Assessment of Safety/Risk vs. Public Health Concerns: Aflatoxins and Hepatocarcinoma

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Abstract

Hepatocellular carcinoma, (HCC) is a serious health problem. It is prevalent in certain parts of the world where food contamination with aflatoxin is common. Aflatoxin, especially AFB₁, has been shown to induce HCC in many species of laboratory and wild animals, including subhuman primates. Carcinogenesis studies have demonstrated that AFB₁ is a potent genotoxic carcinogen. After bioactivation it may covalently bind with protein and with DNA. The former reaction is positively correlated with AFB₁ exposure, and the latter signifies initiation of the carcinogenesis process.

With these biomarkers, epidemiological studies have amply demonstrated the etiological role of aflatoxin in HCC. However, hepatitis B virus also contributes to the development of HCC. Risks and VSD (virtual safe dose) have been estimated from animal and epidemiological studies. These estimates further confirm that AFB₁ is a potent carcinogen. Furthermore, the effects of AFB₁ exposure and hepatitis B are synergistic. Some preventive measures, such as lowering the contamination level of AFB₁ in food and appropriate vaccination programs, have been implemented in many parts of the world. Chemopreventive agents, which may abolish or reduce the effects of AFB₁ are being tested for their effectiveness.

Key words: aflatoxins, primary hepatocarcinoma, biomarkers, carcinogenesis, risk assessment

Introduction

Hepatocellular carcinoma (HCC) is a serious public health problem especially in certain parts of the world, such as southeast Asia and Sub-Saharan Africa. For example, in the People's Republic of China the incidence of HCC was 300,000 deaths per million cancer deaths (1).

These high incidences have been attributed partly to food contaminated with aflatoxins. These toxins are produced by certain species of the fungus *Aspergillus*. The food stuffs most often contaminated are corn and ground nuts and their products.

Aflatoxins first attracted attention because of the turkey "X" disease (2). The disease was so named because in 1960 there was an outbreak of a peculiar disease in turkeys. Those birds having been fed with moldy ground nuts, showed severe hepatic lesions. HCC was observed in some of them (2). The toxins were chemically characterized and were designated aflatoxins (3).

There are several types of aflatoxins: aflatoxin B₁ and B₂ are blue in color, and aflatoxin G₁ and G₂ are green. Aflatoxins B₂ and G₂ are dihydroxylated derivatives of B₁ and G₁. In addition, there are aflatoxins M₁ and M₂, which are hydroxylated deriva-

tives of B₁ and B₂ and are found in the milk of cows fed contaminated fodder. Of these related chemicals, aflatoxin B₁ (AFB₁) is the most common and biologically most active (4). Furthermore, IARC (4) has judged it to be a human carcinogen.

Thousands of studies have been done on aflatoxins to elucidate their carcinogenicity in animals and selected human populations and their relation to human HCC. However, AFB₁, being the most potent and most prevalent, was the center of attention.

Aflatoxin Carcinogenesis

There are two types of carcinogens: genotoxic and nongenotoxic (or epigenetic). AFB₁ is a *genotoxic* agent; its mode of action has been extensively reviewed (5). Aflatoxin is inactive until enzymatically activated to form aflatoxin-8,9-epoxide (6). In other words, it is converted from a procarcinogen to an ultimate carcinogen.

The ultimate carcinogen may be covalently bond to a macromolecule. When the molecule is serum albumin, a lysine adduct is formed (7). When the macromolecule is DNA, a molecule of AFB₁-DNA adduct may be formed. The cell will then become an initiated tumor cell.

The initiated tumor cell may be dormant for a long time. On the other hand, it may become a differentiated neoplasm upon promotion by a promoting agent. As AFB₁ is a complete carcinogen, further biochemical changes may convert the neoplasm to a cancer, through a process of conversion and progression.

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The active AFB₁-DNA adduct has been identified as 8,9-dihydro-8-(N⁷-guanyl)-9-hydroxy-AFB₁ (AFB₁-N⁷-gua) (8, 9). The DNA adduct is excreted in the urine and has been used in many AFB₁ studies in animals and humans as a quantitative indicator of exposure to aflatoxin (10).

In the complete genotoxic carcinogenesis, there is evidence that the *p53* tumor suppressor gene is involved in about half of human cancers (11). Humans exposed to aflatoxins showed *p53* mutations (12, 13, 14).

Although *nongenotoxic carcinogens* do not alter the genetic material; they promote the growth of cells initiated by genotoxic carcinogens. The mechanisms of action include (1) stimulation of cell proliferation through cytotoxicity or hormonal effects; (2) inhibition of intercellular communication, thereby releasing the initiated cells from the restraint exerted by the surrounding normal cells, and (3) immunosuppression. Additional details of the mechanisms of carcinogenesis and some examples of nongenotoxic carcinogens and their mode of action previously reported by Williams and Weisburger (15), Lu (16) and Wang and Groopman (10). One of the nongenotoxic carcinogens is ethanol. It potentiates the AFB₁ carcinogenesis (17).

Epidemiology

Since the first report of serious liver toxicities in turkeys resulting from exposure to aflatoxins as noted above, many studies have been carried out in a great variety of laboratory and wild animals. Hepatic cellular carcinoma (HCC) was observed in most of these animals. Thus Carnaghan (18) reported that 11 ducks fed aflatoxin at 30 ppm in the diet developed HCC. Svoboda et al. (19) reported that 3 of 6 Fischer rats fed aflatoxin at 1.0 ppm in the diet developed HCC. Wogan (20) compiled the results of many studies on various species of animals, including rats, mice, subhuman primates, birds and fishes. The animals with the exception of certain mouse strains, developed HCC.

In light of the extreme potency of aflatoxin in inducing HCC in various species of animals, it became obvious that there might be a connection between the high incidence of human HCC in those parts of the world where food contamination with aflatoxin was noticeable. As a result, many epidemiological studies were conducted to determine the incidence of HCC and the levels of aflatoxins in the food. In the earlier studies, the intake was estimated from chemical analysis of prepared meals or staple foods. The results of 20 such studies were compiled by Bruce (21). He noted that the estimated intakes were the highest in China, followed in decreasing order: Mozambique, Swaziland, Thailand and Kenya.

For various reasons, the data on the levels of aflatoxins were considered imprecise. Therefore, later studies relied on relevant biomarkers of exposure to aflatoxin. The most often used biomarker was a tumor suppressor gene *p53*. It is the most commonly mutated gene detected in human cancers. There was a high frequency of G→T transversions of *p53* in codon 249 (12).

Another biomarker of aflatoxin exposure is urinary AFB₁-N⁷ guanine adduct. The amounts released in 24-hour urine are positively correlated with the exposure to AFB₁ and liver DNA adducts formed in exposed rates (22).

In addition, other biomarkers such as aflatoxin-albumin adduct has also been used, e.g. Wang et al. (23). This is an adduct of aflatoxin with serum albumin. It is not an indicator of mutation, but a fairly reliable one for exposure.

It was increasingly apparent that, as epidemiological studies continued, additional etiological risk factors, such as hepatitis B virus (HBV) and hepatitis C virus (HCV) became known to be involved. HBV and HCV have now been considered human liver carcinogens by IARC, (24). The matter is further complicated by the fact that in many regions of the world where there is heavy aflatoxin contamination, infections with HBV and HCV are also prevalent. It is helpful in epidemiological studies that hepatitis B and C viruses provide surface markers HB_sAg and HC_sAg in the patients and carriers.

Eaton and Gallagher (25) summarized the findings from thirty studies on the status of *p53* and HBV positivity in 631 subjects from many geographical regions. The authors observed a positive correlation between levels of exposure to aflatoxin and the occurrence of HCC among those with or without HBV infection.

Wang et al. (26) reported on 56 cases of HCC patients and 220 healthy controls matched by age, gender and residence. The authors found a synergistic effect between HBV and aflatoxin. Thus, among HB_sAg carriers the incidence of HCC was significantly higher among those with higher levels of exposure to aflatoxin as determined either by aflatoxin-albumin adducts or by urinary aflatoxin metabolites. In addition, carriers of HB_sAg showed a significantly increased risk for HCC.

In a more recent article (27) analyses were made on the *p53* with G→T transversions in codon 249 in patients living along the Gambia river. In that region, the food is considered to be often contaminated with aflatoxins. The results suggest that 19 (36%) of the 53 HCC patients were positive for this biomarker. On the other hand, this was true only in 3 (6%) of 16 cirrhosis patients and none (0%) of 60 non-Gambian Europeans with various liver pathologies. Hence, the authors considered that the results suggest aflatoxin as a risk factor for HCC.

Risks/VSD

Risk is defined as the expected frequency of an adverse effect arriving from exposure to a specific pollutant. A number of mathematical models have been developed to estimate the *risk* at a specific exposure level based on the observed dose-response relation. Alternatively, one may estimate the dose at a specified risk level. When the specified risk level is very low, such as 10⁻⁵ or 10⁻⁶, the dose associated with it is known as the “*virtual safe dose*”, (VSD).

On the basis of a study in rats, the Food Safety Council (28) estimated the VSD at a risk of 10⁻⁶, using five models. The VSDs in ppm and the models used, in parenthesis, are as follows: 3.4×10⁻⁵ (one-hit), 7.9×10⁻⁴ (multistage), 4.0×10⁻² (Weibull), 0.28 (multihit), and 2.5 (probit).

In recent years more epidemiological findings, which are more reliable for estimating risk/VSD, have become available. Thus, Hoseyni (29) estimated, that the risk of death from liver cancer for those exposed to AFB₁, relative to the unexposed population, increases by 0.05% per ng/kg/day exposure to AFB₁. The results also suggest a 25-fold increase in the risk of death from liver cancer among those infected with hepatitis B virus, relative to noncarriers.

Groopman (30), after analyzing the findings reported by Ross et al. (31) concluded that there was a 5-fold increase in the relative risk for HCC cases with detectable AFB₁-N⁷-guanine. The patients who tested positive for HBV_sAg also showed a relative risk of

about 5. It is remarkable that the relative risk was greater than 60 for those with detectable AFB-N⁷-guanine and HBV_sAg. Thus, there was a strong synergism between aflatoxin and hepatitis B.

The Joint FAO/WHO Expert Committee of Food Additives and Contaminants (32) concluded that the “*potency value*” would be 0.3 cancers/year per 100,000 population per ng aflatoxin/kg bw per day in HB_sAg+ individuals. Among those with HbsAg- individuals, the potency value would be 0.01 cancers/100,000 population per ng aflatoxin/kg bw per day. Thus, the Committee’s assessments suggest a strong synergism between aflatoxin and hepatitis.

Discussions

HCC is a serious public health problem especially because of its poor prognosis. Exposure to AFB₁, as noted above, is a definite etiological factor. Numerous studies in animals and humans have shown that it is an extremely potent genotoxic carcinogen with a distinct tendency to affect the hepacytes, thereby inducing HCC.

However, there are other etiological factors. Especially notable are hepatitis B virus infections and to a lesser extent, hepatitis C virus. In fact, there appears to be a strong synergism between HBV and aflatoxin (33, 34).

To reduce the prevalence of HCC, a number of measures have been implemented; others are being tested for effectiveness. For example, improved food processing and storage have been or are being put into place to minimize food contamination by aflatoxins. Government standards have been introduced setting upper limits for contamination. For example, the U.S. Food and Drug Administration has set “*Action Levels*” for aflatoxin at 20

ppb in nuts and foods (35).

In addition, the effectiveness of certain chemoprotective agents are being tested. For example, oltipraz [4-methyl-5-(2-pyrazinyl)-1,2-dithiol-3-thione] is an effective inhibitor of AFB₁-mediated hepatocarcinogenesis in rats. It was tested in a project in Qidong, China. The results suggest that small daily doses or large weekly doses of oltipraz increased the excretion of aflatoxin. (36). Selenium has been shown to inhibit aflatoxin carcinogenesis in the rat (37).

Since hepatitis is also a risk factor of HCC, systematic vaccination has been practiced in a number of countries. More recently, Oda (38) reviewed the “*vertical transmission*” of hepatitis B virus in Japan, and a nationwide selective vaccination strategy using anti-HBs immunoglobulin and HB vaccines was being considered.

Conclusions

1. Aflatoxin B₁, a fungal contaminants of nuts and certain other foods, is a potent genotoxic carcinogen. It induces hepatocarcinoma (HPP) in many species of animals and humans.
2. HCC is a serious disease. To combat this public health problem, governmental and industrial measures are being taken to minimize the aflatoxin contamination of foodstuffs.
3. As hepatitis B, and to a lesser extent hepatitis C, viruses are also etiological factors of HCC, immunizations against these infections are being expanded.
4. Chemopreventive agents against AFB₁-induced HCC are being studied for their effectiveness.
5. The multi-pronged approach to the prevention of HCC may soon greatly diminish this public health problem.

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