14

Aflatoxin B1 - Prevention of Its Genetic Damage by Means of Chemical Agents

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1. Introduction

Mycotoxins are structurally diverse groups largely composed of small molecular weight chemicals, which are generally produced by the mycelial structure of filamentous fungi. These toxins are secondary metabolites mainly synthesized during the end of the mould exponential phase of growth. They appear to have no biological significance with respect to their growth/development or competitiveness, but when ingested by higher vertebrates and other animals they can cause diseases called mycotoxicoses (Kabak et al., 2006; Madrigal-Santillán et al., 2010). Aflatoxin B1 (AFB1), in particular, is a tetrahydrofuran moiety fused to a coumarin ring and was chemically classified as cyclopenta[c]furo[3',2':4,5]furo[2,3h][1] benzopyran-1,11-dione,2,3,6a,9a-tetrahydro-4-methoxy-, (6aR,9aS) (Eaton et al., 1994; Hedayati et al., 2007) (Figure 1).

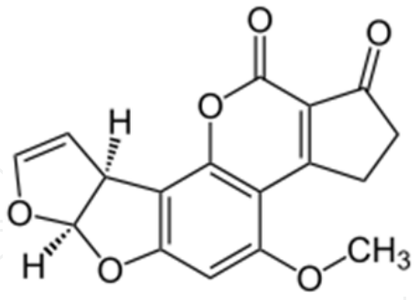

Fig. 1. Chemical structure of aflatoxin B1

The compound is a pale-white to yellow crystalline, odorless solid, soluble in water and in polar organic solvents, such as methanol, chloroform, acetone, acetonitrile, and dimethyl sulfoxide. It has a molecular weight of 312.3, a melting point between 268-269°C, and shows a blue fluorescence in the presence of ultraviolet light (Eaton et al., 1994; Hussein & Brasel, 2001). This secondary metabolite is produced by several strains of filamentous ascomycetes fungi, mainly *Aspergillus flavus* and *Aspergillus parasiticus* (Table 1) which are ubiquitous in the environment and highly resistant to heat and drying. They are saprophytic and frequently live in soil, vegetation, and feeds, acquiring nutrients from dead plants and animal matter.

Their spores are produced in large numbers and are spread widely by air currents. These molds grow within many commodities when temperatures are between 24-35 °C, and the moisture content exceeds 7% -10% (Kogbo et al., 1985; Williams et al., 2004).
The present chapter has the purpose of putting into perspective the worldwide relevance of the AFB1 contamination problem due to its effect on the aspects of economy and health, as well as to review the main strategies developed for coping with such contamination. In particular, we discuss the theoretical grounds and the practical approaches which have been carried out by using antimutagenesis and chemoprevention strategies. In these areas are included a description and discussion of the more relevant agents tested against the genotoxic and carcinogenic damage induced by AFB1.

2. The contamination problem

Aspergillus parasiticus often grows in oily products such as peanuts, walnuts, pistachios, pine nuts, pumpkin seeds and sunflower seeds, while *A. flavus* is commonly found contaminating agricultural fields of grains such as corn, sorghum, rice, barley, rye, and oats, as well as in spices (chili, pepper, mustard, and cloves). All of these commodities or products may be raw material for animal feed, which when ingested may pass into breast milk and can later be found in cheese, yogurt, cream, meat, and egg, constituting a source of secondary contamination for humans (Juan-López et al., 1995).
Fungal invasion and aflatoxin contamination often begin before harvest and can be promoted by production and harvest conditions, genotypes, drought, soil types, and insect activity, among other factors (Cole et al., 1995; Lynch & Wilson, 1991; Mehan et al., 1986, 1991). Therefore, timely harvest and rapid and adequate drying before storage are important factors to avoid or reduce post-harvest contamination, because even moisture generated by insect respiration and local condensation may develop local pockets favorable to aflatoxin growth (Mehan et al., 1986; Williams et al., 2004). This may partially explain differences in the range of contaminated products among countries. For example, in Japan aflatoxins were detected in about 50% of peanut butter and bitter chocolate samples, while their presence was not found in corn products; in contrast, a study in China reported contamination in 70% of corn products (Kumagai et al., 2008; Wang & Liu, 2007).
Aflatoxin contamination may be more severe in developing than in developed countries, yet this is a worldwide problem that could reach as much as 25% of the world's crops (Fink-Gremmels, 1999). In past years, a survey conducted in Midwestern states of the USA found 19.5% of corn samples contaminated with aflatoxin when assayed prior to any induced environmental stress, and 24.7% of them contaminated following stress induction (Russell et al., 1991). Also, Shane (1993) estimated losses in Southeastern USA for around 97 million dollars because of AFB1-contaminated corn with an additional 100 million dollars in production losses at hog farms feeding the contaminated grain.

Kingdom	**Fungi**
Phylum	Ascomycota
Class	Eurotiomycetes
Order	Eurotiales
Family	Trichocomaceae
Genus	*Aspergillus*

Table 1. Taxonomy of *Aspergillus*

There are diverse criteria for assessing the economic impact of aflatoxins. These include loss of human and animal life, health care and veterinary care costs, loss of livestock production, loss of forage crops and feeds, regulatory costs, and research cost focusing on relieving the impact and severity of the aflatoxin problem. However, most reports on the matter are on a single aspect of aflatoxin exposure or contamination.

With regard to the heavy impact of AFB1 contamination, India can be an example of the problem in emerging countries. A study in the Bihar region showed that nearly 51% of the 387 samples tested were contaminated with molds, and that from the 139 samples containing AFB1, 133 had levels above 0.02 mg/kg (Ranjan & Sinha, 1991). In other studies, authors found levels as high as 3.7 mg/kg of AFB1 in groundnut meal used for dairy cattle, as well as 0.05 to 0.4 mg/kg in 21 of 28 dairy feed samples from farms in and around Ludhiana and Punjab (Dhand et al., 1998; Phillips et al., 1996). Also, in raw peanut oil 65-70 % of AFB1 was found in the sediment and 30-35 % in the supernatant oil after centrifugation (Banu & Muthumary, 2010a). In this context, groundnut contamination was estimated to represent about a 10 million dollar loss in India's export within a decade (Hussein & Brasel, 2001; Vasanthi & Bhat, 1998). Regarding the extent of the problem in developing countries, Table 2 shows that a wide range of commodities are contaminated, even to a higher degree than usually allowed (Williams et al., 2004).

In Mexico the main contaminated crop is corn. This is a logical situation considering that the country has one of the highest rates of human consumption of this grain in the world (120 kg per capita per year) with a production of about 10.2 million tons for human consumption and 5 million tons for animal feed and other industries (Plasencia, 2004). One of the most significant episodes of aflatoxin contamination of maize was probably that which occurred in a northern state (Tamaulipas) in 1989, where levels of the toxin above 0.1 mg/kg were reported in practically all the plants harvested (García & Heredia, 2006). This represents a potential high health risk to the population, because corn is a basic food consumed as tortilla, with a consumption of 325 g per day (Anguiano-Ruvalcaba et al., 2005). However, this is not the only food susceptible to AFB1 that may pose a health risk, because a number of other maize-based foods are part of the Mexican diet. In regard to this contamination a few studies have been made. In kernelled corn for human consumption in the city of Monterrey, AFB1 was determined in 36 of the 41 samples tested, with concentrations ranging from 5 to 465 ng/g, with 59% of those samples above the Mexican legal limit of 0.02 mg/kg (Torres-Espinoza et al., 1995). Another study in 66 stored samples of maize and wheat in the state of Sonora showed 13 samples (20%) contaminated with AFB1, although the level was higher than 0.02 mg/kg in only one sample (Ochoa et al., 1989). Some general explanations for the contamination in the country are the following: 1- inadequate pre-harvest and storage management, as well as distribution procedures that may favor the development of *Aspergillus*; 2- corn growing under non-irrigation conditions in many places, predisposing plants to drought stress and mold infection; 3- limited possibilities of modern agricultural practices for low income farmers; 4- legal restriction for the use of transgenic maize manifesting insecticidal proteins or any other trait to reduce aflatoxin contamination; 5-infestation with the microleopterans *Carpophilus freemani*, the sap beetle, *Sitophilus zeamais*, the maize weevil, and *Cathartus quadricollis*, square-necked grain beetle, which may facilitate spore entry in the cobs; 6- growth of pollinated varieties which appear to be more prone to disease development and to the effect of environmental factors in comparison with maize hybrids (Figueroa, 1999; Plasencia, 2004; Zuber et al., 1983).

Country/ commodity/Number	Positive AFB1 samples (%)	Contamination rate (ppb)
Bangladesh		
Maize (95)	67	33.0 (mean)
Brazil		
Corn (96)	38.3	0.2-129.0
Peanut (97)	67	43.0-1099.0
China		
Corn (99)	76	>20.0
Costa Rica		
Maize (100)	80	>20.0
Egypt		
Peanut butter (101)	56.7	>10.0
Hazelnut (102)	90	25.0-175.0
Soybean (104)	35	5.0-35.0
Guatemala		
Incaparina (corn/ cottonseed flour) (106)	100	3.0-214.0
India		
Chilies (109)	18	>30.0
Maize (113)	26	>30.0
Korea		
Barley food (114)	12	26.0 (mean)
Corn food (114)	19	74.0
Malaysia		
Wheat (117)	1.2	>25.62
Mexico		
Corn (118)	87.8	5.0-465.0
Nigeria		
Corn (119)	45	25.0-770.0
Maize-based gruels (120)	25	0.002-19.716
Qatar		
Pistachio (121)	8.7 to 33	>20.0
Senegal		
Peanut oil (122)	85	40.0 (mean)

Table 2. Examples of market sample contamination, frequencies, and concentrations

Besides economical and educational actions that can be carried out to reduce the contamination problem in Mexico, other specific actions can be the following: 1- more research and breeding programs to identify varieties resistant to fungal infection and AFB1 contamination; 2- epidemiological data concerning liver cancer/AFB1 ingestion, as well as determination of AFB1 intake and its excretion in fluids, (particularly because cancer initiation may take about 6 years); 3- adoption of a standard method for measuring AFB1 content at both national and international levels, which must be sensitive, reliable, reproducible, and cost-effective.

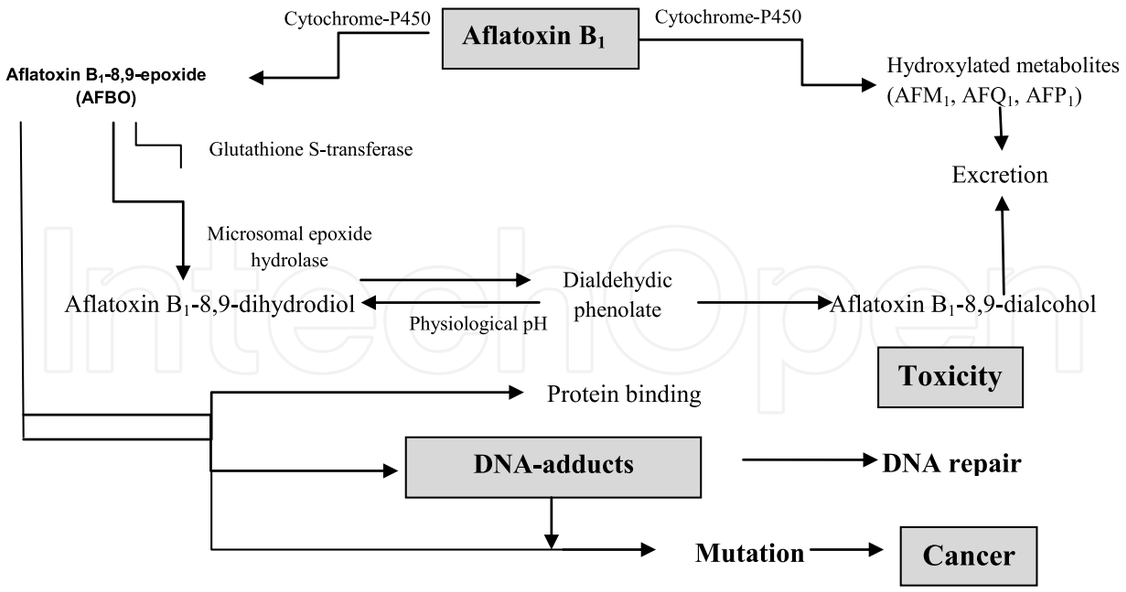

Fig. 2. Biotransformation pathways of aflatoxin B1

3. Toxicity and intervention strategies

AFB1 was first isolated some 40 years ago after outbreaks of disease and death occurred in turkeys and rainbow trout fed on contaminated peanut and cottonseed meals (Williams et al., 2004). From this time onwards a number of investigations have corroborated the strong toxicity of this mycotoxin in mammals, poultry, fish and other animals (Girish & Smith, 2008; Kensler et al., 2011; Madrigal-Santillán et al., 2010; Santacroce et al., 2008). Aflatoxicosis is the poisoning that results from ingesting AFB1, and two general forms of the affection have been identified. One is an acute, severe intoxication, which results in direct liver damage and subsequent illness or death, related with large doses; this type of aflatoxicosis includes symptoms such as hemorrhagic necrosis of the liver, bile duct proliferation, edema, lethargy, and liver cirrhosis The other, a chronic form of the disease, corresponds to a subsymptomatic exposure, which is related with nutritional and immunologic consequences, such as suppression of the cell-mediated immune responses; also, as dose exposure has a cumulative effect, there can be a significant risk increase of developing cancer (Steyn, 1995; Williams et al., 2004).

Studies on the matter have established a species-related susceptibility to health effect by AFB1, and a role of the dose and the duration of the exposure (Neiger et al., 1994; Pestka & Bondy, 1994; Silvotti et al., 1997); nevertheless, it has been clearly shown that AFB1 is a powerful carcinogen for humans and many animal species, including rodents, non-human primates, and fish (Kimura et al., 2004; Santacroce et al., 2008). The main target of the agent is the liver, although tumors may also develop in other organs, such as the lungs, kidney and colon (Wang & Groopman, 1999). Therefore since 1993, The International Agency for Research on Cancer (IARC) has classified it as a high potential carcinogenic agent (Class I) (IARC, 1993). Besides, a strong synergy between aflatoxin and the presence of hepatitis B and C viruses has also been determined, a combination that significantly increases the risk for having liver cancer, as shown in places like Gambia, and Qidong, China (Wang et al., 1996, 2001).

AFB1 is absorbed in the small intestine and distributed by the blood throughout the body. Examination of the physicochemical and biochemical characteristics of the AFB1 molecule has revealed two important sites for toxicological activity. One is the double bond in position C / C-8,9, of the furo-furan ring. The aflatoxin-DNA and protein interaction at this site can alter the functioning of these macromolecules leading to cellular deleterious effects. Another reactive group is the lactone ring in the coumarin moiety, which is easily hydrolyzed and therefore, vulnerable for degradation (Banu & Muthumary, 2010b). AFB1 is metabolically activated by cytochrome P450 enzymes to yield two chemically reactive epoxides: AFB1-8,9-*exo* and -8,9-*endo* epoxides (Figure 2). However, only the 8,9-*exo* isomer reacts readily with DNA, forming the N7-guanine and its derivative AFB1-formamidopyrimidine adduct (Johnson & Guengerich, 1997). These events constitute the basis of AFB1 genotoxicity, which includes promutagenic and mutagenic events that can result in the activation of protooncogenes and the inactivation or loss of tumor suppressor genes. The formed epoxide is very unstable in water but can be handled relatively easily in aprotic solvents. CYP enzymes, on the other hand, also oxidize AFB1 to deactivated products that are generally poor substrates for epoxidation, or to those which after that step do not interact with DNA, including AFM, AFQ, and the *endo*-epoxide (Johnson & Guengerich, 1997; Guengerich et al., 1998).

The genotoxic effects induced by AFB1 have been extensively documented. The chemical is known to inhibit DNA synthesis, as well as DNA-dependent RNA polymerase activity messenger RNA synthesis, and protein synthesis (McLean & Dutton, 1995; Wang & Groopman, 1999). Furthermore, its strong genotoxicity has been demonstrated in many endpoints and model systems which include HeLa cells, *Bacillus subtillis, Neurospora crassa, Salmonella typhimurium,* CHO cells, chromosomal aberrations, sister chromatid exchanges (SCE), micronucleus, unscheduled DNA synthesis, DNA strand breaks, and DNA adducts (Anwar et al., 1994; El-Zawahri et al., 1990; Le Hegarat et al., 2010; Miranda et al., 2007; Theumer et al., 2010).

The above mentioned genotoxicity is in line with the induction of cancer by aflatoxins. Hepatocellular carcinoma is one of the most common malignancies worldwide, and a major risk factor includes dietary exposure to AFB1. Genetic and epigenetic changes are involved in the pathogenesis of the disease, including G:C to T:A transversions at the third base of codon 249 of the tumor suppressor gene *p53*. Besides, chronic infection with hepatitis virus, and the generation of reactive oxygen/nitrogen species can also damage DNA and mutate cancer-related genes, such as *p53*. One of the functions of this gene is to regulate the transcription of protective antioxidant genes, however, when the DNA is damaged, *p53* regulates the transcription of protective antioxidant genes, but with extensive DNA damage it transactivates pro-oxidant genes that contribute to apoptosis. Also, genes from the hepatitis B virus can be integrated in the genome of hepatocellular carcinoma cells, and mutant proteins may still bind to p53 and attenuate DNA repair and apoptosis; thus, it is clear that viruses and chemicals may be involved in the etiology of mutations during the molecular pathogenesis of liver carcinoma (Hussain et al., 2007; Oyaqbemi et al., 2010).

The strong toxicity of AFB1, which may be reflected in financial and social problems, prompted countries to incorporate regulations concerning the levels of mycotoxins in food and feed. In the case of AFB1, the maximum tolerated level varies from 1 to 20 µg/kg. The limit of 4 µg/kg is usually applied in countries that follow the harmonized regulations of the European Free Trade Association (EFTA) and the European Union (EU), and the 20 µg/kg limit is mainly applied in Latin American countries, the United States, and Africa (Guzmán de la Peña & Peña Cabrales, 2005).

Actions to fulfill regulations or to correct possible failures can be taken at the phases of production, storage, and processing. At the initial steps, insect control can be performed, and improvements made in irrigation practices and storage structures as well as in the inoculation of non-aflatoxigenic strains; in the latter step, the actions can refer to the separation of the contaminated product, its dilution with grains lacking AFB1, or its decontamination through a number of physical and chemical methods which are designed to degrade, destruct, inactivate or remove the toxin. The ideal decontamination procedure should be easy to use and inexpensive, and it should not lead to the formation of compounds that are still toxic, or that may reverse to compounds that reform the parent mycotoxin or alter the nutritional and palatability properties of the grain or grain products. This has been a difficult task, and thus a number of methods have been proposed, showing variable results. Examples of these methods are presented in Table 3 (Madrigal-Santillán et al., 2010). The widespread contamination of AFB1, in addition to the complexity and danger of its toxicity, has suggested that not only one form of control and prevention can cope with the problem; this is a conviction that has promoted the development of different strategies. One of these refers to the application of antimutagenesis and chemoprevention procedures, as the basis to avoid or reduce DNA lesions, as well as other molecular and cellular alterations related with the process of cancer initiation. These studies can be carried out by inhibiting the formation of active AFB1 metabolites, avoiding the interaction with target macromolecules, or by accelerating the detoxication and repair processes, among other mechanisms. Comparison of antimutagenic or chemopreventive activities with biochemical and organic quantifications are relevant to confirm the efficacy of the prevention strategy.

Physical methods	**Specific examples**
Inactivation by heat	Vapor pressure Microwave treatment Nixtamalization
Inactivation by radiation	Ultraviolet light Gamma radiation
Elimination by adsorbents	Zeolites Bentonites Aluminosilicates
Chemical methods	
Extraction by organic solvents	Ethanol 95% Acetone 90%
Chemical destruction	Hexane-ethanol Hydrogen peroxide Ammonium hydroxyde Methylamine Sodium hypochlorite

Table 3. AFB1 decontamination procedures

4. Antimutagenesis and chemoprevention

The relationship between chemical exposure and cancer development was observed about 140 years ago when an increase was noted in the cancer mortality rate of workers

managing coal tar. This effect was experimentally confirmed by Yamagiwa and Ichikawa (Weisburger, 2001) in exposed rabbits. Later, with the identification of the DNA structure and the development of new analytical methods, carcinogenesis was clearly shown to be related with alterations in this molecule. Such a relation was confirmed in the 60s, determining the effect exerted by metabolites of specific carcinogens on the structure and function of DNA and studying the activity of carcinogens on various cellular systems. In this context, the term genotoxic was then used for identifying carcinogens acting on DNA, in contrast with others whose effect was exerted through other routes (Weisburger, 2001). At this time, it was evident that the presence of numerous physical, chemical, and biological genotoxic agents could affect human health, and also that these genotoxicants may be present in a wide range of human activities, such as those related with work, food, health or personal habits. This produced a sort of genotoxic saturation, a condition which stimulated a search for knowledge about the agent's molecular, cellular, and metabolic peculiarities as well as detailed characteristics of the xenobiotic-DNA interaction; furthermore, the consequences of such an interaction were investigated, particularly with reference to human health.

A number of educational recommendations were then suggested in order to counteract the genotoxic effects, in addition to establishing regulatory measures related to permissible limits for specific substances; besides, efforts were made for substituting genotoxic drugs with less dangerous ones, including appropriate modification of their molecular structure. In the search for strategies to cope with the deleterious effect of mutagens, agents appeared which may reduce or eliminate such damage, the antimutagens. The basis for studying these substances is the knowledge that carcinogenesis is strongly related with mutagenesis, evidence supporting that carcinogenesis is highly due to the activity of environmental agents, and also, information that genotoxic inhibitors may frequently be found in plants and their products, as well as in other components of the diet; factors which favor their use under the appropriate conditions (Weisburger, 2001). In this context, it is pertinent to refer to Ames (1983) who suggested that a diet insufficient in fruits and vegetables may double the risk of acquiring cancer and cardiac diseases. This statement, as well as a number of reports on the matter increased the interest in determining the potency, toxicity, and mechanism of action of antimutagens as the necessary basis for incorporating the best candidates in preclinical and clinical chemoprevention trials. Moreover, studies on the matter have put into perspective the real beneficial action of this type of agents. Such studies have also considered various aspects that must be understood or solved, such as the fact that most antimutagens act on specific mutagens, and the possibility that the effect can be nullified or even reversed to mutagenicity in regard to the dose, time, and cell/organism tested, a possibility which is also complicated by the interactions that may occur between any compound and the complex human organism (De Flora & Ferguson, 2005; Ferguson, 2010).

The number of antimutagens and how they may act has been growing in recent years. Moreover, it is known that an agent may have more than one mechanism of action, and that two or more antimutagens could act synergistically. For more detailed information on the classification and the antimutagen's mechanism of action the reader may consult specific reports (De Flora et al., 2001; De Flora & Ferguson, 2005); however, for the sake of simplicity, in this revision antimutagens have been classified as desmutagens, impeding or limiting the effect of the mutagen before reaching the DNA molecule, such as the adsorbents that may interfere with the cellular absortion, or as those that avoid or reduce mutagen formation by blocking the biotransformation of premutagens through the inhibition of their

activation at the cytochrome P-450. The other class of antimutagens corresponds to the bioantimutagens agents which may even reduce the level of mutations after the DNA has been damaged. To this group belong sequesters of mutagens and free radicals, agents that enhance the activity of phase II enzymes and the repair system, or those that reduce errors at the DNA replication level (Kada & Shimoi, 1987).

The term chemoprevention was coined by Sporn in the mid 70s, and during the following years it was defined as a procedure for the prevention, inhibition, delay, or reversal of carcinogenesis by means of a variety of agents which include different nutrients, extracts of plants or pharmacologic compounds, among others. The aim of the strategy refers to finding agents with several characteristics: 1-low cost, as related to cost-benefit analyses and to the size of the target population; 2- practicality of use, regarding availability, storage conditions and administration route, besides taking into account the need to be used for long periods of time; 3-efficacy; and 4-safety. The selected chemopreventive agents should protect target molecules, cells, general population and individuals at risk, against the initiation, promotion or progression phases of carcinogenesis (De Flora & Ferguson, 2005). The concept is based in that chronic diseases may have common pathogenic determinants, such as DNA damage, oxidative stress, and chronic inflammation, and that a number of agents have proved to be efficient in blocking such alterations and in improving the quality and span of human life. The more promising candidates are subjected to clinical trials, which should be designed and conducted properly, and should include well characterized agents, suitable cohorts, and reliable biomarkers for measuring efficacy, which can serve as surrogate endpoints for cancer incidence. Phase II chemoprevention trials test promising agents for biomarkers modulation in cohorts of 30 to 200 participants at greater than average risk of the cancer under study; in contrast, phase III trials test agents for their efficacy in cancer prevention in thousands of participants who are generally healthy or who may be at slightly elevated risk (Kelloff et al., 1995; Richmond & O´Mara, 2010).

5. Genotoxicity/antigenotoxicity tests

Genotoxicity/antigenotoxicity tests can be defined as in vitro and in vivo assays designed to detect both, compounds that induce genetic damage by various mechanisms, as well as those that prevent such damage. These tests enable hazard identification with respect to DNA damage and its fixation in the form of gene mutations, chromosomal aberrations or other alterations, all of which are considered essential for heritable effects and for the multi-step process of malignancy (Figure 3). In contrast, the same tests may provide information on the level of protection and the mechanism involved regarding the antigenotoxic agents (Food and Drug Administration [FDA], 2008). In this section we will briefly describe the more basic fundaments regarding some of the tests most used to evaluate the prevention of DNA damage induced by AFB1.

a. The Ames mutation assay, which was developed in *Salmonella typhimurium* in the mid 70s, is based in the use of strains with a mutation in the histidine locus which does not allow the bacteria to synthesize such aminoacid; thereby, reversion to the normal situation constitutes the mutagenic endpoint. The sensitivity of the test has been improved by incorporating mutations to the test organism, making it more permeable to chemicals and more resistant to DNA repair (Dearfield & Moore, 2005). Moreover, several strains which detect different base-pair substitutions have been constructed, thereby allowing the detection of oxidative damage or DNA cross-linking. Besides,

limitation of the bacteria in regard to the absence of metabolic activation by enzymes common to the mammalian metabolism was overcome by adding rat liver microsome homogenate (S9 homogenate) to the bacterial cultures (Ames et al., 1973; Dearfield & Moore, 2005).

b. SCE represent the interchange of DNA replication products at apparently homologous chromosomal loci; such exchanges presumably involve DNA breakage and reunion (Latt & Schreck, 1980). The test can be made in vitro or in vivo. In the first case a number of cellular lines or primary cultures from different organisms can be used, with or without the addition of S9 for inducing metabolic activation. Also, a main step in making the test is to differentially stain the sister chromatids in such a way that they can be clearly visualized as a distinct chromatid in second division metaphases; this is essential for counting the number SCE per chromosome/cell, which is the evaluated genotoxic endpoint. The compound bromodeoxyuridine is usually added to the cultures or intraperitoneally injected to the test animal to visualize the sister chromatids; this compound is a thymidine analogue which is readily incorporated into the DNA chains and acts as a molecular marker during DNA replication, which is reflected as the differentially stained chromatids when colored with a Giemsa stain (Latt & Schreck, 1980).

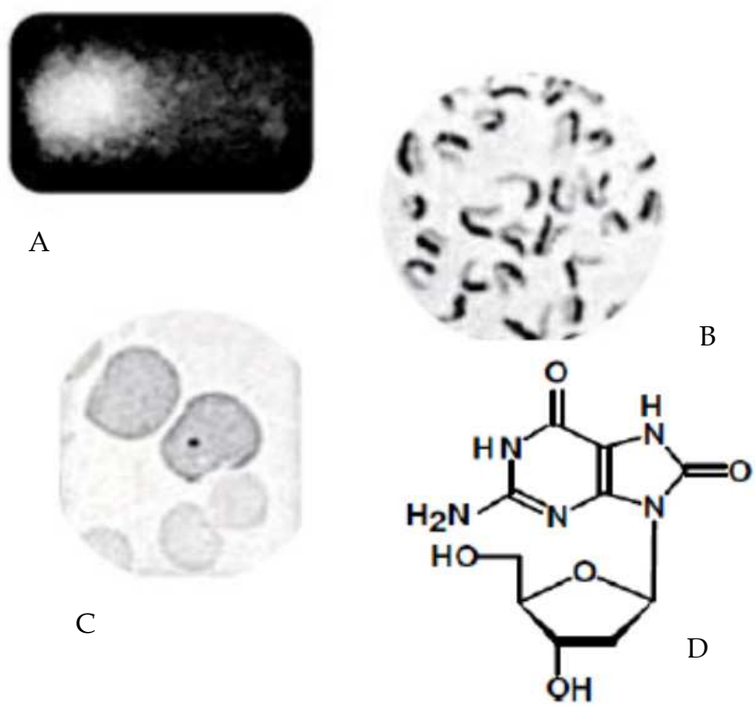

Fig. 3. Examples of genotoxic lesions. A) DNA damage observed with the comet assay, B) Sister cromatid exchanges in mouse bone marrow cells, C) micronuclei in mouse erythrocyte, D) DNA adduct (8-hydroxy-2´-deoxiguanosine)

c. Chromosomal aberrations are generally classified under two types: a numerical one and a structural one, and although both types are useful for the analysis of genotoxicity, the structural type of anomalies, is probably the more utilized. This type of aberrations is

usually classified as chromosome and chromatid lesions, and more specifically, to deletions and fragments as well as various forms of chromosome intrachanges and interchanges. The studied cells and/or organisms can be selected based on their growth ability, stability of the karyotype, chromosome number, and spontaneous frequency of alterations (US Enviromental Protection Agency [EPA], 1998; Organisation for Economic Co-operation and Development [OECD], 2010). The in vivo or in vitro assay requires proliferating cells which are treated with the tested substance during an appropriate exposure time, and at the end, the test requires a hypotonic treatment, followed by the cell fixation and staining. Also, evaluating chromosomal aberrations in an initial round of cellular proliferation is important in order to have precise quantification of aberrations due to possible losses in subsequent divisions; therefore, the differential staining procedure applied to determine SCE can also be used to specifically identify cells in the first cellular division.

d. Micronuclei are genotoxic lesions that may originate from acentric chromosome fragments or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell divisions (Organisation for Economic Co-operation and Development [OECD], 2007). The test can be designed to detect the activity of chemicals with clastogenic and aneugenic potential, in cells that have undergone cell divisions during or after exposure to the test substance. Because of easier performance and microscopic detection, as well as high sensitivity for detecting mutagens/antimutagens, it has partially substituted the chromosomal aberration analysis. The assay can be made in vitro and in vivo, as well as in animals or plants. In cultured cells, the incorporation of cytochalasin, a cytokinesis blocker that allows micronuclei evaluation in synchronic binucleated cells, has become popular. In mouse in particular, micronuclei can be scored in both immature polychromatic erythrocytes or mature, normochromatic erythrocytes. Moreover, the test can be applied for examining exfoliated cells, and its sensitivity can be increased by means of flow cytometry which allows the analysis as many as 500000 events (Dertinger et al, 2011; Fenech et al., 2011).

e. The single cell gel electrophoresis assay, also known as comet assay, is a sensitive technique for detecting and analyzing DNA breakage in a variety of organs and various plant and animal cells. The basic principle resides in the migration of DNA in an agarose matrix under electrophoretic conditions, which depends on the level of breakage. When viewed under a microscope, a cell may have the appearance of a comet, with a head (the nuclear region) and a tail containing DNA fragments which have migrated in the direction of the anode. The length and the frequency of comets depend on the genotoxic potential of the tested agent (Oshida et al., 2008). The advantage of the comet assay is that it allows any viable eukariote cell to be analyzed for DNA damage, by detecting single or double strand breaks, alkali-labile sites that are expressed as single-strand breaks, and single-strand breaks associated with incomplete excision repair. Quantitative analysis for DNA damage has yielded several parameters, including tailed nuclei, tail length, percentage of DNA in the tail, and tail moment; besides, specific enzymes can be added to the test to analyze oxidative damage or the test can be integrated with the FISH assay for evaluating specific gene position/movement (Hartmann et al., 2003; Kumaravel et al., 2009).

f. An adduct corresponds to a stable complex formed when a chemical is covalently linked to a macromolecule, such as protein or DNA. The measurement of adducts in

body fluids is highly sensitive and specific to determine the effect of the studied xenobiotic. DNA adducts have been clearly shown to be relevant to the disease process in prospective studies (Bonassi & Au, 2002). In relation to our present review, the adducts 8,9-dihydro-8-(N(7)-guanyl-)-9-hydroxyaflatoxin, as well as the AFB1-formamidopyrimidine compound, among others, are thought to be involved in the mutations caused by AFB1. The detection of these compounds can be made in various organs, as well as in serum and urine, by means of a variety of methods that include HPLC, ELISA, accelerator mass spectrometry, and liquid chromatography/electrospray ionization/mass spectrometry (Sharma & Farmer, 2004; Wang et al., 2008).

6. Chlorophyllin

Chlorophyll was detected as an antimutagenic agent since the early 80s. However, very soon afterwards most research on the matter was focused on the effect of chlorophyllin (CHL) (Figure 4), mostly because this chemical is a water-soluble, sodium and copper salt, chlorophyll derivative. In regard to its protective effect on AFB1 damage, one of the first studies with CHL was made by quantifying the number of revertants of specific base-pair mutants in *Neurospora crassa* (Whong et al., 1988). The study showed strong inhibition of the antimutagen on the AFB1-mutagenicity determined in growing cultures of the mold, a result which prompted the group to continue their studies but now in *Salmonella typhimuriun*, strain TA98, as a model (Whong et al., 1988). The authors used the plate-incorporation test in this bacteria and found a concentration-dependent inhibition of AFB1 mutagenicity when the cells were treated with the tested substances, concurrently; in their assay they observed that 860 nmol/plate of CHL completely abolished the mutagenicity of the toxin. However, when other approaches were tested, negative effects were detected; therefore, the authors suggested that CHL acted before the mutagen entered the bacterial cell by suppressing metabolic activation or by scavenging the mutagen.

The preventive capacity of CHL on the AFB1-DNA damage was confirmed by means of the arabinose-resistant *Salmonella* forward mutation assay (Warner et al., 1991); the results of the study were obtained in a preincubation test, and showed an inhibitory effect of CHL with and without the addition of S9 mix. With 2.5 mg/plate or less, the authors reported an almost complete inhibition of the aflatoxin mutagenicity.

In the following years, a number of studies were made, particularly in rainbow trout but also in microorganisms. Their purpose was to confirm the CHL antimutagenic potential by means of various models, as well as to investigate the involved mechanism(s) of action and to evaluate its cancer chemopreventive capacity (Breinholt et al., 1995a, 1995b; Dashwood et al., 1991). Results of these studies confirmed the in vitro efficiency of CHL when liver microsomes were added to the system, not only against the damage induced by AFB1, but also on the mutagenic effect induced by its precarcinogenic metabolite, 8,9-epoxide. In rainbow trout, the authors found an inhibitory effect of CHL on the precarcinogenic hepatic DNA adduction induced by AFB1, as well as a significant lowering in the number of liver tumors. In agreement with this finding, a study made in rat concluded that a concurrent administration of both compounds engendered an important reduction in the level of liver AFB1-DNA adducts (Kensler et al., 1998a), and another study showed a significant inhibitory effect of CHL (60%) on the morphological transformation of BALB/3T3 cells (Wu et al., 1994). In regard to the

mechanism of action, a strong complex formation between mutagen and antimutagen was proposed.

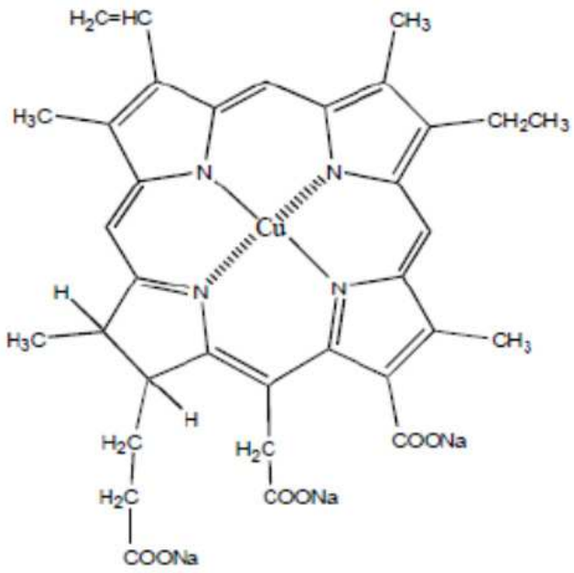

Fig. 4. Chemical structure of chlorophyllin

Based on the described positive experimental studies, efforts were initiated to determine the CHL chemopreventive capacity in humans. Qidong, People's Republic of China is a high risk region for hepatocellular carcinoma probably related with the consumption of AFB1 contaminated food; here, a randomized, double-blind, placebo-controlled trial was made to determine whether CHL administration altered the disposition of aflatoxin. CHL consumption at each meal led to an overall 55 % reduction in a median urinary level of the biomarker, aflatoxin-N^7-guanine, compared with individuals taking placebo (Egner et al., 2001). The determined adduct biomarker derives from the carcinogenic metabolite, aflatoxin 8,9-epoxide; thus, the authors suggested that prophylactic interventions with CHL or supplementation of diets with chlorophyll rich foods may be useful to prevent the development of hepatocellular carcinoma or other environmentally induced cancers. This type of studies was supported by reports on the experimental effect of CHL in rats (Simonovich et al., 2007). The authors observed the inhibition of AFB1-albumin adducts and of AFB1-N^7-guanine adducts, as well as the inhibition of AFB1 uptake when quantified in feces, besides a decrease in the number of colonic aberrant crypt foci induced by the aflatoxin. However, no modification in the activity of phase II enzymes was found.

In summary, a number of in vitro and in vivo studies have supported the antigenotoxic and chemopreventive capacity of CHL against the damage induced by AFB1, activities which can be related with the formation of a strong non-covalent complex, although additional mechanisms, such as its antioxidant potential, cannot be discarded. However, a word of caution about safety in using CHL is pertinent in light of the negative or controversial results that have been published in regard to the compound: for example, its effect as both inhibitor or promoter of genetic damage depending on the tested approach (Cruces et al.,

2003, 2009), its induction of embryo lethality in mouse (Garcia-Rodriguez et al., 2002), or its possible effect as a tumor promoter in rats (Nelson, 1992).

7. Oltipraz

Dithiolethiones are five-membered cyclic sulfur-containing compounds found in cruciferous vegetables, which have shown radioprotective, chemopreventive, chemotherapeutic, and antiviral activities (Kensler, 1997). In the context of our interest, a study made with lyophilized cabbage or cauliflower demonstrated a significant reduction in the rate of AFB1-induced carcinogenesis in rat (Boyd et al., 1982); subsequently, this chemopreventive effect has been thoroughly evaluated in regard to the drug oltipraz (OL), 5-(2-pirazinyl)-4-methyl-1,2-dithiole-3-thione (Figure 5). The mentioned dithiolethione was initially used because of its antischistosomal capacity in animals and humans, but later it was widely evaluated because of the detected potential to abolish or reduce the liver carcinogenesis induced by AFB1.

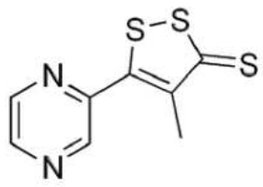

Fig. 5. Chemical structure of oltipraz

In an earlier study, rats were fed for 4 weeks with OL and gavaged with AFB1 during the second and third week of the assay (Kensler et al., 1987). In this report, the authors determined a significant reduction of focal areas with hepatocellular alterations achieved with the administration of OL, an effect shown by the staining of liver sections for gamma-glutamyl transpeptidase. The research concluded that dietary concentrations of OL as low as 0.01 % powerfully inhibited the formation of presumptive liver preneoplastic lesions. Similarly, the ameliorating effect of OL on the AFB1- toxicity induced in rats was shown by a decrease in the mortality rate, in the levels of serum alanine amino transaminase and sorbitol dehydrogenase, and also because of the normal growing rate propitiated by OL during the aflatoxin treatment (Liu et al., 1988). A few years later, another study made in rats, clearly confirmed the OL protective effect on AFB1-hepatocarcinogenesis (Roebuck et al., 1991). In this report, 11 % of hepatocellular carcinoma and 9 % hepatocellular adenomas were observed in AFB1-treated, diet-fed rats, in contrast with no tumor development in OL treated animals. Moreover, rats in the OL group had a significantly longer life span and an increased survival free of liver tumors in comparison with animals under aflatoxin treatment; besides, the authors found at least 65% reduction in the liver aflatoxin-N^7-guanine adducts in the OL-fed animals, a finding which suggested that the protection against hepatocarcinogenesis might have resulted from the marked decrease in this type of hepatic DNA adducts. Studies about the chemopreventive efficacy of OL were also made in other animals, such as the marmoset monkey and the tree shrew. These studies gave rise to variable, but positive results (Bammler et al., 2000; Li et al., 2000).

With respect to humans, several studies have been made to examine the chemopreventive effect of OL. Phase IIa and phase IIb clinical trials were performed in a rural township in China (Kensler et al., 1998b; Wang et al., 1999). These trials were randomized, placebo-

controlled, double-blind studies of people who had ingested AFB1 via their usual diet. For the rationale of the study the authors considered that the AFB1 epoxides are substrates for glutation S transferase (GST), an enzyme which catalyzes the conjugation of the epoxide with reduced glutation, thus mitigating the formation of DNA adducts. In fact, the results showed an increase in the activity of GST related with a sustained low dose of OL (125 mg/day), also yielding a high level of the AFB1 urinary metabolite, mercapturic acid; however, an intermittent, high dose of OL inhibited the activation of phase I enzymes, as reflected by a lowering in the excretion of the metabolite AFBm1. Nevertheless, the results of another study made in the same population suggested that prevention of oxidative DNA damage by OL was not a relevant mechanism to explain its effect against AFB1 (Glintborg et al., 2006).

The above-mentioned reports, as well as others, have suggested that the major preventive action of OL is through the activation of phase II enzymes, and secondarily on the inhibition of the carcinogen metabolism through the blocking of CYP enzymes. In addition to this, OL has been suggested to increase the nucleotide excision repair, which represents one of the major pathways for eliminating carcinogen DNA adducts; however, this effect has not been confirmed, as negative reports have also been published (O´Dwyer et al., 1997; Sparfel et al., 2002). With respect to GST, the mechanism by which OL enhances its level has been studied. Activation of such a cytoprotective enzyme seems to be related with a complex cellular signaling which includes the interaction of the Kelch ECH-associating protein (keap1) with the transcription factor NF-E2-related factor 2 (Nrf2), especially with the participation of the antioxidant response element-mediated regulation of Nrf2 (ARE) (Yates & Kensler, 2007).

At this time, other dithiolethiones seem even more promising than OL in preventing cancer. One such example is the compound 3H-1,2-dithiole-3-thione, which has shown a potent induction of phase II enzymes, a powerful inhibition of AFB1-induced hepatic toxicity including the formation of hepatic preneoplastic lesions, and inhibition of hepatic AFB1-DNA adducts; besides, the chemical was found to cause significant increases of GST and/or NQO1 in 12 tissues in addition to the liver, and it is probably not an inducer of CYP enzymes (Kensler et al., 1987; Roebuck et al., 2003; Zhang & Munday, 2008).

Future studies may include a structure-activity relationship among dithiolethiones, mainly to identify structural features that convey potent activation of Nrf2 and induction of phase II enzymes, the identification of mechanisms involved, as well as new biomarkers for evaluating their in vivo efficacy. Also, toxicity evaluations and clinical trials, especially with new dithiolethiones should be valuable.

8. Vitamins

These are organic compounds essential for the normal growth and development of a multicellular organism. A human fetus begins to develop from the nutrients it absorbs, including a certain amount of vitamins which facilitate the chemical reactions in different tissues. Vitamins have diverse biochemical functions such as the hormone-like regulation of mineral metabolism, regulation of cell and tissue growth as well as differentiation, besides acting as antioxidants, or precursors of enzyme cofactors. A number of efforts have been made to evaluate the useful impact of these compounds on the damaging effects induced by AFB1. Published studies have been made in in vitro and in vivo models, which include investigations in bacteria, cultured cells, experimental animals, or humans. The applied approaches go from the determination of their capacity to prevent various types of AFB1

genotoxic alterations, including adduction, to their modulatory effects on the AFB1 metabolism, or their participation as anticarcinogenic agents.

In regard to vitamin A, initial reports about its protective capacity were made by means of the Ames *Salmonella*/mammalian microsomes test. Such studies showed a relevant concentration-dependent decrease of the mutagenicity induced by AFB1. The effects were determined in strains TA98 and TA100, where the authors considered that the observed capacity of the vitamin could be related with the inhibition of AFB1 metabolism or with an increased breakdown of the active metabolite (Busk & Ahlborg, 1980; Raina & Gurtoo, 1985). Other strains (TA102 and TA1535) were also tested and revealed positive results (Qin & Huang, 1986). In Chinese hamster V79 cells, (Huang et al., 1982) found a similar effect. In this assay, dose and time dependent inhibition of AFB1-induced SCE, as well as correction of the cell cycle delay produced by the toxin was achieved by adding vitamin A to the cultures. Moreover, Qin et al. (1985) confirmed the indicated finding and extended it to determine a similar effect of the vitamin over the amount of chromosomal aberrations induced by AFB1. A few years later, S9 fractions obtained from mice with a high vitamin A liver level were found to be less potent in activating AFB1 than those with a low liver level; also, the first ones proved to be related with a stronger reduction of SCE in mice administered aflatoxin with respect to the effect in mice with a low vitamin A level, which therefore confirmed the role of such vitamin to ameliorate the genotoxic damage (Quin & Huang, 1985). In this period, Suphakarn et al. (1983) also determined an enhancement of liver and colon cancer in rats with a vitamin A deficient diet and exposed to AFB1. The authors evaluated factors such as liver morphology, enterohepatic recirculation, level of reduced glutathione in liver, and conjugating capacity to GST, and they suggested that their results may have been related with the influence of the vitamin on the binding of AFB1 to cellular macromolecules, partially influenced through enzymatic mechanisms. With the purpose of learning more about the preventive effect of vitamin A, Webster et al. (1996) applied the approach of modulating its ingested amount. They found that rats with a deficiency of vitamin showed a high level of DNA single strand breaks induced by AFB1, as well as a decrease in various repair enzymes subsequent to DNA damage, although correction of these two parameters was achieved with vitamin supplementation. In regard to the capacity of AFB1 for inducing DNA adducts, an in vitro assay using a microsome catalyzed reaction showed that the addition of vitamin A to the system produced a dose-dependent inhibition of the adduction (Firozi et al., 1987). Similar results were found studying woodchuck hepatocytes (Yu et al., 1994).

The information indicated above suggested to researchers that the main action of vitamin A (as well as of other vitamins) was on the initiating step of AFB1 carcinogenesis, yet there still remained studies to be done so as to clarify the issue on the preventive biochemical action of the vitamin (Bhattacharya et al., 1989; Decoudu et al., 1992).

The antigenotoxic and antitoxic potential of vitamin A was determined in experimental mice. In these animals, a decrease in the toxin-induced clastogenicity in both mitotic and meiotic chromosomes was reported, as well as inhibition in sperm abnormalities (Sinha & Darmshila, 1994). Besides, the antigenotoxic effect of vitamin A was also found in human lymphocytes (Alpsoy et al., 2009); in these cells the authors reported a significant, dose-dependent reduction of the SCE induced by 5 uM of AFB1, with the lowest protective concentration being 0.5 uM.

With respect to the anticarcinogenic potential, a report established such an effect in a 2-year follow up of AFB1-administered rats where it was observed that most animals fed a diet

devoid of the vitamin developed liver cancer, contrary to few cases in rats which received it (Nyandieka & Wakhisi, 1993). However, a study aimed at determining the vitamin inhibitory effect on liver preneoplastic foci showed negative results, a finding which was probably related with an excess of the vitamin in the assay; this, nonetheless, helped to stress the relevance of selecting appropriate experimental conditions in chemoprevention studies (Gradelet et al., 1998).

Finally, although there is a deplorable scarcity of studies in humans, AFB1-albumin adducts were quantified in a high risk Ghanaian population, where a relationship was determined between a high mycotoxin level with decreased levels of the vitamins A and E, suggesting then, that such deficiency may significantly influence the incidence of adverse health effects (Tang et al., 2009).

Vitamin C and E are other compounds tested against the genotoxic damage induced by AFB1. With respect to these chemicals, a study made by means of the *Salmonella typhimurium* test (strains TA98 and TA100) showed that although both vitamins prevented the expression of AFB1-induced mutagenesis, vitamin E was more potent, and also that its effect was related with the metabolism of the mycotoxin, whereas vitamin C was involved in both the metabolic and post-metabolic levels of the AFB1 mutagenesis assay (Raina & Gurtoo, 1985). This result was congruent with the protective, dose-dependent effect determined for both vitamins against the SCE induced by AFB1 in cultured human lymphocytes (Alpsoy et al., 2009). In this study, the order of protective efficacy was vitamin C-vitamin E-vitamin A. In regard to vitamin E, however, the indicated positive results were contrary to those reported by Karekar et al. (2000) who applied two short term genotoxicity assays — the Ames test and the Drosophila wing spot test — and they found no antimutagenic response of the vitamin; moreover, woodchuck hepatocytes that were treated with four doses of [3H]AFB1 or with different combinations of the toxin and vitamins C and E for 6 h resulted in an effect of vitamin C for inhibiting AFB1-DNA binding; contrarily, an enhancement of covalent binding of AFB1 to DNA by vitamin E was observed (Yu et al., 1994). Also, negative results were found when evaluating the protection of such vitamin in SCE induced by AFB1 in V79 cells (Deng et al., 1988). These results clearly suggest the need for further research to understand the complex role of these vitamins in the mutagenesis and carcinogenesis of the aflatoxin. Such a complex response was also reported in rats fed on a variable diet of vitamin E (Cassand et al., 1993). Animals on a diet supplemented with a low amount of the vitamin (0.5 IU) increased P-450 IIB and IIIA enzyme activity, whereas a higher vitamin supplemented diet (5 IU) reduced these specific activities. However, lipid peroxidation was increased in the vitamin E free diet animals and strongly decreased in the supplemented group. Nevertheless, in a subsequent study (Karakilcik et al., 2004) a significant increase was found in the level of various liver enzymes in rabbits fed a diet with AFB1, while such activities were lower in the groups receiving the mycotoxin plus vitamins C or E, whether alone or combined. In spite of these controversial reports on antigenotoxicity, another study made in rats to determine the preventive capacity of vitamins C and E on the development of liver cancer gave strong positive results, because only few animals under vitamin treatment suffered the illness along the 24 months of the assay (Nyandieka & Wakhisi, 1993).

On the other hand, some studies aimed to test the effect of specific types of vitamin B have given inconclusive results. In the case of riboflavin, an earlier assay using the *Salmonella typhimurium,* strain T100, rat-liver microsome system, concluded that with lower

concentrations of AFB1 the effect of the vitamin was very strong (Bhattacharya et al., 1987). However, another in vitro and in vivo study suggested a variable role of the compound with respect to the AFB1 metabolic activation, an effect which was related with the tested amount of the vitamin (Prabhu et al., 1989). Then, in a subsequent report made in rats under riboflavin supplementation, a clear, positive effect was determined on the DNA damage induced by AFB1 by quantifying the reversion of DNA single strand breaks (Webster et al., 1996). Folic acid has also been evaluated. In this case, a survey made in high risk Chinese individuals concluded that increased folate levels may be inversely associated with the development of liver damage and hepatocellular carcinoma (Welzel et al., 2007).

9. Probiotics and microbial cell wall components

In the context of the exposed theme, biological decontamination seems attractive because it works under mild, environmentally friendly conditions. The AFB1 detoxification potential of probiotics such as yeast and lactic acid bacteria, among other microorganisms, has been evaluated in light of their adsorbent capacity that prevents the transfer of aflatoxin to the intestinal tract of humans and animals (Wu et al., 2009). This effect has been reported for various species of *Lactobacillus,* including *L. casei, L. plantarum, L. fermentum, and L. rhamnosus*; moreover, the participation of teichoic acids has been suggested to play a key role in the binding ability of some species toward AFB1 (Fazeli et al., 2009; Gratz et al., 2007; Hernandez-Mendoza et al., 2009). Although the antimutagenic capacity of fermented foods and probiotics is known, few studies in respect to aflatoxin have been done with bacteria; an example is the report made in Caco-2cells treated with *Lactobacillus rhamnosus* strain GG, which showed protection against AFB1-induced reductions in transephitelial resistance, as well as reductions in DNA fragmentations assessed by extracting DNA and separating intact and damaged DNA by the use of gel electrophoresis (Grats et al., 2007). In regard to probiotics, there is an interesting study made on ninety healthy young men from Guangzhou, China whose diet was supplemented with a probiotic mixture that induced a reduction of the biologically effective dose of aflatoxin exposure, suggesting an effective dietary approach to decrease the risk of liver cancer. This conclusion was reached after quantifying the urinary excretion of AFB1-N7-guanine in the evaluated population (El-Nezami et al., 2006).

Research on the yeast *Saccharomyces cereviciae* (Sc) has confirmed its decontaminating ability through its binding with AFB1, which may depend on the used strain and other experimental conditions (Shetty et al., 2007). Besides, the potential of Sc to ameliorate the effects of aflatoxicosis was clearly established in broiler chicks or Japanese quail by evaluating a number of biochemical and organic parameters (Parlat et al., 2001; Stanley et al., 1993). The above-mentioned information is congruent with the antigenotoxic effect observed in mice fed with AFB1 contaminated corn (Madrigal-Santillán et al., 2006). In this study, the animals were experimentally fed with the tested chemical for six weeks; the results observed in the groups treated with the yeast showed a significant improvement in the weight loss induced by AFB1, and a decrease of more than 60 % in the level of micronuclei induced by the toxin in normochromatic erythrocytes, as well as a similar reduction in the level of SCE in mouse bone marrow cells, effects that were related with the adsorbing capacity of the yeast. Besides, the study revealed a recovery to normal parameters in about three weeks without the aflatoxin administration, which suggest the usefulness of periodical monitoring of commodities at risk.

The cell wall of yeast, as well as that of other microorganisms is composed of polyssacharides, mainly mannans, glucans, and glucomannans, some of which have been studied for their AFB1 protective effect. Mannan is a highly branched olygossacharide constituted by a main chain of α-(1,6)-D-mannoses linearly attached, and with α-(1,2) and α-(1,3)-D-mannose branches. In a first report mice were fed AFB1-contaminated corn, and AFB1 treated grain plus three doses of mannan (including the appropriate control groups). The assay lasted four weeks and the measurements included, weight, micronuclei, cytotoxicity index, and SCE (Madrigal-Santillán et al., 2007). Results showed that mice fed AFB1 had a significant weight decrease, as well as a significant increase in the rate of MNNE and SCE, while animals fed the combined regime presented a 25 % weight increase with respect to animals treated with AFB1 alone, as well as a reduction in the level of MNNE and SCE (about 70 % with the high two doses). In a subsequent report, the authors confirmed the protective effect of mannan in mouse hepatocytes which were analyzed with the comet assay at 4, 10 and 16 h of exposure (Madrigal-Santillán et al., 2009). In such study, the best preventive effect of mannan was found at 10 h with the high tested dose (700 mg/kg). Moreover, the authors proposed a supramolecular complex between mannan and the aflatoxin based on the melting points, and the UV spectra of the crystals from the independent compounds and a co-crystalization of both chemicals.

Glucans are a heterogeneous group of glucose polymers, consisting of a backbone of β(1,3)-linked β-D-glucopyranosyl units with β(1,6)-linked side chains of varying distribution and length (Akramiene et al., 2007). Besides its immunostimulant effect, the compound has been reported to have chemoprotective potential against a number of mutagenic agents (Akramiene et al., 2007; Mantovani et al., 2007); also, interaction of glucans with AFB1 including the participation of hydroxyl, ketone, and lactone groups was proposed as the basis for the formation of hydrogen bonds and van der Waals interactions (Yiannikouris et al., 2006). However, in spite of such information, very few studies have been made in regard to the antigenotoxic potential of glucans on the damage induced by AFB1. An investigation similar to the described above by Madrigal-Santillán et al. (2009) but testing the antigenotoxicity of glucan and glucomannan in mice hepatocytes showed a positive effect for the two agents (Madrigal-Santillán, 2004). DNA damage was quantified by means of the comet assay at 4, 10 at 16 h after the chemicals exposure. Glucan showed a protective effect with the two low doses tested (400, and 700 mg/kg), reaching about 40% as the highest reduction of the damage induced by AFB1; glucomannan, however, showed a significant response with all the three tested doses, reaching an inhibition as high as 80 % at 10 h of treatment.

10. Miscellaneous agents

Our purpose in this section is not to show an extensive list of the agents tested against the genotoxicity of AFB1, but rather the variability of such agents, which goes from single compounds to mixtures with different complexity. These investigations may be motivated by the mutagenic potency of AFB1, which make it a relevant candidate for demonstrating the capacity of antimutagens, as well as by the need for finding efficient agents to prevent the serious damage that such mutagen can provoke.

Ellagic acid and a phenolic extract obtained from the bean (*Phaseolus vulgaris*) are examples of phenolic compounds studied for their use in controlling the mutagenicity of AFB1. In both cases *Salmonella typhimurium* (strain TA98 and TA100) was used as the test model, and the

obtained results showed a concentration-dependent antimutagenic effect, which was more clearly expressed when the compounds were tested at the same time. The authors suggested the formation of a chemical complex between the involved agents as an explanation for the protective effect (Loarca-Piña et al., 1998; Cardador-Martinez et al., 2002).

Green and black teas are known as efficient antimutagenic and anticarcinogenic agents. In the case of AFB1, tea polyphenols from both teas were tested against its mutagenicity by means of the *Salmonella typhimurium* assay (strain TA98). In the report the authors determined a sharp decrease toward the mutagenic effect of the aflatoxin (Weisburger et al., 1996). Besides, results obtained in rat bone marrow cells treated with AFB1 in vivo revealed that the administration of green tea 24 h before administering the mutagen produced a significant reduction in the number of structural chromosomal aberrations (Ito et al., 1989). A confirmation of the green tea protective effect was determined in 352 human blood and urine samples that corresponded to a 3-month trial of individuals under green tea consumption (Tang et al., 2008). The authors measured AFB1-albumin adducts, AFBM1, and AFB1-mercapturic acid, and concluded that green tea effectively modulates the metabolism and metabolic activation of AFB1.

A number of plant flavonoids were tested against the effect of AFB1 by means of the *Salmonella typhimurium* assay (TA98 and TA100 strains), and some of them showed an efficient antimutagenic capacity: kaempferol, morin, fisetin, biochanin A, and rutin (Francis et al., 1989). Also, it was reported that kolaviron, a flavonoid from the seeds of *Garcinia kola* was able to inhibit the amount of micronuclei and the hepatic oxidative damage induced by AFB1 in rats (Farombi et al., 2005).

The determination of ammonia as antimutagen is included in this review considering that the chemical has been used as one of the agents to decontaminate AFB1; therefore, confirmation of its utility through genetic endpoints seems interesting. In the described report, mice were fed for four weeks with AFB1 contaminated corn and concomitantly treated with ammonium hydroxide (Marquez-Marquez et al., 1993). The results showed a significant reduction in the rate of micronucleated normochromatic erythrocytes starting from the first week of the assay, and at the fourth week of treatment the inhibition reached 60 %; besides, at the last week of the test, the quantification of SCE showed an inhibition of 55 % in comparison with the level determined in the AFB1 treated group.

Coffee is a beverage of habitual consumption that has shown controversial results concerning its genotoxic/antigenotoxic potential; however, there is an interesting study by Abraham (1991) who evaluated the inhibitory effect of standard instant coffee on the number of mice bone marrow micronuclei. Mice were orally administered coffee 2 and 20 h before injecting the carcinogen, and observations made at 28 and 48 h showed a dose-dependent decrease in the rate of micronuclei, with a reduction of more than 60 % with the high tested dose (500 mg/kg).

In regard to constituents of apiaceous vegetables, such as carrots, parsnips, celery or parsley, Peterson et al. (2006) used a methoxyresorufin O-demethylase assay and a trp-recombination assay in *Saccharomyces cereviciae*, and found that 5-methoxypsoralen, and 8-methoxypsoralen reduced the CYP1A2-mediated mutagenesis of AFB1. In the same context, it was reported the hepatoprotective effect of ethanolic extract of *Phyllantus amarus* on AFB1-induced damage in mice, as well as the protective effect of soybean saponins against the aflatoxin in the *Salmonella typhimurium* assay, and a significant decrease of DNA-adduct formation in human liver hepatoma cells (Jun et al., 2002; Naaz et al., 2007).

11. Conclusions

In light of the serious effects that AFB1 contamination can originate, the authors agree that different socio-economical and toxicological approaches should be carried out for its elimination or control, including specific strategies regarding regulatory, supervisory, educational, scientific and technologic issues. Basic knowledge on the metabolism and the molecular and cellular fate of AFB1 is presently known, and various models have been used to test the effects of a number of chemopreventive agents, some of which have shown promising results, suggesting then, the pertinence of continuing with such strategy. However, it is reasonable to have a deeper knowledge on the chemical characteristics of each AFB1 metabolite, as well as on their interactions with macromolecules and cells, and to identify the more sensitive biomarkers for the assayed damage; this will be of help in designing more appropriate experimental projects, or clinical trials with the best candidates detected, in addition to preventing the selected genotoxic damage with more efficacy. At present, only a few agents have been tested in humans for evaluating their capacity of protection against AFB1 damage, although numerous chemicals have been evaluated in an almost isolated experimental form and have presented favorable results; therefore, extensive studies on these agents should be carried out so as to gain knowledge on their safety, efficacy, and mechanism of action, in order to select those more suitable for chemopreventive purposes.

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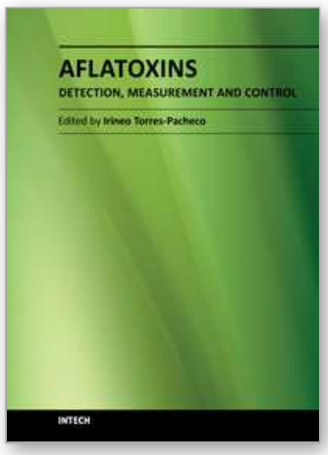

Aflatoxins - Detection, Measurement and Control

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This book is divided into three sections. The section called Aflatoxin Contamination discusses the importance that this subject has for a country like the case of China and mentions examples that illustrate the ubiquity of aflatoxins in various commodities The section Measurement and Analysis, describes the concept of measurement and analysis of aflatoxins from a historical prespective, the legal, and the state of the art in methodologies and techniques. Finally the section entitled Approaches for Prevention and Control of Aflatoxins on Crops and on Different Foods, describes actions to prevent and mitigate the genotoxic effect of one of the most conspicuous aflatoxins, AFB1. In turn, it points out interventions to reduce identified aflatoxin-induced illness at agricultural, dietary and strategies that can control aflatoxin. Besides the preventive management, several approaches have been employed, including physical, chemical biological treatments and solvent extraction to detoxify AF in contaminated feeds and feedstuffs.

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