115.3 (3.3) | 839.6 ± 92.1 (3.7) |

**Table 1B. Combined effect of AFB1 and sulforaphane or D3T treatment on liver GST activity**

| Treatment Group + AFB1 | SD Male   | SD Female  | Fischer Male | Fischer Female |
|------------------------|-----------|------------|--------------|----------------|
| Corn Oil               | 224.9 ± 17.8 (-1.3) | 267.7 ± 32.6 | 328.1 ± 25.6 (1.2) | 325.9 ± 46.2   |
| SF (0.7 mmol/kg)       | 323.2 ± 50.8 | 419.8 ± 70.0 (1.4) | 480.1 ± 63.9 (1.3) | 381.2 ± 55.2   |
| SF (1.4 mmol/kg)       | 514.3 ± 63.1 | 487.8 ± 108.1a | 499.9 ± 66.8a | 411.6 ± 86.8c  |
| D3T Vehicle            | 342.3 ± 41.0 (1.2) | 277.0 ± 32.8 | 387.4 ± 22.1 (1.3) | 274.6 ± 23.0 (1.2) |
| D3T (0.3 mmol/kg)      | 951.5 ± 83.1 (1.3) | 963.4 ± 128.9 (1.3) | 1178.5 ± 127.2 | 1023.8 ± 68.1 (1.2) |

Values = \( \bar{x} \pm SD \) for groups of n=5, unless noted where n=\(^a4\), \(^b2\), or \(^c3\). Values in parentheses indicate fold change (increase or decrease in GST activity) relative to (A) the corresponding vehicle-treated control or (B) the corresponding non-AFB1-treated group (\(p<0.05\)).

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Table 2. Effect of sulforaphane or D3T on the level of AFB1-N7-guanine in liver DNA

| Treatment Group + AFB1 | SD Male | SD Female | Fischer Male | Fischer Female |
|------------------------|---------|-----------|--------------|----------------|
| Corn Oil               | 8.1 ± 3.3 | 15.5 ± 1.9 | 14.4 ± 5.3   | 15.6 ± 3.7      |
| SF (0.7 mmol/kg)       | 4.6 ± 2.0 (43%) | 7.5 ± 1.6 (52%) | 11.7 ± 2.5 (19%)c | 11.1 ± 4.5 (29%)d |
| SF (1.4 mmol/kg)       | 2.3 ± 1.0 (71%) | 4.1 ± 0.3a (74%) | 9.9 ± 1.3a (32%)d | 8.9 ± 3.3b (43%) |
| D3T Vehicle            | 9.9 ± 5.3 | 11.3 ± 7.3 | 17.0 ± 6.8   | 20.1 ± 3.0      |
| D3T (0.3 mmol/kg)      | 1.1 ± 0.6 (89%) | 1.4 ± 0.3 (88%) | 2.1 ± 0.7 (87%) | 2.1 ± 0.4 (89%) |

Values = x ±SD for groups of n=5, unless noted where a n=4 or b 3. Values in parentheses indicate percent reduction in adduct levels relative to the corresponding vehicle-treated group (p<0.05 except where noted when c p<0.2 or d p≤0.1).
Figures:

**Figure 1.** Experimental protocol. Sulforaphane and D3T were evaluated for their effects on GST activity and levels of AFB$_1$-N$^7$-guanine in liver DNA of male and female Fischer and Sprague-Dawley rats. ● Indicates start of AIN67A diet; ↓ Indicates gavage with sulforaphane or D3T; * Indicates treatment with AFB$_1$. Each group consisted of 10 animals per strain; 5 males and 5 females.

**Figure 2.** Effect of sulforaphane (SF) and D3T on liver GST levels. Rats were treated with as described in Material and Methods and cytosolic GST activity determined 24 h after the last dose. * indicates significantly different from vehicle-treated ($p<0.05$).

**Figure 3.** Effect of sulforaphane (SF) or D3T on the levels of AFB$_1$-N7-guanine in liver DNA isolated from Sprague-Dawley (A) or Fischer (B) rats. Animals received SF or D3T prior to administration of AFB$_1$ as described in Material and Methods. SF was dissolved in corn oil and D3T was dissolved in PBS/DMSO/Cremophor (D3T vehicle), both were administered *via* gavage.
FIGURE 1
FIGURE 2

GST activity (nmol/min/mg)

Corn Oil  D3T  SF 0.7 mmol/kg  SF 1.4 mmol/kg  D3T 0.3 mmol/kg

Sprague-Dawley Male
Sprague-Dawley Female
Fischer Male
Fischer Female

Vehicle
SF 0.7 mmol/kg
SF 1.4 mmol/kg
D3T 0.3 mmol/kg

* * *
FIGURE 3

A. Sprague Dawley

B. Fischer

AFB₁-N⁷-guanine/10⁶ nucleotides

Male
Female

Corn oil  D3T vehicle  0.7 SF (mmol/kg)  1.4 SF (mmol/kg)  D3T

A. Sprague Dawley

B. Fischer

AFB₁-N⁷-guanine/10⁶ nucleotides

Male
Female

Corn oil  D3T vehicle  0.7 SF (mmol/kg)  1.4 SF (mmol/kg)  D3T
References:

Busby, W. F., and Wogan, G. N. (1984). Aflatoxins. In Chemical Carcinogens (C. D. Searle, Ed.), pp. 945-1136. American Chemical Society, Washington, D.C.

Dinkova-Kostova, A. T., Holtzclaw, W. D., and Kensler, T. W. (2005). The role of Keap1 in cellular protective responses. Chem Res Toxicol 18, 1779-1791.

Egner, P. A., Gange, S. J., Dolan, P. M., Groopman, J. D., Muñoz, A., and Kensler, T. W. (1995). Levels of aflatoxin-albumin biomarkers in rat plasma are modulated by both long-term and transient interventions with oltipraz. Carcinogenesis 16, 1769-1773.

Egner, P. A., Groopman, J. D., Wang, J. S., Kensler, T. W., and Friesen, M. D. (2006). Quantification of aflatoxin-B1-N7-Guanine in human urine by high-performance liquid chromatography and isotope dilution tandem mass spectrometry. Chem Res Toxicol 19, 1191-1195.

Fahey, J. W., Haristoy, X., Dolan, P. M., Kensler, T. W., Scholtus, I., Stephenson, K. K., Talalay, P., and Lozniewski, A. (2002). Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo[a]pyrene-induced stomach tumors. Proc Natl Acad Sci U S A 99, 7610-7615.

Fahey, J. W., and Talalay, P. (1999). Antioxidant functions of sulforaphane: a potent inducer of Phase II detoxication enzymes. Food Chem Toxicol 37, 973-979.

Fahey, J. W., Zhang, Y., and Talalay, P. (1997). Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc Natl Acad Sci U S A 94, 10367-10372.

Fatemi, F., Allameh, A., Dadkhah, A., Forouzandeh, M., Kazemnejad, S., and Sharifi, R. (2006). Changes in hepatic cytosolic glutathione S-transferase activity and expression of its class-P during prenatal and postnatal period in rats treated with aflatoxin B1. Arch Toxicol 80, 572-579.

Groopman, J. D., Busby, W. F., Jr., and Wogan, G. N. (1980). Nuclear distribution of aflatoxin B1 and its interaction with histones in rat liver in vivo. Cancer Res. 40, 4343-4351.

Groopman, J. D., Croy, R. G., and Wogan, G. N. (1981). In vitro reactions of aflatoxin B1-adducted DNA. Proc Natl Acad Sci USA 78, 5445-5449.

Groopman, J. D., DeMatos, P., Egner, P. A., Love-Hunt, A., and Kensler, T. W. (1992a). Molecular dosimetry of urinary aflatoxin-- N 7 -guanine and serum aflatoxin--albumin adducts predicts chemoprotection by 1,2-dithiole-3-thione in rats. Carcinogenesis 13, 101-106.

Groopman, J. D., Jackson, P. E., Turner, P., Wild, C. P., and Kensler, T. W. (2002). Validation of exposure and risk biomarkers: Aflatoxin as a case study. In Progress in Nucleic Acid Research and Molecular Biology, pp. 307-318. CRC Press LLC.

Groopman, J. D., Kensler, T. W., and Wild, C. P. (2008). Protective interventions to prevent aflatoxin-induced carcinogenesis in developing countries. Annu Rev Public Health 29, 187-203.

Groopman, J. D., Roebuck, B. D., Kensler, T. W., and D'Amato, R. (1992b). Molecular dosimetry of aflatoxin DNA adducts in humans and experimental rat models. In Relevance of Animal Studies to the Evaluation of Human Cancer Risk: Proceedings of a Symposium held December 5-8, 1990 in Austin, Texas, pp. 139-155. Wiley-Liss, Inc., New York.

Gross-Steinmeyer, K., Stapleton, P. L., Tracy, J. H., Bammler, T. K., Strom, S. C., and Eaton, D. L. (2010). Sulforaphane- and phenethyl isothiocyanate-induced inhibition of aflatoxin B1-mediated genotoxicity in human hepatocytes: role of GSTM1 genotype and CYP3A4 gene expression. Toxicol Sci 116, 422-432.
Gurtoo, H. L., and Motycka, L. (1976). Effect of sex difference on the in vitro and in vivo metabolism of aflatoxin B1 by the rat. *Cancer Res* **36**, 4663-4671.

Habig, W. H., Pabst, M. J., and Jakoby, W. B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* **249**, 7130-7139.

Harris, A. J., Shaddock, J. G., Manjanatha, M. G., Lisenbey, J. A., and Casciano, D. A. (1998). Identification of differentially expressed genes in aflatoxin B1-treated cultured primary rat hepatocytes and Fischer 344 rats. *Carcinogenesis* **19**, 1451-1458.

Kensler, T. W., Chen, J. G., Egner, P. A., Fahey, J. W., Jacobson, L. P., Stephenson, K. K., Ye, L., Coady, J. L., Wang, J. B., Wu, Y., Sun, Y., Zhang, Q. N., Zhang, B. C., Zhu, Y. R., Qian, G. S., Carmella, S. G., Hecht, S. S., Benning, L., Gange, S. J., Groopman, J. D., and Talalay, P. (2005). Effects of glucosinolate-rich broccoli sprouts on urinary levels of aflatoxin-DNA adducts and phenanthrene tetraols in a randomized clinical trial in He Zuo township, Qidong, People's Republic of China. *Cancer Epidemiol Biomarkers Prev* **14**, 2605-2613.

Kensler, T. W., Egner, P. A., Davidson, N. E., Roebuck, B. D., Pikul, A., and Groopman, J. D. (1986). Modulation of aflatoxin metabolism, aflatoxin- N7-guanine formation, and hepatic tumorigenesis in rats fed ethoxyquin: Role of induction of glutathione S-transferases. *Cancer Res.* **46**, 3924-3931.

Kensler, T. W., Groopman, J. D., Eaton, D. L., Curphey, T. J., and Roebuck, B. D. (1992). Potent inhibition of aflatoxin-induced hepatic tumorigenesis by the monofunctional enzyme inducer 1,2-dithiole-3-thione. *Carcinogenesis* **13**, 95-100.

Kensler, T. W., Qian, G. S., Chen, J. G., and Groopman, J. D. (2003). Translational strategies for cancer prevention in liver. *Nature Reviews* **3**, 321-329.

Kensler, T. W., Roebuck, B. D., Wogan, G. N., and Groopman, J. D. (2010). Aflatoxin: A 50 Year Odyssey of Mechanistic and Translational Toxicology. *Toxicol Sci* **2010**, 3.

Langouet, S., Furge, L. L., Kerriguy, N., Nakamura, K., Guillouzo, A., and Guengerich, F. P. (2000). Inhibition of Human Cytochrome P450 Enzymes by 1,2-Dithiole-3-thione, Oltipraz and Its Derivatives, and Sulforaphane. *Chem.Res.Toxicol.* **13**, 245-252.

Roebuck, B. D., Curphey, T. J., Li, Y., Baumgartner, K. J., Bodreiddigari, S., Yan, J., Gange, S. J., Kensler, T. W., and Sutter, T. R. (2003). Evaluation of the cancer chemopreventive potency of dithiolethione analogs of oltipraz. *Carcinogenesis* **24**, 1919-1928.

Rogers, A. E., Kula, N. S., and Newberne, P. M. (1971). Absence of an effect of partial heptectomy on aflatoxin B1 carcinogenesis. *Cancer Res.* **31**, 491-495.

Wogan, G. N., and Newberne, P. M. (1967). Dose-response characteristics of aflatoxin B1 carcinogenesis in the rat. *Cancer Res.* **27**, 2370-2376.

Zhang, Y., Kensler, T. W., Cho, C. G., Posner, G. H., and Talalay, P. (1994). Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proc Natl Acad Sci U S A* **91**, 3147-3150.