

Do Gastroenterologists Consider Aflatoxins as Origin of Digestive System Cancers?

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Abstract

Aflatoxins are important etiological factors for cancers in the digestive system that have not been extensively studied. The present review describes reports of the presence of aflatoxins in cancers of the esophagus, stomach, pancreas, liver, colon and rectum. The presence of AFB₁-FAPY adducts and mutations in codon 249 of the tumor suppressor gene p53 in colorectal cancer tumors and Ki-ras activation by point mutation in pancreas cancer are reliable criteria to accept AFs as an important etiological factor. The Ministries of Health of the different countries should perform more preventive practices on crops in the field and in warehouses; they should chemically analyze aflatoxins in fresh and industrialized foods for humans and feeds for animals to avoid the presence of these dangerous toxins.

Keywords: Aflatoxins; Carcinogens; Digestive system; Food contamination

Introduction

Gastroenterologists, especially hepatologists, know the nature and properties of aflatoxins: they are well accepted mutagens, teratogens, immuno-depressors and carcinogens for humans [1] present in multiple human foods, but they are not given adequate attention.

Aflatoxins

Aflatoxins (AFs) are the most frequent carcinogen in food for humans and animal feed, such as vegetables (chilli-pepper [2], maize [3], oilseeds such as nuts [4], peanuts [5], etc.), their derivative products (industrialized chili sauces [6], tortillas [7], beers [8], dry fruits [9], peanut butter [10], etc.) and aflatoxins of animal derivative foods (eggs [11], milk [12, 13], human maternal milk [14], cheese [15,16], hens breast [17], liver [18], sausages [19], pâté [11], etc.). Many cosmetics have aflatoxins from their vegetable sources (almond oils [20] and cosmetic creams [21], hair dyes [22] made from chili pepper, etc.) and AFs can enter through the skin or the scalp.

AFs are secondary metabolites, polyketides that chemically correspond to a bisdihydrodifuran or tetrahydrobisfuran united to a coumarin substituted by a cyclopentanone or a lactone [23-25]. AFs are divided into two subgroups [24-27].

- Bisfuran-coumarin-cyclopentanones, which include AFs of series B (AFB₁, AFB₂, AFB_{2a}), M (AFM₁, AFM₂, AFM_{2a}), Q (AFQ₁) and P (AFP₁) and aflatoxicol (AFL) that interconverts with AFB₁.
- Bisfuran-coumarin-lactones, which contain AFs of series G (AFG₁, AFG₂, AFG_{2a}).

Only AFB₁, AFB₂, AFG₁ and AFG₂ are naturally synthesized by toxigenic fungi. The other AFs (M₁, M₂, P₁, Q₁, G_{2a}, B_{2a} and AFL) are products of microbial or animal metabolism [27-31].

Animal livers protect the organism by lowering the toxicity of AFB₁ *via* the addition of an OH- group to form hydroxylates (AFM₁, AFP₁, AFQ₁ and AFL); this step facilitates AF solubility in water and their disposal *via* urine, faeces and milk. AFB₁ and AFG₁ have a double bond at the 8, 9 position that oxidizes and forms AFB₁-exo-8,9-epoxide, an unstable molecule, which produces dihydrodiol AFB₁ and becomes linked to the N⁷-guanine of DNA [32] to form active carcinogens called AFB₁-DNA adducts. AFB₂ and AFG₂ [33] lack a double bond, which affects their toxicity. The bond changes that conversion.

AFB₁ to AFB₂ are well studied [32, 34] and the biotransformation and biosynthetic routes of AFB₁ have been previously described [18-21]. Aflatoxins damage the digestive system and the active carcinogen AFB₁ formamido-pyrimidine (AFB₁-FAPY adduct) has been recovered, identified and quantified in cancer tumors from the liver, intestines, colon and rectum [35]; and has also been recovered in cervical cancer [36], the pancreas and malignant human breast tumors [35]. The effects of aflatoxin B₁ (AFB₁) intake, genetic polymorphisms of AFB₁ metabolic enzymes and interactions between the polymorphisms and intake of AFB₁ have been investigated regarding the risk of gastric cancer in Korea [37]. The results suggested that dietary AFB₁ exposure might be associated with a risk of gastric cancer, but the authors did not measure AFB₁-DNA adducts, which are the active carcinogens. However, the effect of AFB₁ on gastric carcinogenesis may not be modulated by genetic polymorphisms of AFB₁ metabolic enzymes [37]. Other studies on the digestion process of maize tortilla showed that pH was an important factor to activate the carcinogen [38]. Aflatoxins have a synergetic relationship with some viruses, such as hepatitis B virus [39] and human papilloma virus (HPV), particularly more with 16 HPV than 18 HPV [36].

Aflatoxins can cause damage in most living creatures, from viruses to humans [40-42] and they also damage plants [43]. The damage caused by aflatoxins to plants has been reported as a reduction in seed germination, suppression of the synthesis of chlorophyll and development of radicles in *Lepidium sativum* [44] and lettuce [45].

Aflatoxins inhibited the growth of algae such as *Chlorella pyrenoidosa* [46], among their effects on molds [47]. AFB₁ inhibited the growth of four isolates of its own producing fungi *Aspergillus flavus*, *A. awamori*, *Penicillium chrysogenum* and *P. duclauxi*. Aflatoxins also affect fungi by inhibiting the sporulation of *Aspergillus niger*, *Penicillium expansum*, *Cladosporium herbarum*, *Mucor hiemalis*, *Rhizopus nigricans* and *Thamnidium elegans* with distinct deformations [48, 49].

Experimental

Biomarkers

The early identification of AF metabolites in human fluids [50] stimulated the development of biomarkers [51]. The availability of specific antibodies helps detect the AF metabolites in human urine [52-54].

Aflatoxins are recognized biomarkers for the risk of cancer as a result of nearly 57 yrs of study and they represent one of the most extensive data sets that assist the future studies of other environmental agents [27].

The application of biomarkers, validated in epidemiological studies, is useful to prevent high-risk human populations from cancer. The experimental and human studies of aflatoxin biomarkers are based on their biochemistry and toxicology. This systematic approach provides encouragement for preventive interventions [55].

An exposure biomarker is the present and past measurement of the exposures of AFs and their metabolites or products in the human body or fluids. The biomarkers of internal doses and of doses of biologically effective AF are generally hydroxylated metabolites and the AF-DNA adducts formed from epoxide derivatives [56]. Biomarkers in etiological research are used for prevention in high risk populations because experimental studies have established time links between AF biomarker modulation and the risk of disease.

In the body, AFBs, mainly AFB₁, are biotransformed into various metabolites, especially active AFB₁-exo-8, 9-epoxide. The AFB₁ 8, 9-epoxide AFB₁ and other metabolites interact with various biomolecules in the body, including DNA, RNA and the various metabolic pathways, such as protein synthesis, glycolytic pathway and the electron transport chain involved in ATP production in cells. AFB interacts with DNA to form AFB-DNA adducts causing DNA mutations and breakages. AFB₁ and its metabolites induce the up regulation of nuclear receptors, such as pregnane X receptor (PXR), constitutive androstane receptor (CAR) and aryl hydrocarbon receptor (AhR), through gene expression that regulates metabolizing enzymes, such as CYP450 involved in Phase I and Phase II metabolism of xenobiotics. AFB₁ activates these nuclear receptors to produce the metabolizing enzymes.

Cytochrome P450 controls AF adduct formation in the AFB₁ metabolic pathway. The 8, 9-epoxide AFB₁ is an electrophilic metabolite that covalently reacts with DNA to form adducts that have the carcinogenic and mutagenic activities of AFB₁. The 8, -epoxide AFB₁ links to N⁷ of the guanine residues of DNA to form the 8, 9-dihydroxy-8-(N⁷) guanyl-9-hydroxy AFB₁ adducts (AFB₁-Gua), which are the most abundant [57-59]. The positively charged imidazol ring of AFB₁-Gua promotes depurination, leading to an apurinic base. This ring opens to form a chemically and biologically more stable adduct, the formamidopyrimidine; the 2, 3-dihydro-2-(N-formyl)-2', 5',6'-

triamino-4'-4'-oxy-N-pyrimidyl-3-hydroxy-AFB₁ (AFB₁-FAPY) adduct can be present several times in DNA replication in a variety of organs [60-61] and represents the long term accumulation of years, of the persistent secondary adduct of the carcinogen in the DNA. The AFB₁-FAPY adduct is indicative of persistent exposure to AFB and this may be contributing to the cancer risk [35]. Aflatoxins may in fact be a risk factor for cancer induction in a variety of organs in man, in the same manner as that of cigarette smoking. The detection of AFB₁-DNA adducts in "normal" individuals and the lack of adducts in some cancer patients indicates that either a) some individuals lack the specific P450 isozymes required to produce the epoxide; b) they have high levels of the glutathione transferase isozyme required for detoxification; c) they have extremely efficient repair systems; d) they have never been exposed to AFB₁ or have adduct levels below the minimum detection level of this system (30 pg AFB₁) [35]. The activation of the metabolic pathway of AFB₁ has been described previously [59].

For these reasons, it is important to determine the presence of free AFs (AFB₁, AFB₂, AFG₁, AFG₂) as an exposure measure of a person to food AFs and to measure the metabolic hydroxylates (AFM₁, AFM₂, AFP₁ and AFL) as biomarkers of internal doses and effective biological doses in control samples, as well as the presence of the AFB₁-Gua and AFB₁-FAPY adducts, which are etiological agents of human cancers, particularly hepatocellular carcinoma (HCC).

Epigenetics

The term epigenetics refers to heritable changes in gene expression (active versus inactive genes) that do not involve changes to the underlying DNA sequence, thus a change in phenotype without a change in genotype. Epigenetics refers to external modifications to DNA that turn genes "on" or "off". These modifications do not change the DNA sequence, but instead, they affect how cells "read" genes and affect gene expression rather than altering the genetic code itself. The 8, 9-epoxide AFB₁, AFB and other metabolites also affect epigenetic mechanisms, including DNA methylation, histone modifications, maturation of miRNAs as well as the daily formation of single nucleotide polymorphisms (SNPs) where AFB exposure may facilitate the process and induce G:C to T:A transversions at the third base in codon 249 of TP53, causing the p53 mutations reported in HCC [62]. The transversion is not an epigenetic mechanism because it changes the DNA sequence, but it affects the epigenetic mechanisms. There is a multiplicative effect on HCC risk resulting from the mutational effect of aflatoxin on TP53, as monitored by detection of plasma 249 (ser), with concomitant chronic infection with HBV. The changes in epigenetic mechanisms affect gene expression, cellular differentiation and growth. AFB₁ also through epigenetic mechanisms, promotes tumorigenesis, angiogenesis, invasion and metastasis in HCC. However, formation of the small amounts of AFB₁ from AFB₂ is suspected to cause the carcinogenicity of AFB₂ in humans and animals. Chronic AF exposure leads to the formation of reactive 8, 9-epoxide AFB₁ metabolites in the body that could activate and de-activate the various epigenetic mechanisms leading to the development of various cancers [63]. Aflatoxins as etiological factors of some human cancers from the digestive system will be described.

Aflatoxins in Buccal cancer

There are no conclusive studies that prove the relationship between AFs and buccal cancers although these toxins are present in many foods chewed in the mouth, such as cereals, chilli peppers and oilseeds. Some chemoprotection trials have been performed on buccal mucosal

cells, but the results are preliminary and inconclusive. More studies are needed to clarify the etiology of these buccal cancers.

Aflatoxins in Oesophageal cancer

The accepted epidemiological risk factors for esophageal cancer development are Barrett's esophagus, obesity, tobacco, opium consumption, hot tea drinking, poor oral health and low intake of fresh fruit and vegetables [64], with no mention of AFs.

There are a few reports about aflatoxin contamination of wheat flour and the risk of esophageal cancer in a high risk area of Golestan province in North-eastern Iran. There was a positive relationship between the AF level of white flour samples and the risk of esophageal cancer in Iran. The humidity and temperature of silos were the most important determinants of AF contamination of white flour [65]. There are some reports about human immunodeficiency virus (HIV) transmission frequency being positively associated with maize consumption in Africa. HIV and esophageal cancer deaths were significantly related to maize but were inversely related to the percentage of Muslims and rice consumption [66]. Regarding the relationship between cancer and food, these authors suggests that fumonisin contamination rather than aflatoxin is the most likely factor in maize promoting HIV and esophageal cancer; this finding could be true, as fumonisins promote cancer, but to determine if the real carcinogen is AFB₁, a study on AFB₁-DNA adducts is needed [66, 67].

In the last 30 yrs, the incidence of esophageal and gastric adenocarcinoma has steadily increased approximately seven-fold, a greater increase than that of several malignancies, including melanoma, breast cancer and prostate cancer. The main risk factors for gastroesophageal junction cancer are a history of GERD and obesity [68].

Aflatoxins in Stomach cancer

In general, cancer begins when an error (mutation) occurs in a cell's DNA. The mutation causes the cell to grow and divide quickly and to be more resistant to death than the normal cells. Later, metastasis occurs invading nearby tissues and organs. Knowing that AFs are recognized mutagens, it is highly probable that they can cause stomach cancer, but it has not been proven. There is a strong correlation between a diet high in smoked and salted foods and stomach cancer in the main part of the stomach. As the use of refrigeration for preserving foods has increased around the world, the rates of stomach cancer have declined [69].

The risk factors for cancer in the gastroesophageal junction are associated with having gastrointestinal reflux disease (GERD) and, less strongly, with obesity and smoking. GERD is a condition caused by frequent backflow of stomach acid into the esophagus [70, 71]. There is a report, with AFs chemical analysis, about an Italian woman with intense abdominal pain and heavy inflammation caused from the ingestion of beef contaminated with aflatoxins [72]. In well-developed countries aflatoxins exposure should be considered in case of unexplained abdominal symptoms. Cancer risk cannot be assessed, nevertheless individual counselling and periodical medical examinations are recommended.

The factors that increase the risk of stomach cancer have been previously reported [68,73] and include eating foods contaminated with aflatoxins, a diet high in salty and smoked foods and low in fruits and vegetables, a family history of stomach cancer, infection with

Helicobacter pylori, long-term stomach inflammation, pernicious anemia, smoking and stomach polyps. The effects of AFB₁ intake, genetic polymorphisms of AFB₁ metabolic enzymes and interactions between the polymorphisms and intake of AFB₁ have been investigated regarding the risk of gastric cancer in Korea [37]. The study included 477 gastric cancer patients and 477 age and sex-matched control subjects. The probable daily intake of AFB₁ was significantly higher among gastric cancer patients than among control subjects and increased AFB₁ intake was significantly associated with an elevated risk of gastric cancer (odds ratio 1.94; 95% confidence interval 1.43 to 2.63). The results suggested that dietary AFB₁ exposure might be associated with a risk of gastric cancer, but the authors did not measure AFB₁-DNA adducts, which are the active carcinogens.

Moreover, there was no interaction between AFB₁ intake and the genotypes of metabolic enzymes that affect gastric cancer risk [37]. Other studies showed that, in the digestion process of maize tortilla with AFs, a neutral pH was an important factor to activate the carcinogen; the stomach has an acidic pH \pm 2.5 in which the AFB₁ is not activated, with the AF mutagenicity tested with an Ames test, which might protect the individual from the formation of stomach cancer [38]. Thus, dietary AFB₁ exposure might be associated with a risk of gastric cancer [37].

Aflatoxins in Liver cancer

Aflatoxins are well recognized as a cause of liver cancer [35,39,41,58], with additional important toxic effects, such as immune depression and interference of protein metabolism and with multiple micronutrients basic to health. These effects have been well studied in animals but less so in humans, but some of the effects observed in animals also occur in humans. The prevalence and human exposure to aflatoxins is approximately 4.5 billion persons living in developing countries, who are chronically exposed to largely uncontrolled amounts of the toxin [67].

The aflatoxin exposure and toxic effects result in changes in nutrition and immunity that affect health, including HIV infection, which accounts for approximately 40% of the burden of disease in developing countries where a short lifespan is prevalent. Food systems and economics make the management of aflatoxins impractical in developing-country settings, but the strategy of using food additives to protect farm animals from the toxins may also provide effective and economical new approaches to protecting human populations [67].

Oncogenes and tumour suppressor gene p53

Oncogenes, such as N-ras, c-myc or c-fos, are over-expressed, but their mutations are rare and there is little evidence of a direct implication for pancreatic cancer [74]. A specific mutation in codon 249 of the p53 gene is present in regions with hepatocellular carcinomas (HCC) and high exposure to AF [75]. This mutation, induced by the reactive forms of AFB₁, is a "hotspot" for the mutation induced by AFB₁, specifically the transversion GC \rightarrow TA [59]. In Gambia, this mutation was detected in DNA in cases of HCC and was not frequent in the control cases [76-79]. Transversions G \rightarrow T or transitions G \rightarrow A are produced in the third base of codon 249 of the p53 gene and in the first or second base of codon 12 of the H-ras gene [80-85].

AFB₁ in liver cancer in rats was identified with an increase in the expression of c-myc and c-Ha-ras and in one of the tumors, an amplification and adjustment of the c-Ha-ras oncogene was observed

[86]. When 12 tumors induced by AFB₁ were examined in rats, the genomic DNA of the 10 tumors was transformed and 2/8 had activated Ki-ras [87]. When male Fisher rats were exposed to AFB₁ and AFG1, four liver tumors were induced, three had activated N-ras and one had a transversion G→A in codon 12 of Ki-ras [88].

The tumor suppressor p53 plays an important role in the development of HCC, as well as other oncogenes and tumor suppressor genes. The identification of a specific mutation in the tumor suppressor gene p53 in HCC of world regions with high AF exposure has helped identify AF as a biomarker [89].

The mutation of the tumor suppressor p53 gene was found in 53% of the HCC cases in Mexico, a country with high exposure to AFB₁, while in populations with low exposure to this toxin, the mutations were observed in 26% of the cases [90]. In Senegal where people are exposed to high concentrations of AFB₁ in foodstuffs, the 249 codon mutation of the p53 gene was found in 10/15 of HCC tumors [91]. The mutation index in the p53 gene is higher in tumors with a prevalence of Hepatitis B Virus (HBV) compared with tumors with Hepatitis C Virus (HCV) and non-viral HCC and independent of the AF exposure [92].

Aflatoxins in Colorectal cancer

The most complete study on aflatoxins in colorectal cancer was reported [35] with the amount of AFB₁-FAPY adducts in different sections of the colon and rectum. The link between constipation and colorectal cancer is natural due to the union of the food carcinogen and the internal surface of the colon, while in non-constipated cases, the excretion of the food carcinogens is more likely. The possibility of endogenous aflatoxin production by a species of *Aspergillus* or other aflatoxin-producing fungi colonizing the gut is another possibility [35]. Another report refers to the acute effects of aflatoxin in rat proximal and distal colon spontaneous contractions [93].

Aflatoxins in Pancreas cancer

There are reports of aflatoxin B1 (AFB₁)-DNA adduct detection in formalin-fixed tissue from an acute poisoning incident in Southeast Asia [94]. Tumor tissues tended to have higher adduct levels than normal tissue from the same individual and the levels generally increased with patient age. In samples analyzed by HPLC, the adducts present had chromatographic properties of AFB₁-FAPY, the open-ring form of the AFB₁-guanine adduct.

In addition to a very high frequency of Ki-ras activation by point mutation [95], such as those caused by AF, there is evidence of an autocrine loop involving the overexpression of transforming growth factor alpha and the epidermal growth factor receptor [96, 97]. These abnormalities may be linked as there is *in vitro* evidence that activation of ras oncogenes causes upregulation of TGF alpha expression [98]. The same phenomenon occurs when aflatoxins mutate DNA.

Results and Discussion

Protection mechanisms: Glutathione (GSH)

GSH is an important antioxidant in plants, animals, fungi and some bacteria and archaea, preventing damage to important cellular components caused by reactive oxygen species, such as free radicals, peroxides, lipid peroxides and heavy metals [99].

Glutathione S-transferase (GST) enzymes

The GSTs are a family of enzymes that protect the organism. In Phase I of cytochrome P450, there are reactions to generate hydrosoluble products. In Phase II, the glutathione S-transferases (GSTs) allow these metabolites to combine with polar endogenous molecules to form conjugation products that are excreted quickly [100]. With this reaction, the solubility of dangerous compounds increases for their excretion [101]. In this same Phase II of the enzymatic de-intoxication, many electrophilic metabolites, such as xenobiotic derivatives and endogenous molecules, such as aflatoxins, have carcinogenic and genotoxic effects [102-104].

Glutathione S-transferase and aflatoxins

AFB₁ induces enzymatic conjugation reactions mediated by GST to inactivate the 8, 9-epoxide AFB₁. Spontaneously, 8, 9-epoxide AFB₁ is hydrolyzed to 8, 9 dihydrodiol and conjugates with GSH to form AFB₁ glutathione transferase (AFB₁-SG) [105]. The conjugate AFB₁-SG is the most abundant biliary metabolite and it is excreted by urine [106]. The induction of GST and aldehyde-AFB₁ reductase (AFAR) reduces the formation of adducts AF-ADN and AF-proteins and blocks cancer initiation in rats and this formation of AFB₁ to ADN is avoided in different species. The ingestion of antioxidants in the diet increases the levels of GST and more AFB-SG is eliminated in the urine of treated animals [106].

Conclusion

Aflatoxins are the most frequent carcinogen daily ingested in multiple foods and they are an important etiological factor for cancers in the digestive system that have not been thoroughly studied. The presence of AFB₁-FAPY adducts and mutations in codon 249 of the tumor suppressor gene p53 in many cancer tumors such as colorectal cancer tumors and Ki-ras activation by point mutation in pancreas cancer, are criteria to accept AFs as an important etiological factor. The Ministries of Health of the different countries should perform more preventive practices on crops in the field and in warehouses; they should chemically analyze aflatoxins in fresh and industrialized foods for humans and feeds for animals to avoid the presence of these dangerous toxins.

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