Of Mice, Rats, and Men: Could Nrf2 Activation Protect against Aflatoxin Hepatocarcinogenesis in Humans?

David L. Eaton1,2 and Christopher M. Schaupp1

Abstract

In this issue, Johnson and colleagues provide a remarkable demonstration of the potential for "chemoprevention" of cancer from mutagenic chemicals. The authors demonstrated complete protection of rats from a carcinogenic treatment regimen with the potent dietary mutagen and hepatocarcinogen, aflatoxin B1 (AFB) by pretreatment with a synthetic oleanane triterpenoid, 1-[2-cyano-3-,12-dioxooleana-1,9(11)-dien-28-oyl]imidazole (CDDO-Im). This study is notable for two reasons: (i) Activation of the Nrf2/Keap1/ARE "antioxidant response" pathway by CDDO-Im conferred complete protection against AFB-induced hepatocellular carcinomas in the Fisher F344 rat (a strain frequently used in life-time carcinogenicity bioassays), and (ii) extensive AFB–DNA adduct formation was seen in all animals at early time points, including those treated with CDDO-Im, albeit at lower levels (~30% of the untreated animals), suggesting a strong divergence in the association between early DNA-damaging events, and tumor formation later in life. The authors suggest that this provides compelling experimental support for the concept of carcinogenic "thresholds" for mutagenic chemicals, because the treatment reduced persistent, mutagenic adducts (AFB–FAPyr adducts) only by 70%, but nearly completely eliminated tumors after approximately 2 years and preneoplastic lesions 6 weeks after the last dose of AFB. Cancer Prev Res; 7(7): 653–7. ©2014 AACR.
populations at high risk for aflatoxin-induced hepatocellular carcinoma. Indeed, Kensler and colleagues have been exploring this avenue for the past decade in clinical trials in Qidong, China, a region of the world with remarkably high incidence of liver cancer due apparently to a combination of endemic hepatitis B viral infections and very high dietary exposure to AFB (2). Using two different, well-established Nrf2 activators, sulforaphane (SFN; an Nrf2 agonist, delivered as the natural glucosinolate derivative, glucoraphanin, from a broccoli sprout tea extract), and the antischistosomal drug, oltipraz, Kensler and colleagues evaluated whether daily treatment for 2 weeks with glucoraphanin or 8 weeks with oltipraz, could reduce the extent of aflatoxin biomarkers, determined through evaluation of AFB–N7-guanine adducts and aflatoxin-mercapturate in the urine and/or a surrogate biomarker, AFB–albumin adduction, in serum (6–8). On the basis of mechanistic studies in rats, the proposed mechanism of protection conferred by Nrf2 agonists occurs via upregulation of GSTA5 (6–8). The remarkable resistance of mice to the hepatocarcinogenic (GSH; Fig. 1). They also examined whether treatment with Nrf2 activators increased the elimination of AFB-mercapturate, a breakdown product of the AFB–GSH conjugate. Although there was, not surprisingly, a great deal of inter-subject variability, daily treatment with oltipraz increased AFB-mercapturic acid excretion, and treatment with glucoraphanin hinted at a slight decrease in AFB–N7-guanine adducts in the urine, though the results with glucoraphanin did not reach statistical significance (7, 8).

However, the question remains: can treatment with CDDO-Im be as effective in preventing aflatoxin-induced human liver cancer as it is in rats? Although earlier chemointervention trials suggested some possible effects, other studies are not so encouraging. There is convincing evidence that induction of GST genes, specifically the rat alpha class GST, rGstA5, by Nrf2 agonists is the principal, if not sole, mechanism by which Nrf2 agonists decrease AFB–DNA adduct formation and, thus, presumably the cancers that ultimately develop from the DNA damage. Indeed, the remarkable resistance of mice to the hepatocarcinogenic...
effects of AFB is due solely to the constitutive expression of mGstA3 in the liver. This particular GST has a catalytic efficiency toward AFB-8,9-epoxide (AFBO) that is at least 10,000 times greater than any human alpha class GST (9). Surprisingly, both human and non-human primate alpha class GST enzymes display no measurable catalytic activity toward AFBO (10–12). Microsomal and cytosolic enzyme activities using human liver tissue effectively activate AFB to the genotoxic epoxide, but cytosolic fractions of human liver are unable to form a detectable amount of glutathione conjugate (13) under conditions identical to experiments in which mouse and Nrf2-activated rat liver cytosols form extremely high levels of AFB–GSH. Interestingly, one human mu class GST protein, GSTM1-1, does have very low, but measurable, catalytic activity toward AFBO (14, 15), and the GST activity of hepatic cytosolic fraction from the non-human primate M. fascicularis was shown to be from mu and not alpha class GSTs by Nrf2 agonists would have any effect on AFB–DNA adducts, because there is no human alpha class GSTs by Nrf2 agonists would have any effect on AFB–DNA adducts, because there is no human alpha class GSTs lack catalytic activity, neither alpha class GST nor GSTM1 genes seem to be inducible by chemical Nrf2 activators. Consistent with this observation is the apparent lack of any ARE consensus sequences in the 5′ flanking region of human GSTA1 (19) or, preliminarily, any other human alpha, mu, or pi class GST (20), which raises some doubt as to whether the orthologous human genes are inducible via the Nrf2 pathway.

Is it possible that CDDO-Im is providing protection against AFB-induced tumors by inhibiting early-stage carcinogenic processes other than AFB–DNA adduct formation? That is most certainly a possibility. However, Kessler and colleagues (21) have recently shown that pretreatment of GstA3-knockout mice—which are highly sensitive to the acute toxicity and genotoxic effects of aflatoxin (22)—with CDDO-Im had no significant effect on AFB–DNA adduct formation in these mice, which argues somewhat against “non–GST”-mediated mechanisms behind the anticarcinogenic effects of CDDO-Im toward AFB-induced liver tumors.

Perhaps the most intriguing results from this study come from the nearly complete protection against both early preneoplastic lesions (GSTP + foci) and later hepatocellular carcinoma, even though there was clearly a great deal of genomic damage through AFB adduction in the CDDO-Im rats. CDDO-Im treatment substantially reduced the amount of AFB–DNA adducts formed, as indicated by both a large increase in the amount of AFB-mercapturate excreted in the urine and a significantly lower amount of AFB–N7-guanine adducts eliminated in the urine of the CDDO-Im–treated rats relative to untreated rats. Although the treatment clearly induced rGstA5 gene expression, which significantly enhanced GSH conjugation of the genotoxic AFB–epoxide, the treatment did not completely eliminate AFB-DNA adduct formation, though it did completely eliminate tumor formation and nearly all early lesions. The authors argue that these results provide compelling evidence that an organism must experience relatively large amounts of DNA damage—i.e., a "threshold for carcinogenic response"—before the DNA damage ultimately gives rise to hepatocellular carcinoma(s).

There are a relatively large amount of data on aflatoxins demonstrating an essentially linear relationship between administered dose and the extent of AFB–DNA adducts, as well as an inferred linear relationship between AFB–DNA adducts and eventual tumor development (13, 23). A recent article by Williams (24) reported preliminary evidence of linearity in liver tumor response following AFB treatment even at very low doses. Although the recent findings of Johnson and colleagues certainly demonstrate that there is a strong deviation from linearity between AFB–DNA adduct burden and consequent hepatocellular carcinoma development, the failure of tumors to develop in the CDDO-Im–treated animals may be due in part to CDDO-Im–specific effects on downstream events that interfere with the ultimate progression of early-initiated cells. In addition to its canonical role as a regulator of antioxidant defense, Nrf2 has been shown to interact with myriad signaling pathways...
within the cell, including those controlling cellular growth, proliferation, nutrient sensing (PI3K/AKT/mTOR pathway), and differentiation (Notch1), as well as modulating the expression of genes involved in inflammation, gluco-neogenesis, β-oxidation, and lipogenesis (25). Furthermore, CDDO-Im has been shown to block NF-kB (a transcription factor responsible for the production of cytokines and subsequent inflammatory response) signaling through direct inhibition of its cytoplasmic inhibitor IκB kinase (26). Thus, it is possible that if CDDO-Im treatment altered other cellular processes, such as inflammation, that enhance the elimination of initiated cells or otherwise interfere with promotion and progression of initiated cells, the apparent "threshold" for AFB–DNA damage needed to result in hepatocellular carcinoma (i.e., the lack of linearity between AFB–DNA adduct burden and tumor development) as presented here may not apply to very low, chronic exposure to AFB, which could, in fact, be linearly related to tumor development.

Nevertheless, Johnson and colleagues provide the most compelling experimental evidence to date supporting the concept of a threshold for AFB-induced DNA adductions and tumor development. In light of the debate surrounding linear versus threshold approaches to risk assessment for carcinogenesis at low doses, the implications of these findings cannot be understated. Currently, the Environmental Protection Agency’s Carcinogen Assessment Guidelines (27) require that the agency use a linearized (“nonthreshold”) approach for determining the "threshold" for AFB–DNA damage needed to result in promotion and progression of initiated cells, the apparent assumption that tumor response would also be linear at low doses. Although the data presented by Johnson and colleagues strongly suggested that AFB–DNA adduct formation and tumor response are not linearly related, no dose–response assessment was done in this study. Given the relatively small sample size and the high dose of AFB received, these data alone cannot eliminate the possibility that there is a proportional relationship between AFB–DNA adducts and tumor response at much lower doses, as suggested by the data from the trout carcinogenesis bioassay reported by Williams (24). It will be interesting to see whether the remarkable protection against tumor development provided by CDDO-Im in AFB-treated rats applies to other mutagenic carcinogens and additionally, whether CDDO-Im affords protection from neoplastic promotion and progression in other models of chemical mutagenesis.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References


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