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Review

Aflatoxin contamination of foods in developing countries: Implications for hepatocellular carcinoma and chemopreventive strategies

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Hepatocellular carcinoma (HCC) is the fifth leading cause of cancer mortality in the world and, in certain parts of Asia and Africa, it accounts for about 70% of cancer deaths. Chronic hepatitis B virus (HBV) infection and dietary exposure to aflatoxin B_1 (AFB $_1$) are the two major risk factors in multi factorial aetiology of HCC. Multiple lines of evidence indicate synergistic interaction between these two agents in the development of HCC. Several mechanisms of interaction have been suggested including activation of cytochrome P450s by HBV infection leading to the metabolism of inactive AFB_1 to the mutagenic AFB $_1$-8,9-epoxide as well as the generation of reactive oxygen species by HBV and AFB_1 sensitising the cells to AFB_1-induced p53 249^{ser} mutations. The poor survival rate achieved by the current surgical procedures and chemotherapy treatment has motivated a number of scientific investigations to elucidating the molecular events involved in HCC thus providing the scientific rationale for prevention strategies, including primary and chemoprevention approaches. Recent findings have implicated intracellular signalling cascades involving nuclear factor kappa B (NF-κB) and nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) as molecular targets of a wide range of chemopreventive agents. The new findings thus raise the intriguing possibility that chemopreventives modulating these molecular targets in the liver might provide a novel therapeutic approach to the development of liver cancer.

Key words: Hepatocellular carcinoma, chronic hepatitis B virus, aflatoxin B_1, chemoprevention.

IINTRODUCTION

Hepatocellular carcinoma (HCC) accounts for 5.5% of all cancer cases world wide (Kensler et al., 2003) and is one of the most common cancers in Asia, Africa and in groups of Asian- and Hispanic- Americans. HCC attacks people at an early age in high risk zones. The highest occurrence and the youngest people with this disease are in the hyper endemic areas of China, Taiwan, Thailand and sub-Saharan Africa. There appears to be an increasing trend of HCC in these regions in recent years. For instance data from death certificates in Thailand from 1993 to 2003 reveal that liver cancer mortality in Sa Kaeo Province of Thailand increased from 3.1 to 26.1 per 100,000 populations between 1993 and 2003. In Thailand overall rates increased from 9.0 to 19.8 per 100,000 population between 1996 and 2003. According to electronic hospital records, the total number of patient encounters (in-patient admissions and out-patient visits) for liver cancer in the two main hospitals in Sa Kaeo Province increased by 56% (14% annually) between 1999 and 2003. The number of cases of HCC increased from 42 in 2001 to 73 in 2003 (Amon et al., 2005).

Epidemiological studies have identified chronic infection with hepatitis B virus (HBV) and dietary aflatoxin exposure as two major etiological risk factors for the development of HCC (Kew, 2003). The synergistic interaction between HBV and aflatoxins especially aflatoxin B_1 (AFB_1) has been observed in both animals

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(Bannasch et al., 1995) and humans (Lunn et al., 1997; Wang et al., 2001).

Primary prevention involving vaccination for HBV in babies and food safety procedures to limit aflatoxin contaminations has proved important strategy for reducing HCC in developing countries. Furthermore, understanding of the mechanisms of AFB_1-induced hepatocarcinogenesis has provided a scientific basis to reduce HCC risk (Wild and Hall, 2000) and has allowed other strategies such as chemoprevention with dietary naturally occurring agents to modulate the noxious effects of aflatoxins.

The present article updates the occurrence of aflatoxins in foods in developing countries, reviews the recent advances in the molecular mechanisms of aflatoxins and HBV as related to the development of liver carcinogenesis and presents a detailed discussion on the preventive interventions involving the use of targeted chemopreventive agents that focus on the mechanism of action of AFB_1.

HISTORY OF AFLATOXINS

Aflatoxins, a group of closely related heterocyclic compounds were first discovered in 1960 in England after the outbreaks of turkey disease that resulted in deaths (Blount, 1961) and of cancer development in rainbow trout fed on rations formulated from peanut and cottonseed meals (Halver, 1965). Subsequently the toxin was isolated (Vander Zijden, 1962). Aflatoxins are produced predominantly by *Aspergillus flavus* and *Aspergillus parasiticus*. Recent studies revealed that *A. nominus* and *A. tamari* strains are capable of producing the toxin (Goto et al., 1996, 1997) . Very recently, Ito et al. (2001) isolated another strain, *A. pseudotamarii*, capable of producing aflatoxin. At temperatures between 24 and
35°C and when the moisture content exceeds 7% (10% with ventilation) aflatoxins will grow within many commodities (Williams et al., 2004) . The fungi contaminate a vast array of dietary staples and agricultural products such as rice, corn, cassava, peanuts and spices. Most commodities in the developing countries are therefore easily contaminated due to the environmental condition, poor processing and lack of proper storage facilities.

AFLATOXIN B1: TOXICOLOGY AND METABOLISM

Of the aflatoxins, AFB_1 is the most prevalent, the most occurring and also the most potent. Acute dietary exposure to AFB_1 has been implicated in epidemics of acute hepatic injury (Sudakin, 2003). The liver is the primary site of biotransformation of ingested AFB_1. The predominant human CYP450 isoforms involved in human metabolism of AFB_1 are CYP 3A4 and CYP 1A2 (Figure 1) . Both enzymes catalyze the biotransformation of AFB_1 to the highly reactive *exo*- 8,9-epoxide of AFB_1 (Guengerich et al., 1998). CYP 1A2 is also capable of catalyzing the epoxidation of AFB_1 to yield a high proportion of *endo* epoxide and hydroxylation of AFB_1 to form aflatoxin M1 (AFM1), which is a poor substrate for epoxidation (Guengerich et al., 1998), less potent than AFB_1 (Wild and Turner, 2002) and generally considered detoxification metabolite while CYP 3A4 can also form AFQ1 a less toxic detoxification metabolite. CYP 3A5 metabolizes AFB_1 mainly to the *exo* epoxide and some AFQ1 (Wang et al., 1998). Polymorphism studies with CYP 3A5 reveal that this isoform is not expressed by most people and in particular about 40% of African-Americans do not express this enzyme (Wild and Turner, 2002). Studies with Gambian children reveal that Aflatoxin can cross the placenta and be transported into the new born (Wild et al., 1993). Thus CYP 3A7 a major cytochrome P450 in human fetal liver, has the capacity to activate AFB_1 to the 8,9- epoxide (Kitada et al., 1989).

Epoxidation of AFB_1 to the exo-8,9- epoxide is a critical step in the genotoxic pathway of this carcinogen (Figure 1). The epoxide is highly unstable and binds with high affinity to guanine bases in DNA to form afltoxin -N^7-guanine (Guengerich, 2001). The afltoxin-N^7-guanine has been shown to be capable of forming guanine (purine) to thymine (pyrimidine) transversion mutations in DNA (Bailey et al., 1996). Studies *in vitro* and animal models as well as epidemiological studies have revealed a high incidence of this transversion mutation occurring at codon 249 of the p53 tumor suppressor gene (Mace et al., 1997; Li et al., 1993) a region corresponding to the DNA binding domain of the corresponding protein (Sudakin, 2003).

Glutathione pathway has been shown to play a major role in the detoxification of AFB_1 (Johnson et al., 1997; Farombi et al., 2005 a). The AFB_1 8,9 *exo* and *endo* epoxides can be conjugated with glutathione resulting in the formation of AFB- mercapturate catalyzed by glutathione S-transferase (GST) (Johnson et al., 1997). The *exo* and *endo* epoxide can also be converted non-enzymatically to AFB_1-8,9-dihydrodiol which in turn can slowly undergo a base-catalysed ring opening reaction to a dialdehyde phenolate ion (Guengerich et al., 1998). AFB_1 dialdehyde can form Schiff bases with lysine residues in serum albumin forming aflatoxin-albumin complex (Sabbioni and Wild, 1991). Furthermore, aflatoxin dialdehyde can be reduced to a dialcohol in a NADPH-dependent catalyzed reaction by aflatoxin aldehyde reductase (AFAR) (Hayes et al., 1993; Knight et al., 1999).

AFLATOXINS AND FOOD CONTAMINATION

Food represents an unavoidable source of human exposure to certain mycotoxins and data from many developing countries show that a wide range of dietary staples and agricultural products are contaminated with a

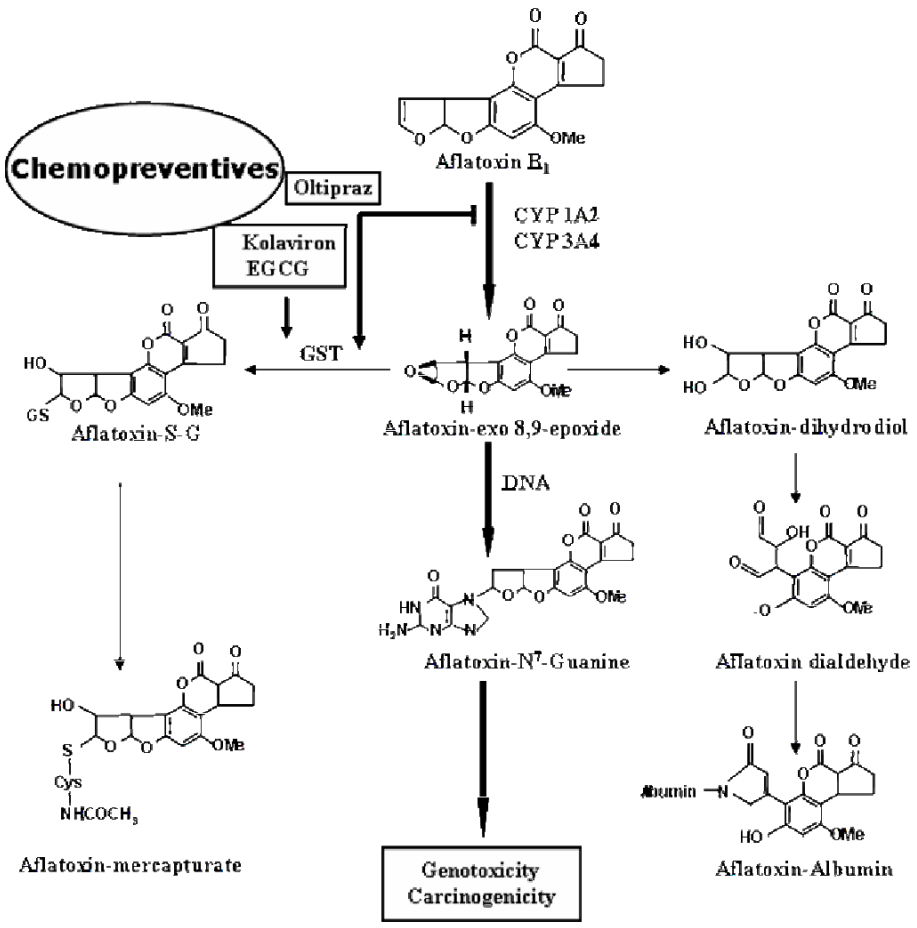

Figure 1. Major mechanism of biotransformation of aflatoxin B_1 (AFB_1) to AFB_1-exo 8, 9-epoxide leading to the formation of AFB_1-DNA adduct (AFB_1-N^7-guanine), detoxification product AFB_1-mercapturate catalysed by glutathione S-transferase (GST) and proposed site of action of selected chemopreventives.

significant proportion of these toxins, particularly AFB_1. Monitoring and regulatory programs have been put in place by many countries in view of the potential hazard of aflatoxins to human health. Levels ranging from zero to 50 ppb have been set as permissible levels for aflatoxin content in foods and feeds (Patterson, 1983). Most countries including the USA have a regulatory level around 20 ppb in foods; however in 1999 European Economic Community established a lower limit of 2.0 ppb for AFB_1 and 4.0 ppb for total aflatoxins (Mishra and Chitrangada, 2003).

Maize and peanuts are good substrates for the growth of aflatoxins. For the purpose of this review data on contamination rate of these two food products in different developing countries will be presented. However, discussion on some other food products is unavoidable. In a survey of groundnut samples from 21 selected markets in the 10 regions of Ghana, Awuh and Kpodo (1996) reported high levels of the aflatoxigenic fungus in these samples. Total aflatoxin levels ranging from 5.7 to 22, 168 ppb were identified with damaged kernel samples. An assessment of three-year surveillance program in Bangladesh revealed the extent of mycotoxin contamination of key foods and feeds grown. The levels of contamination of maize, roasted and raw groundnuts, and poultry feed were considerably high, with average total AFB_1 contents of 33, 13, 65, and 7 μg/kg, respectively, and maximum AFB_1 contents of 245, 79, 480, and 160 μg/kg, respectively (Dawlatana et al., 2002). AFB_1, contamination levels ranging from 0.2 to 129 μg/kg was detected in corn samples collected at wholesale markets in different regions in Brazil (Vargas et al., 2001). Also peanuts and their products were found to be contaminated with aflatoxin ranging from 43 to 1099 μg/kg (Freitas and Brigido, 1998). In samples from Guangxi, a high risk area of HCC China, AFB_1 in corn was the predominant toxin detected in terms of quantity and frequency with concentration ranging from 9 to 1396 ppb (Li et al., 2001). Recently aflatoxin more than the recommended value (30 ppb) was detected in peanut,

Table 1. Food contamination by aflatoxins in certain developing countries.

Country	Commodity	Contamination rate	References
Bangladesh	Maize	33 μg/kg	Dawlatana et al., 2002
	Groundnut	65 μg/kg	Dawlatana et al., 2002
Botswana	Peanut	to 64 μg/kg	Siame et al., 1998
	Peanut butter	0.3 to 23 μg/kg	Siame et al., 1998
Brazil	Corn	0.2-129 ppb	Vargas et al., 2001
	Peanuts and products	43-1099 ppb	Freitas et al., 1998
China	Corn	9-1396 ppb	Li et al., 2001
Egypt	Haze nut	25-175 ppb	Williams et al 2004; Abdel-Hafez et al., 1993
	Soybean	5-35 ppb	Williams et al 2004; el Kadt et al 1993
	Wall nut	15-25 ppb	Williams et al 2004; Abdel-Hafez et al., 1993
Gambia	Ground nut source	162 ppb	Williams et al 2004; Hudson et al., 1992
Ghana	Kernels	5.7-22,168 ppb	Awuah and Kpodo, 1996
India	Pistachio nuts	15 to 259 μg/kg	Candlish et al., 2001
	Dry slices of quince	96 to 8164 μg/kg	Sharma and Sumbali 1999
	Maize	>30 ppb	Bhat et al., 1997; Vasanthi and Bhat 1998
Malaysia	Wheat flour	25.6-289 μg/kg	Abdullah et al. 1998
	Peanut	1-378 μg/kg	Ali et al., 1999
Nepal	Peanut, corn flakes Peanut butter, Vegetable oil	>30 ppb	Koirala et al., 2005
Nigeria	Yam chips	4-186 μg/kg	Bankole and Adebanjo, 2003
	Pre harvest maize	3-138 μg/kg	Bankole and Mabekoje, 2004
	Shelled melon	5-20 μg/kg	Bankole et al., 2004
	Corn and corn based snacks	25-770 ppb	Adebajo et al., 1994
Philippines	Rice bran and rice hull	0.27-11 μg/kg	Sales and Yoshizawa, 2005
	Corn	130 μg/kg	Ali et al., 1999
Senegal	Peanut	40 ppb	Ndiaye et al., 1999
Sudan	Peanut butter and peanut	87.4 - 197.3 μ/kg	Omer et al., 1998
Thailand	Corn	73 μ/kg	Lipigorngoson et al., 2003
	Peanut oil	102 μg/kg	Lipigorngoson et al., 2003
Turkey	Red pepper	1.1 - 97.5 ppb	Erdogan, 2004

cornflakes samples and peanut butter/vegetable oil in Nepal (Koirala et al., 2005).

In Nigeria, a survey of market dietary staples shows that some of the commodities are contaminated by aflatoxins. Aflatoxins were detected in 54% of yam chips in Ogun and Oyo states of Nigeria with concentrations ranging between 4-186 μg/kg (Bankole and Adebanjo, 2003). In another survey in the same region aflatoxins ranging between 3 to 138 μg/kg were detected in pre harvest maize (Bankole and Mabejoje, 2004). Furthermore Bankole et al. (2004) reported the contamination of shelled melon seeds sold in markets in the south western as well as the northern part of Nigeria with mycotoxins. AFB_1 was detected at levels above 5 μg/kg in 32.2% of samples, while only 3.5% of the samples contained the toxin above the 20 μg/kg Nigerian tolerance level in foods.

In a report by Williams et al. (2004), it was revealed that in Egypt haze nut (25-175 ppb), soybean (5-35 ppb) and wall nuts (15-25 ppb) were contaminated with aflatoxins. In Gambia, groundnut source was contaminated with aflatoxin at a concentration of 162 ppb. In Uganda, maize (1-100 ppb) peanut and cassava (> 100 ppb) were contaminated with aflatoxins. Exposure to aflatoxins varies between countries in the developing world principally due to consumption of different diets. A summary of various commodities and aflatoxin contamination rates in different developing countries is presented in Table 1.

HEPATOCELLULAR CARCINOMA (HCC)

Risk factors for HCC

The major risk factors for the development of HCC are viral hepatitis HBV and/or HCV. Aflatoxin contamination of foods and alcohol are also considered as major risk factors in HCC. Minor risk factors such as smoking, polluted pond water, oral contraceptives and androgenic anabolic steroids have been shown to play certain role in

HCC development (Kensler et al., 2003). The relevance and importance of these factors varies in different geographic locations. HBV has been shown to play pivotal role in Chinese, South East Asia and sub-Saharan African patients with HCC. Whereas HCV is common in HCC patients in developed countries such as Japan, Italy and France (Tang, 2001) as well as in areas with intermediate incidence of HCC like southern Europe. In northern Europe and the United States, HCC is often related to other factors such as alcohol liver disease (Pang et al., 2005). In this review, attention will be focused on HBV which is more relevant in the developing countries.

AFB1 and HBV: A deadly duo in HCC

A synergistic interaction between AFB_1 exposure and HBV infection on HCC risk has been reported in several epidemiological studies (Wang et al., 1996; Ross et al., 1992). Studies on HCC in Swaziland and Guangxi Province of China have revealed the possible synergistic interaction between AFB_1 and HBV (Peers et al, 1987; Yeh et al., 1989). In animal model, interaction of AFB_1 and HBV has also been demonstrated. In a transgenic mice over expressing the large envelope polypeptide of HBV and fed with AFB_1 model, Sell et al. (1991) showed that these mice produced more rapid and extensive hepatocyte dysplasia and HCCs than mice unexposed to these agents. Further evidence for a more synergistic interaction between AFB_1 and HBV came from the studies of interaction between AFB_2 and another member of *Hepadnaviridae* family, the woodchuck hepatitis virus (WHV) (Bannasch et al., 1995). De Flora et al. (1989) showed that infection of woodchucks with WHV enhanced the activation of AFB_1 to the reactive AFB_1-8,9-epoxide. Furthermore using urinary AFB_1 metabolite and aflatoxin- albumin adducts as biomarkers, studies in Shangai, China and Taiwan showed a synergistic interaction between exposure to AFB_1 and HBV carrier state (Kew, 2003). Chen et al. (2001) in a study involving adolescents in Taiwan demonstrated a positive association between hepatitis B surface antigen (Hbs-AG) status and AFB_1-albumin supporting the synergistic interaction between HBV and AFB_1. However, other studies failed to find an association between HbsAg status and albumin adducts (Wild et al., 2000; Wang et al., 1996)

Genetic polymorphism seems to play an important role in the interaction between HBV and AFB_1. GST M1 and T1 phase II detoxification genes involved in the detoxification of AFB_1-8,9-epoxide has been identified. In a cohort study in Taiwan, Sun et al. (2001), found a statistically significant relationship between detectable levels of AFB_1-albumin adducts in serum and risk of HCC among chronic HBsAg carriers. In addition they found that the effect of aflatoxin exposure on HCC risk was more pronounced among chronic HBsAg carriers with the GSTT1 null genotype than those who were non-null The interaction between serum AFB_1-albumin adduct level and GSTT1 genotype was also significant.

The tumor suppressor gene p53 has been implicated in the synergistic interaction between AFB_1 and HBV. p53 is the most commonly mutated gene in human cancers . A guanine (G) to thymine (T) transversion at the third position of codon 249 of the p53 gene (249^{ser}) is commonly found in HCC from patients in regions with dietary aflatoxin exposure. *In vitro* studies have supported this finding demonstrating that AFB_1 can induce this mutation (Puisieux et al., 1991; Aguilar et al., 1993). In a study in China, all the patients with 249^{ser} mutations showed evidence of chromic HBV infection (Ming et al., 2002) . Similarly in Taiwanese patients with HCC, all the 249^{ser} mutations occurred in patients positive for HBsAg (Lunn et al., 1997). On the contrary, Stern et al. (200) in a study involving Guangxi, People's Republic of China found little evidence for an HBV-aflatoxin interaction modulating the presence of the p53 249^{ser} mutation or any type of p53 mutation.

MOLECULAR MECHANISMS OF HCC

Role of transcription factor nuclear factor-κB (NF-κB) in HCC

Transcription factors are nuclear proteins activated by the cell transduction pathways in response to a variety of stimuli. They bind to specific DNA sequences on the promoter of target genes, and translate short-term biochemical signals generated by signalling cascades into long term changes in gene expression (Liu et al., 2002). A causal link between constitutive activation of NF-κB and hepatocarcinogenesis via transcriptional regulation of genes involved in cellular transformation, proliferation, survival, invasion and metastasis has been defined by several investigations. NF-κB belongs to a family of dimeric transcription factors composed of p50 (NF-κB1) and p65 (RelA) subunit (Arsura and Cavin, 2005). In addition, c-rel, relB and p52 (NF -κB) subunits have also been identified (Baldwin, 1996). In most cells there is a preponderance of p50/p65 heterodimer NF-κB over others. In resting state, NF-κB is sequestered in the cytoplasm by the chaperon family of specific inhibitory proteins termed IκBs. Several members of the IκB regulatory family has been characterized, including Iκ B-α, IκB-β and IκB-γ. IκB molecules are mostly cytosolic. IκB-α has been implicated in the regulation of NF-κB activity during oncogenic transformation of liver cells (Arsura et al., 2000). In response to viral infection, DNA damage, carcinogenic insult and other proinflammatory responses, inhibitor kappa kinase (IKK) which comprises of two catalytic subunits, IKK-α (IKK1) and IKK-β (IKK2), phosphorylates IκB which leads to liberation of NF-κB

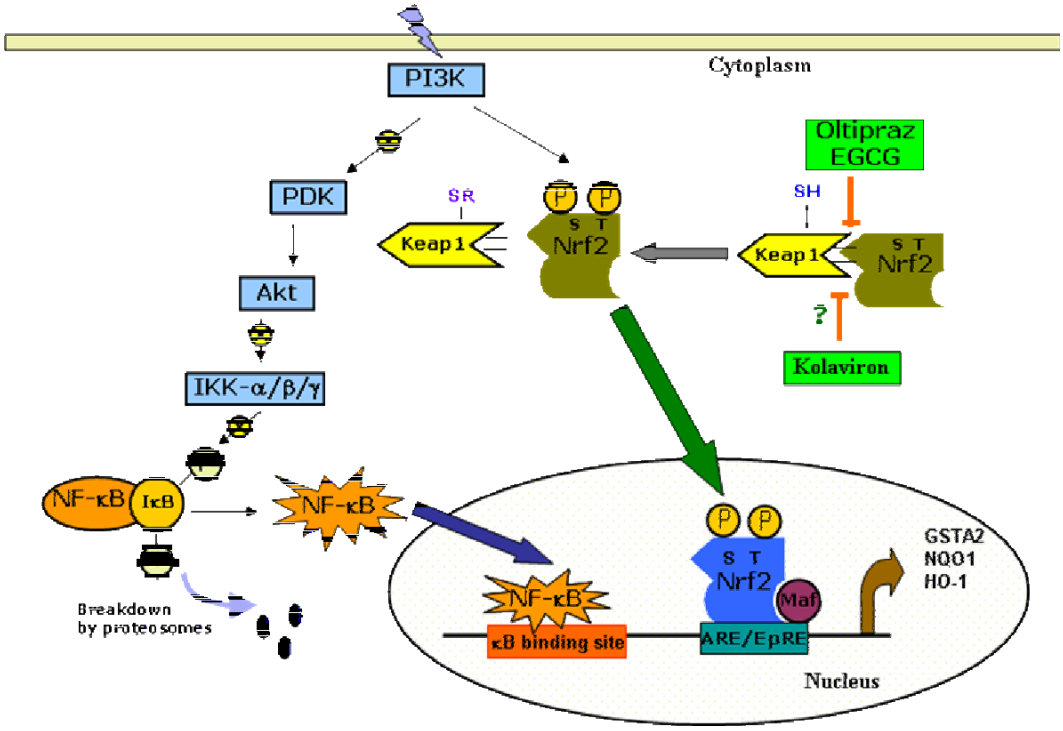

Figure 2. The schematic representation of signaling cascades involving NF- B and Nrf2. The activation of NF- B begins when specific receptors are stimulated at the cell surface and recruitment of adaptor proteins which targets the external signal for specific transduction pathways controlled by various kinases. Phosphatidyl inositol 3-kinase (PI3-K) has been identified as one of the key kinases. PI3-K activates Akt/protein kinase B via phosphorylation by 3-phosphoinositide-dependent protein kinase-1 (PDK1). Akt mediates NF- B activation via the I B kinase (IKK) multiprotein complex. IKK promotes the phosphorylation of I B with further ubiquitination and subsequent degradation by 26S proteasome. NF- B is released and subsequently transported to the nucleus where it binds to specific promoter regions of various genes. Epigallocatechin gallate (EGCG) can inhibit the activities of P13-K and Akt. Nrf2 is kept in the cytoplasm by a Kelch-like-ECH-associated protein 1 (Keap 1). Phase 2 enzyme inducers can cause covalent modification of these cysteine residues which leads to the dissociation of Nrf2 from Keap 1. Dissociation of Keap 1-Nrf2 can also be facilitated by P13-K and other upstream kinases. Nrf2 is subsequently translocated into the nucleus where it interacts with a small Maf protein, forming a heterodimer that binds to ARE which leads to the stimulation of ARE-driven expression of gene that encode phase-2 detoxifying enzymes such as glutathione S-transferase alpha2 (GSTA2), NADPH_quinone oxidoreductase (NQO1) and heme oxygenase (HO-1). Oltipraz, EGCG and possibly Kolaviron can facilitate the release of Nrf2 from Keap 1.

and results in subsequent translocation of NF-κB to the nucleus where it can perform its functions (Figure 2).

Other transcription factors known to be involved in cancer development and cell proliferation regulation include activator protein (AP-1) and the signal transducers and activators of transcription (STATs) (Liu et al., 2002). AP-1 is a dimmer formed of proteins of the Jun family (c-Jun, JunB, JunD) and the Fos family (c-Fos, Fos-B, Fra 1, Fra 2). Early activation of AP1, NF-κB and STAT has been shown to possibly contribute to the acquisition of a transformed phenotype during hepatocarcinogenesis (Liu et al., 2002).

Molecular mechanisms of HBV mediated HCC

Several mechanisms involving interaction of HBV viral DNA into the host genome to induce chromosomal instability (Murakami et al., 2005) and insertional mutations resulting from genome integration of HBV at specific site leading to activation of endogenous genes such as retinoic acid B-receptor, cyclin A and TRAP1 genes (Minami et al., 2005; Gozuacik et al., 2001) have been proposed for HBV-mediated hepatocarcinogenesis.

However, a major contributory mechanism involves modulation of cell proliferation through the expression of viral proteins particularly X protein (HBx). Shirakata et al. (1989) demonstrated *in vitro* the expression of HBx can transform rodent hepatocyes and subsequently it was shown that it induces HCC in mice (Kim et al., 1991). In addition, studies have shown that ectopic expression of HBV large envelope in transgenic mice determines the accumulation of toxic levels in HBsAg that is followed by liver injury, inflammation and HCC formation (Chisari et al., 1989).

It has been demonstrated that HBx does not bind to DNA directly but it is capable of co-activating the transcription of some viral and cellular genes (Pang et al.,

2005). Thus HBx has been shown to transactivate and up-regulates the expression of class III promoters, protooncogens (Twu et al., 1993), NF- κB, AP-1 and ATF/CREB as well as other viral genes such as HBV enhancers in the nucleus (Weil et al., 1999; Henkler et al., 1998; Choi et al., 1999).

Lucito and Schneider (1992) demonstrated that the expression of HBx in hepatocytes promoted transcriptional activation of NF -κB by mechanism involving degradation of IκB-α and p 105 (Chirillo et al., 1996). Subsequently it was shown that HBx interacts with IκB-α and transports it to the nucleus thereby preventing it from re-association with DNA-bound NF-κB (Weil et al., 1999).

Studies have demonstrated the role of mitochondia and reactive oxygen species (ROS) in the mechanisms by which HBx mediates liver diseases associated with HBV. Waris et al. (2001) demonstrate that HBx directly and physically interacts with an outer mitochondrial voltage-dependent anion channel (VDAC3) and that this association leads to a decrease in the mitochondrial membrane potential and causes the elevation of ROS. This sequence of activities leads to the activation of NF-κB and STAT-3. Further evidence underscoring the role of ROS in HBx induced liver cancer came from the studies of Meyer et al. (1992). In their study, they showed that both $MHBs^t$ a hepatitis B surface antigen derivative as well as HBx activated NF-κB and these activities were inhibited by NAC and PDTC.

Additional mechanism of action of HBx involves its interaction with p53. HBx has been reported to bind to the C-terminus of p53 forming a protein-protein complex thereby inactivating several critical p53-dpendent activities. Further studies showed that HBx can inhibit sequence of specific DNA binding and transcriptional activating properties of p53 (Pang et al., 2005). *In vivo* studies involving the use of transgenic mice expressing HBx protein also demonstrate that HBx can repress p53-mediated transcriptional activation (Ueda et al., 1995).

Molecular mechanism of AFB_1-mediated HCC

AFB_1, the most potent of the aflatoxins, has been implicated in the aetiology of HCC by numerous studies. Studies have also demonstrated that the concurrent infection with HBV during aflatoxin exposure increased the risk of HCC. In mechanistic terms, a number of molecular pathways have been proposed linking AFB_1 with HBV.

It has been proposed that HBV infection directly or indirectly may induce the specific CYP that metabolise AFB_1 to the reactive metabolite. In transgenic mice model, the induction of phase I enzymes was demonstrated (Gemechu-Hatewu et al., 1997). In addition, it was reported that Gambian children and adolescents chronically infected with HBV have higher concentration of AFB_1 adducts than uninfected individuals (Chen et al., 2001; Turner et al., 2000). Furthermore, induction of phase 2 detoxification enzymes such as the GST families has been described in AFB_1 and HBV hepatocarcinogenesis (Yu et al., 1997; Sun et al., 2001).

As an alternative mechanism to the formation of 8,9-epoxide from activation of AFB_1, formation of ROS has been demonstrated in several models (Shen et al., 1996; Yang et al., 2000; Lee et al., 2005). ROS has been shown to be mutagenic and may thus contribute to the process of cancer formation. HBV has also been shown to generate ROS (Liu et al., 1994). Synergistic interaction of both AFB_1 and HBV via ROS formation may be a major mechanism by which they induce HCC. Furthermore, carcinogens have been shown to activate NF-κB via ROS production. It may be possible that AFB_1 induces liver cancer via ROS-induced activation of NF-κB. Experimental studies to test this hypothesis are necessary.

PRIMARY PREVENTION OF HCC

Primary prevention strategies of HCC involve the elimination or reduction in exposure to agents implicated in the formation of the disease. HBV vaccination has been advocated in this regard. It has been estimated that approximately 70% of HCC in developing countries is attributable to HBV (Wild and Hall, 2000) therefore vaccination could prevent more than 250,000 cases per year in these areas of the world. In addition, reduction of exposure to aflatoxin at the individual and community level will also help in preventing HCC. Aflatoxins contaminate dietary staple foods such as groundnuts and maize. Reduction of exposure can be addressed at the community level either pre- or post-harvest by limiting fungal contamination of crops; approaches may involve low technology post-harvest measures to limit fungal growth or genetic engineering of crops to be resistant to fungal infection or toxin biosynthesis. The details of primary intervention strategies in HCC have been reviewed by other investigators (Wild and Hall, 2000; Williams et al., 2004).

CHEMOPREVENTIVE STRATEGIES

Although the above strategies will play certain role in the prevention of HCC, they have some limitations. For instance it has been estimated that there are currently 360 million chronic HBV carriers worldwide but HBV vaccine which seems to be crucial in preventing the scourge of HCC is still not incorporated into many national immunisation programs. Currently there is no proven effective systemic chemotherapy for HCC. Alternative treatments such as transcatheter arterial che-momobilization, percutaneous intratumoral ethanol injec-

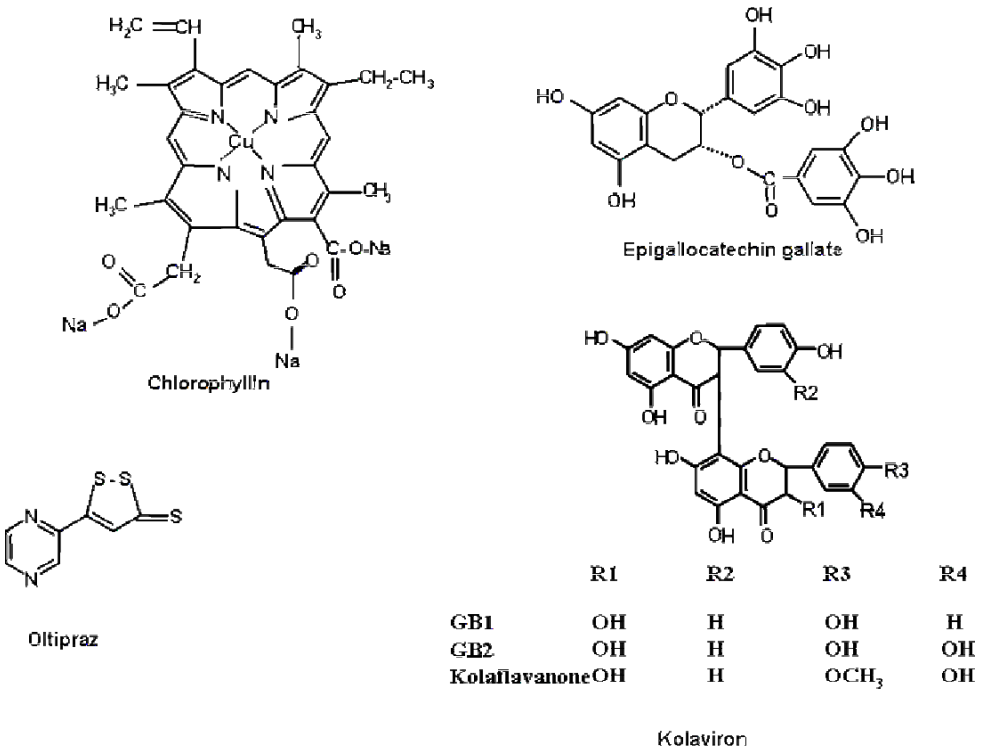

Figure 3. Structures of selected chemopreventive agents.

tion, and radiofrequency ablation are mainly palliative in nature and are only applicable to patients with tumors localised in the liver (Poon et al., 2002). On the other hand it has been suggested also that dietary change on the part of individuals could also assist in preventing HCC. However this may not be feasible since people prefer prescription to proscription. Therefore HCC remains a disease for which alternative therapeutic modalities must be developed. In the developing world where the burden of liver cancer is highest, targeted chemoprevention offers the most appropriate solution for individuals living in areas where HCC is endemic.

Definition of chemoprevention

The term chemoprevention was originally coined by Michael Sporn who utilized retinoids to halt experimental carcinogenesis (Sporn and Roberts, 1984). Chemoprevention refers to the use of relatively non-toxic specific chemical substances either of natural or synthetic origin or their mixtures to suppress, delay, impede, arrest or reverse the process of carcinogenesis. Most chemopreventive agents, according to Lee Wattenberg (1985), can be broadly classified into blocking agents and suppressing agents.

Blocking agents prevent the carcinogens from reaching the target sites, undergoing metabolic activation or subsequently interacting with crucial cellular macromolecules such as DNA, RNA and proteins. Suppressing agents on the other hand, inhibit the premalignant transformation and malignant formation of initiated cells during the stage of promotion and progression. Chemopreventive agents in addition have been shown to induce a set of detoxification phase 2 antioxidant enzymes such as GST through the activation of intracellular signalling mediated by the nuclear transcription factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) (Lee and Surh 2005). Also these agents may modulate other transcription factors such as NF-κB thereby preventing carcinogenesis.

EXAMPLES OF CHEMOPREVENTIVE AND HEPATOPROTECTIVE AGENTS

Oltipraz

Oltipraz, originally developed as an antischistosomiasis drug, has received the attention of several investigators in cancer chemoprevention in the last one and half decade. It is structurally similar to the dithiolethiones usually found in cruciferous vegetables (Figure 3). In a randomised clinical study with low levels of aflatoxin exposure, treatment with oltipraz offered complete protection against hyperplastic nodules and hepatocellu-

lar cancer compared to the placebo group (Roebuck et al., 1991). In experimental animal model, treatment with oltipraz protected against the development of hepatic aflatoxin-DNA adducts and also enhanced the activity of GST (Bolton et al., 1993). In this study, it was hypothesised that oltipraz reduced the formation of aflatoxin-N^7-guanine adducts by enhancing the conjugation of AFB_1-8,9-epoxide with glutathione and thereby preventing binding of the metabolite to DNA. Other studies *in vitro* and *in vivo* as well as human studies suggest the oltipraz has inhibitory effect on certain Phase 1 enzyme such as CYP 1A2 and CYP 3A4 (Langouet et al., 2000; Langouet et al., 1995). In a healthy human volunteer study, oral administration of oltipraz at a dose of 125 mg for eight days was associated with a significant reduction in CYP 1A2 activity (Sofowora et al., 2001).

In another randomized, placebo-controlled, double-blind phase II trial in People's Republic of China (at high risk for development of HCC in part due to consumption of foods contaminated with aflatoxins) revealed the chemopreventive activity of oltipraz. One month of weekly administration of 500 mg oltipraz led to decrease in phase 1 metabolite aflatoxin M1 excreted in urine compared with administration of a placebo, while daily intervention with 125 mg oltipraz led to a increase in aflatoxin-mercapturic acid excretion suggesting that intermittent, high-dose oltipraz (500 mg) inhibited phase 1 activation of aflatoxins, and sustained low- dose oltipraz (125 mg) increased phase 2 conjugation of aflatoxin, yielding higher levels of aflatoxin-mercapturic acid with both mechanisms contributing to chemopreventive effects of this drug (Wang et al., 1999).

Molecular mechanism of action of oltipraz indicates that it enhances phase 2 enzymes by activating the antioxidant response elements (ARE) via translocation of Nrf2 to the nucleus. Studies have shown that treatment with oltipraz disrupt the interaction between Keap1 and Nrf2, allowing Nrf2 to translocate to the nucleus where it forms heterodimers with small MAF-familiy protein associated with ARE to induces the expression of GST (Petzer et al., 2003; Kwak et al., 2001a) and other ARE related genes such as heme oxygenase 1 (HO-1) (Kwak et al 2001 b).

Chlorophyllin

Chlorophyllin (CHL) (Figure 3) is a water -soluble form of chlorophyll which forms an essential constituent of human diet. It is used extensively as a food colorant and has numerous medicinal applications including acceleration of wound healing (Young and Bergei, 1980). It exists as a mixture of sodium and copper salt and it is marketed as an over-the- counter drug for controlling odour (Kephart, 1955). CHL is an effective anticarcinogen in experimental models including aflatoxin-induced hepatocarcinogenesis (Breinholt et al., 1995a). CHL is thought to form molecular complexes with carcinogens, thereby blocking their bioavailability (Breinholt et al., 1995 b). It was recently evaluated as a chemopreventive agent in a population at high risk for exposure to aflatoxin and subsequent development of HCC.

In a clinical trial carried out in Quidong, administration of CHL to volunteers three times a day led to a 50% reduction in the median level of urinary excretion of aflatoxin- N^7-guanine compared to placebo group. This excreted DNA adduct biomarker is derived from the ultimate carcinogenic metabolite of AFB_1, aflatoxin-8, 9-epoxide, and is associated with increased risk of developing liver cancer (Egner et al., 2003). During this intervention study, no toxicities were observed coupled with excellent compliance. Thus, CHL may be considered as a safe and effective agent suitable for use in individuals unavoidably exposed to aflatoxins. It has therefore been suggested that supplementation with green leafy vegetable foods rich in chlorophylls might be a more practical means of administration of CHL (Kensler et al., 2003).

Green tea

Green tea (GT) and its polyphenols (GTP) have been shown to be a safe and effective chemopreventive agents in various *in vitro* and *in vivo* animal models for inhibition of carcinogen- induced mutagenesis and tumorigenesis at several target organ sites including AFB_1-induced liver tumors (Lambert and Yang, 2003). Quin et al. (2000) investigated the chemoprevention of hepatocarcinogenesis by green tea in rats treated with AFB_1 and CCl_4 as the initiator and promoter, respectively. Feeding of GT during initiation or promotion inhibited the number of glutathione S-transferase placental form- and gamma- glutamyl transpeptidase-positive hepatic foci by 30-40% and the area and volume by 50%. GT treatment throughout the period inhibited the number of both types of hepatic foci by 60% and the area and volume by 75-80%. Cell proliferation was inhibited (35%) by GT given during promotion, whereas inhibition was 65% when GT was given during initiation or throughout the period suggesting that GT feeding inhibits initiation and promotion steps of AFB_1 hepatocarcinogenesis and that the inhibition of cell proliferation is responsible for the suppression of promotion.

GTP is the secondary metabolite in tea plants and accounts for about 30-36% weight of the water extractable materials in tea leaves. The major GTP components include (-)- epigallocatechin gallate (EGCG) (Figure 3), which is the most abundant, amongst others (Graham, 1992). In humans, inverse relationships between the level of green tea consumption and the risk of development of cancer have been observed (Nakachi et al., 2000; Fujiki et al., 2002).

Recently in a randomized, double blinded, and placebo controlled phase IIa chemoprevention trial with GTP involving 124 participants, modulation of urinary excretion of GTP and oxidative DNA damage biomarker, 8-hydroxydeoxyguanosine (8-OHdG), was assessed in urine samples collected from individuals (Luo et al., 2005). In this study EGC and epicatechin (EC) levels, components of green tea displayed significant and dose-dependent increases in urine of individuals administered with green tea. In addition, 8-OHdG levels decreased significantly in both GTP treated groups. The outcome of this study indicate that urinary excretions of EGC and EC can serve as practical biomarkers for green tea consumption in human populations and also suggest that chemoprevention with GTP is effective in diminishing oxidative DNA damage.

Molecular mechanisms of chemopreventive action of EGCG involve its ability to induce specific phase 2 enzymes via the activation of Nrf2. In a rat liver model, Chou et al. (2000) demonstrated the induction of GST activity by EGCG in a dose- and time-dependent manner. Specifically, GSTM2 was increased significantly with a maximal induction of 2.0-fold. Recent studies indicate the induction of Nrf2 dependent genes by EGCG (Shen et al., 2005, Xu et al., 2005).

Kolaviron

The nut of *Garcinia kola* Heckel (Family: Guttiferae) is native to Nigeria and Ghana and is highly valued in these countries and other parts of west and central Africa. The seed commonly known, as 'bitter kola' is eaten by local people and it is believed to aid digestion and it is therefore referred to as false kolanut. The seeds play important role during traditional and social ceremony in these regions of the world

Phytochemical studies revealed that biflavonoids are the major constituents of *G. kola*. Kolaviron, a fraction of the defatted ethanol extract, containing *Garcinia* biflavonoid GB-1 GB-2 and kolaflavanone (Figure 3) was isolated by Iwu (1985). Subsequently other compounds such as garcinoic acid and garcinal were isolated from the seed (Terashima et al., 2002). Recently, Han et al. (2005) elucidated the complete NMR assignment of the potent antibacterial biflavonid GB1 from the seeds of *G. kola*.

The chemopreventive and hepatoprotective activities of kolaviron have been well investigated *in vitro*, *in vivo* as well as in cell line models. Kolaviron was reported to significantly prevent hepatotoxicity mediated by galactosamine, amanita toxin (Iwu et al., 1987) paracetamol (Akintonwa et al., 1990) and thioacetamide (Iwu et al., 1990) in animal models. Results of investigations in our laboratory have revealed the protective effects of kolaviron against hepatotoxicity and oxidative stress induced by 2-acetylaminofluorene and carbontetrachloride (Farombi et al., 2000; Farombi, 2000). Recently, we showed the chemopreventive effect of kolaviron against AFB_1 hepatotoxicity and genotoxicity (Farombi et al., 2005b).

Kolaviron had been reported to interfere with hepatic drug metabolizing enzymes (Braide, 1991). We also demonstrated that while kolaviron preserved the activities of some representative phase1 enzymes, it enhanced the activities of major phase II enzymes such as GST, uridyldiphosphoglucuronosyl transferase (UDPGT) (Farombi, 2000; Farombi et al., 2005b) and DT-diaphorase (Farombi et al., 2005b). The induction of phase II enzymes by kolaviron has also been confirmed by the studies of Nwankwo et al. (2000) in Hep G2 cells. The authors demonstrated that GST isozyme α-1 and α-2 were induced by 2.2 and 2.5 fold levels respectively for their messages as determined by reverse transcription polymerase chain reaction (RT-PCR) and northern analysis and 2 fold increase in GST- α protein by western blotting. These studies suggest that induction of GST by kolaviron may play a prominent role in its chemopreventive activities.

As a mechanism contributing to the chemopreventive effects of kolaviron, antioxidant and free radical properties of this agent have been investigated. The ability of kolaviron to scavenge hydrogen peroxide, superoxide anion and hydroxyl radicals *in vitro* (Farombi et al., 2002) and suppression of lipid peroxidation *in vivo* (Farombi et al., 2000, Farombi, 2000) have been demonstrated. Recently, Kolaviron was shown to reduce background levels of protein oxidation biomarkers 2 amino-adipic semialdehyde in both plasma and liver and decreased oxidative damage to DNA in the rat liver (Farombi et al., 2004a). Furthermore kolaviron demonstrated significant inhibition of hydrogen peroxide induced strand breaks as well as oxidative DNA damage in both human lymphocytes and rat liver cells (Farombi et al., 2004b). Very recently, we demonstrated the metal chelating properties of kolaviron as a mechanism contributing to its chemopreventive potential. Our study showed that kolaviron (10-60 μmol/L) inhibited the Cu^{2+}-induced oxidation of rat serum lipoprotein in a concentration-dependent manner and elicited significant chelating effect on Fe^{2+} (Farombi and Nwaokeafor, 2005).

Since the edible *G. kola* nut from which kolaviron is obtained occupies a prominent position in the social customs of the people in Nigeria and other parts of West Africa where HCC is common, this novel hepatoprotective agent may, represent an alternative cheaper and natural chemopreventive agent to the antischistosomiasis drug, oltipraz, though with certain draw backs and qualify for clinical trials in the treatment of HCC.

CONCLUSION AND FUTURE PERSPECTIVES

Since the discovery of aflatoxins about 45 years ago, an avalanche of research activities has been conducted in

various models which have cascaded into characterising it as a human hepatocarcinogen. Advances in the molecular biotransformation mechanisms of AFB_1 and synergistic interaction with HBV have afforded opportunities for attacking liver cancer. HBV accounts for 70% of cases of HCC in developing countries and presently vaccination against this virus is being employed coupled with reduction of exposure to dietary AFB_1 at the individual levels and during pre and post harvest of crops. These applications have yielded some results previously but chemoprevention as an additional remedy may offer a more lasting solution. Chemoprevention provides opportunities to create molecular detours, if not roadblocks, to limit and retard the carcinogenic process. Understanding of the molecular mechanisms of AFB_1 and HBV as related to HCC will assist in sourcing for promising chemopreventive agents. Efforts should be geared towards searching for novel liver chemopreventive agents targeting transcription factors such as NF-κB and other signal transduction molecules such as Nrf2. Inhibitors of molecular events leading to activation of NF-κB and enhancement of genes acting via Nrf2 have been shown to prevent against neoplastic disorders. In light of these findings, the use of chemopreventive agents and liver hepatoprotectants inhibiting NF-κB and activating Nrf2 may prove to be beneficial in the prevention and treatment of human HCC.

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REFERENCES

Abdullah N, Nawawi A, Othman I (1998). Survey of fungal counts and natural occurrence of aflatoxins in Malaysian starch-based foods. Mycopathologia. 143:53-8.

Adebajo LO, Idowu AA, Adesanya OO (1994).Mycoflora, and mycotoxins production in Nigerian corn and corn-based snacks. Mycopathologia. 126:183-92.

Aguilar F, Hussain SP, Cerutti P (1993). Aflatoxin B1 induces the transversion of G-->T in codon 249 of the p53 tumor suppressor gene in human hepatocytes. Proc Natl Acad Sci USA 90:8586-90.

Akintonwa A, Essien AR (1990). Protective effects of *Garcinia kola* seed extract against paracetamol-induced hepatotoxicity in rats. J. Ethnopharmacol. 29:207-211.

Ali N, Hashim NH, Yoshizawa T. (1999). Evaluation and application of a simple and rapid method for the analysis of aflatoxins in commercial foods from Malaysia and the Philippines. Food Addit Contam. 16:273-80.

Amon JJ, Nedsuwan S, Chantra S, Bell BP, Dowell SF, Olsen SJ, Wasley A. (2005). Trends in liver cancer, sa kaeo province Thailand. Asian Pac J Cancer Prev. 6:382-6.

Arsura M, Cavin LG (2005). Nuclear factor-kappaB and liver carcinogenesis. Cancer Lett. 229:157-69.

Arsura M, Mercurio F, Oliver AL, Thorgeirsson SS, Sonenshein GE (2000). Role of the IkappaB kinase complex in oncogenic Ras- and Raf-mediated transformation of rat liver epithelial cells. Mol. Cell Biol. 20:5381-91.

Awuah RT, Kpodo KA (1996). High incidence of Aspergillus flavus and aflatoxins in stored groundnut in Ghana and the use of a microbial assay to assess the inhibitory effects of plant extracts on aflatoxin synthesis. Mycopathologia. 134:109-14.

Bailey EA, Iyer RS, Stone MP, Harris TM, Essigmann JM (1996). Mutational properties of the primary aflatoxin B1-DNA adduct.Proc Natl Acad Sci USA. 93:1535-9.

Baldwin AS Jr. (1996). The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev Immunol. 14:649-83.

Bankole SA, Mabekoje OO (2004). Occurrence of aflatoxins and fumonisins in preharvest maize from south-western Nigeria. Food Addit Contam. 21:251-5.

Bankole SA, Ogunsanwo BM, Mabekoje OO (2004). Natural occurrence of moulds and aflatoxin B1 in melon seeds from markets in Nigeria Food Chem Toxicol. 42:1309-14.

Bannasch P, Khoshkhou NI, Hacker HJ, Radaeva S, Mrozek M, Zillmann U, Kopp-Schneider A, Haberkorn U, Elgas M, Tolle T (1995). Synergistic hepatocarcinogenic effect of hepadnaviral infection and dietary aflatoxin B1 in woodchucks. Cancer Res. 55:3318-30.

Bannasch P, Khoshkhou NI, Hacker HJ, Radaeva S, Mrozek M, Zillmann U, Kopp-Schneider A, Haberkorn U, Elgas M, Tolle T (1995). Synergistic hepatocarcinogenic effect of hepadnaviral infection and dietary aflatoxin B1 in woodchucks.Cancer Res. 55:3318-30.

Bhat RV, Vasanthi S, Rao BS, Rao RN, Rao VS, Nagaraja KV, Bai RG, Prasad CA, Vanchinathan S, Roy R, Saha S, Mukherjee A, Ghosh PK, Toteja GS, Saxena BN (1997). Aflatoxin B1 contamination in maize samples collected from different geographical regions of India--a multicentre study. Food Addit Contam. 14:151-6.

Bolton MG, Munoz A, Jacobson LP, Groopman JD, Maxuitenko YY, Roebuck BD, Kensler TW (1993). Transient intervention with oltipraz protects against aflatoxin-induced hepatic tumorigenesis. Cancer Res. 53:3499-504.

Braide VB (1991). Antihepatotoxic biochemical effects of kolaviron, a biflavonoid o garcinia kola seeds. Phytotherap Res. 5:35-37.

Breinholt V, Hendricks J, Pereira C, Arbogast D, Bailey G (1995a). Dietary chlorophyllin is a potent inhibitor of aflatoxin B1 hepatocarcinogenesis in rainbow trout. Cancer Res. 55:57-62.

Breinholt V, Schimerlik M, Dashwood R, Bailey G (1995b). Mechanisms of chlorophyllin anticarcinogenesis against aflatoxin B1: complex formation with the carcinogen. Chem Res Toxicol. 8:506-14.

Candlish AAG, Pearson SM, Aidoo KE, Smith JE, Kelly B, Irvine H (2001). A survey of ethnic foods for microbial quality and aflatoxin content. Food Addit Contam. 18:129-36.

Chen SY, Chen CJ, Chou SR, Hsieh LL, Wang LY, Tsai WY, Ahsan H, Santella RM (2001). Association of aflatoxin B(1)-albumin adduct levels with hepatitis B surface antigen status among adolescents in Taiwan. Cancer Epidemiol Biomarkers Prev. 10:1223-6.

Chirillo P, Falco M, Puri PL, Artini M, Balsano C, Levrero M, Natoli G (1996). Hepatitis B virus pX activates NF-kappa B -dependent transcription through a Raf-independent pathway. J. Virol. 70:641-6.

Chisari FV, Klopchin K, Moriyama T, Pasquinelli C, Dunsford HA, Sell S,Pinkert CA, Brinster RL, Palmiter RD (1989). Molecular pathogenesis of hepatocellular carcinoma in hepatitis B virus transgenic mice. Cell. 59:1145-56.

Choi BH, Park GT, Rho HM (1999). Interaction of hepatitis B viral X protein and CCAAT/ enhancer-binding protein alpha synergistically activates the hepatitis B viral enhancer II/pregenomic promoter. J. Biol Chem. 274:2858-65.

Chou FP, Chu YC, Hsu JD, Chiang HC, Wang CJ (2000). Specific induction of glutathione S -transferase GSTM2 subunit expression by epigallocatechin gallate in rat liver. Biochem Pharmacol. 60:643-50.

Dawlatana M, Coker RD, Nagler MJ, Wild CP, Hassan MS, Blunden G (2002). The occurrence of mycotoxins in key commodities in

Bangladesh: surveillance results from 1993 to 1995. J. Nat. Toxins. 11:379-86.
De Flora S, Hietanen E, Bartsch H, Camoirano A, Izzotti A, Bagnasco M, Millman I. (1989). Enhanced metabolic activation of chemical hepatocarcinogens in woodchucks infected with hepatitis B virus. Carcinogenesis. 10:1099-106.
Egner PA, Munoz A, Kensler TW (2003). Chemoprevention with chlorophyllin in individuals exposed to dietary aflatoxin. Mutat. Res. 523-524:209-16.
el-Kady IA, Youssef MS (1993). Survey of mycoflora and mycotoxins in Egyptian soybean seeds. J. Basic Microbiol. 33:371-8.
Erdogan A (2004). The aflatoxin contamination of some pepper types sold in Turkey. Chemosphere. 56:321-5.
Farombi EO, Adepoju BF, Ola-Davies OE, Emerole GO (2005b). Chemoprevention of aflatoxin B1-induced genotoxicity and hepatic oxidative damage in rats by kolaviron, a natural bioflavonoid of Garcinia kola seeds. Eur. J. Cancer Prev. 14:207-14.
Farombi EO, Akanni OO, Emerole GO (2002). Antioxidative and scavenging activities of kolaviron *in vitro*. Pharmaceutical Biology 40**:**107-116.
Farombi EO, Hansen M, Ravn-Haren G, Moller P, Dragsted LO (2004a). Commonly consumed and naturally occurring dietary substances affect biomarkers of oxidative stress and DNA damage in healthy rats. Food Chem. Toxicol. 42:1315-22.
Farombi EO, Moller P, Dragsted LO (2004b). Ex-vivo and *in vitro* protective effects of kolaviron against oxygen-derived radical-induced DNA damage and oxidative stress in human lymphocytes and ratliver cells. Cell Biol. Toxicol. 20:71-82.
Farombi EO, Nwankwo JO, Emerole GO (2005a). The effect of modulation of glutathione levels on markers for aflatoxin B1-induced cell damage. Afr. J. Med. Sci. 34:37-43.
Farombi EO, Nwaokeafor IA (2005). Anti-oxidant mechanisms of kolaviron: studies on serum lipoprotein oxidation, metal chelation and oxidative membrane damage in rats. Clin. Exp. Pharmacol. Physiol. 32:667-74.

Farombi EO, Tahnteng JG, Agboola AO, Nwankwo JO, Emerole GO (2000). Chemoprevention of 2-acetylaminofluorene-induced hepatotoxicity and lipid peroxidation in rats by kolaviron--a Garcinia kola seed extract. Food Chem, Toxicol. 38:535-41.
Farombi EO (2000). Mechanisms for the hepatoprotective action of kolaviron: studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbontetrachloride-treated rats. Pharmacol. Res. 42:75-80.
Freitas VP, Brigido BM (1998). Occurrence of aflatoxins B1, B2, G1, and G2 in peanuts and their products marketed in the region of Campinas, Brazil in 1995 and 1996. Food Addit. Contam. 15:807-11.
Fujiki H, Suganuma M, Imai K, Nakachi K (2002). Green tea: cancer preventive beverage and/or drug.Cancer Lettl. 188:9-13.
Gechu-Hatewu M, Platt KL, Oesch F, Hacker HJ, Bannasch P, Steinberg P (1997). Metabolic activation of aflatoxin B1 to aflatoxin B1-8, 9-epoxide in woodchucks undergoing chronic active hepatitis. Int. J. Cancer. 73:587-91.
Goto T, Peterson SW, Ito Y, Wiclow DT (1997). Mycotoxin producing ability of *A.tamarii.* Mycotoxins 44:17-20
Goto T, Wicklow DT, Ito Y (1996). Aflatoxin and cyclopiazonic acid production by a sclerotium-producing Aspergillus tamarii strain. Appl. Environ. Microbiol. 62:4036-8.
Gozuacik D, Murakami Y, Saigo K, Chami M, Mugnier C, Lagorce D,Okanoue T, Urashima T, Brechot C, Paterlini -Brechot P (2001). Identification of human cancer-related genes by naturally occurring Hepatitis B Virus DNA tagging. Oncogene. 20:6233-40.
Graham HN (1992). Green tea composition, consumption, and polyphenol chemistry. Prev Med. 21:334-50.
Guengerich FP (2001). Forging the links between metabolism and carcinogenesis. Mutat. Res. 488:195-209.
Guengerich FP, Johnson WW, Shimada T, Ueng YF, Yamazaki H, Langouet S (1998). Activation and detoxication of aflatoxin B1.Mutat. Res. 402:121-8.
Han QB, Lee SF, Qiao CF, He ZD, Song JZ, Sun HD, Xu HX (2005). Complete NMR assignments of the antibacterial biflavonoid GB1 from Garcinia kola. Chem. Pharm. Bull, (Tokyo). 53:1034-6.
Hayes JD, Judah DJ, McLellan LI, Kerr LA, Peacock SD, Neal GE (1991). Ethoxyquin-induced resistance to aflatoxin B1 in the rat is associated with the expression of a novel alpha-class glutathione S-
transferase subunit, Yc2, which possesses high catalytic activity for aflatoxin B1-8,9-epoxide. Biochem. J. 279:385-98.
Henkler F, Lopes AR, Jones M, Koshy R (1998). Erk-independent partial activation of AP-1 sites by the hepatitis B virus HBx protein. J. Gen. Virol. 79:2737-42.
Hudson GJ, Wild CP, Zarba A, Groopman JD (1992). Aflatoxins isolated by immunoaffinity chromatography from foods consumed in The Gambia, West Africa. Nat. Toxins. 1:100-5.
ItoY, Peterson SW, Wiclow DT, Goto T (2001). *Aspergillus pseudotamarii*, a new aflatoxin producing sp. In Aspergillus section Flavi. Mycol. Res. 105:233-239.
Iwu MM (1985). Antihepatotoxic constituents of *Garcinia kola* seeds. Experientia 41: 699-700.
Iwu MM, Igboko OA, Okunji CO, Tempesta S (1990). Antidiabetic and aldose reductase activities of biflavanones of *Garcinia kola*. J. Pharm. Pharmacol. 42:290-292.
Iwu MM, Igboko OA, Onwuchekwa U, Okunji CO (1987). Evaluation of the antihepatotoxic activity of the biflavonoids of *Garcinia kola* seeds. J. Ethnopharmacol. 21:127-142.
Johnson WW, Ueng YF, Widersten M, Mannervik B, Hayes JD, Sherratt PJ, Ketterer B, Guengerich FP (1997). Conjugation of highly reactive aflatoxin B1 exo-8,9-epoxide catalyzed by rat and human glutathione transferases: estimation of kinetic parameters. Biochemistry. 36:3056-60.
Kensler TW, Qian GS, Chen JG, Groopman JD (2003). Translational strategies for cancer prevention in liver. Nat. Rev. Cancer. 3:321-9.
Kephart JC (1955). Chlorophyll derivatives- their chemistry, commercial preparation and uses. Econ. Bot. 9:3-38.
Kew MC (2003). Synergistic interaction between aflatoxin B1 and hepatitis B virus in hepatocarcinogenesis. Liver Int. 23:405-9.
Kim CM, Koike K, Saito I, Miyamura T, Jay G (1991). HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature. 351:317-20.
Kitada M, Taneda M, Ohi H, Komori M, Itahashi K, Nagao M, Kamataki T (1989). Mutagenic activation of aflatoxin B1 by P-450 HFLa in human fetal livers. Mutat. Res. 227:53-8.
Knight LP, Primiano T, Groopman JD, Kensler TW, Sutter TR (1999). cDNA cloning, expression and activity of a second human aflatoxin B1 metabolizing member of the aldo-keto reductase superfamily, AKR7A3. Carcinogenesis. 20:1215-23.
Koirala P, Kumar S, Yadav BK, Premarajan KC (2005). Occurrence of aflatoxin in some of the food and feed in Nepal. Indian J. Med. Sci. 59:331-6.
Kwak MK, Egner PA, Dolan PM, Ramos-Gomez M, Groopman JD, Itoh K, Yamamoto M, Kensler TW (2001a). Role of phase 2 enzyme induction in chemoprotection by dithiolethiones. Mutat. Res.480-481:305 15.
Kwak MK, Itoh K, Yamamoto M, Sutter TR, Kensler TW (2001b). Role of transcription factor Nrf2 in the induction of hepatic phase 2 and antioxidative enzymes *in vivo* by the cancer chemoprotective agent, 3H-1,2-dimethiole-3-thione. Mol. Med. 7:135-45.
Lambert JD, Yang CS (2003). Mechanisms of cancer prevention by tea constituents. J. Nutr. 133:3262S-3267S.
Langouet S, Coles B, Morel F, Becquemont L, Beaune P, Guengerich FP, Ketterer B, Guillouzo A (1995). Inhibition of CYP1A2 and CYP3A4 by oltipraz results in reduction of aflatoxin B1 metabolism in human hepatocytes in primary culture. Cancer Res. 55:5574-9.
Langouet S, Furge LL, Kerriguy N, Nakamura K, Guillouzo A, Guengerich FP (2000). Inhibition of human cytochrome P450 enzymes by 1,2-dithiole-3-thione, oltipraz and its derivatives, and sulforaphane. Chem Res Toxicol. 13:245-52.
Lee JK, Choi EH, Lee KG, Chun HS (2005). Alleviation of aflatoxin B1-induced oxidative stress in HepG2 cells by volatile extract from Allii Fistulosi Bulbus. Life Sci. 77:2896-910.
Lee JS, Surh YJ (2005). Nrf2 as a novel molecular target for chemoprevention.Cancer Lettl. 224:171-84.
Li D, Cao Y, He L, Wang NJ, Gu JR (1993). Aberrations of p53 gene in human hepatocellular carcinoma from China. Carcinogenesis. 14:169-73.

Li FQ, Yoshizawa T, Kawamura O, Luo XY, Li YW (2001). Aflatoxins and fumonisins in corn from the high-incidence area for human hepatocellular carcinoma in Guangxi, China. J. Agric. Food Chem. 49:4122-6.

Lipigorngoson S, Limtrakul P, Suttajit M, Yoshizawa T (2003). In-house direct cELISA for determining aflatoxin B1 in Thai corn and peanuts. Food Addit. Contam. 20:838-45.

Liu P, Kimmoun E, Legrand A, Sauvanet A, Degott C, Lardeux B, Bernuau D (2002). Activation of NF-kappa B, AP-1 and STAT transcription factors is a frequent and early event in human hepatocellular carcinomas. J. Hepatol. 37:63-71.

Liu RH, Jacob JR, Hotchkiss JH, Cote PJ, Gerin JL, Tennant BC (1994). Woodchuck hepatitis virus surface antigen induces nitric oxide synthesis in hepatocytes: possible role in hepatocarcinogenesis. Carcinogenesis. 15:2875-7.

Lucito R, Schneider RJ (1992). Hepatitis B virus X protein activates transcription factor NF-kappa B without a requirement for protein kinase C. J. Virol. 66:983-91.

Lunn RM, Zhang YJ, Wang LY, Chen CJ, Lee PH, Lee CS, Tsai WY, Santella RM (1997). p53 mutations, chronic hepatitis B virus infection, and aflatoxin exposure in hepatocellular carcinoma in Taiwan. Cancer Res. 57:3471-7.

Luo H, Tang L, Tang M, Billam M, Huang T, Yu J, Wei Z, Liang Y, Wang K, Zhang ZQ, Zhang L, Sun S, Martin C, Wang JS (2005). Phase IIa chemoprevention trial of green tea polyphenols in high-riskindividuals of liver cancer: modulation of urinary excretion of green tea polyphenols and 8-hydroxydeoxyguanosine. Carcinogenesis. Jun 1;

Mace K, Aguilar F, Wang JS, Vautravers P, Gomez-Lechon M, Gonzalez FJ, Groopman J, Harris CC, Pfeifer AM (1997). Aflatoxin B1-induced DNA adduct formation and p53 mutations in CYP450-expressing human liver cell lines. Carcinogenesis 18:1291-7.

Meyer M, Caselmann WH, Schluter V, Schreck R, Hofschneider PH, Baeuerle PA (1992). Hepatitis B virus transactivator MHBst: activation of NF-kappa B, selective inhibition by antioxidants and integral membrane localization. EMBO J. 11:2991-3001.

Minami M, Daimon Y, Mori K, Takashima H, Nakajima T, Itoh Y, Okanoue T (2005). Hepatitis B virus-related insertional mutagenesis in chronic hepatitis B patients as an early drastic genetic change leading to hepatocarcinogenesis. Oncogene 24:4340-8.

Ming L, Thorgeirsson SS, Gail MH, Lu P, Harris CC, Wang N, Shao Y, Wu Z, Liu G, Wang X, Sun Z (2002). Dominant role of hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China. Hepatology 36:1214-20.

Murakami Y, Saigo K, Takashima H, Minami M, Okanoue T, Brechot C, Paterlini-Brechot P (2005). Large scaled analysis of hepatitis B virus (HBV) DNA integration in HBV related hepatocellular carcinomas Gut. 54:1162-8.

Nakachi K, Matsuyama S, Miyake S, Suganuma M, Imai K (2000). Preventive effects of drinking green tea on cancer and cardiovascular disease: epidemiological evidence for multiple targeting prevention. Biofactors 13:49-54.

Nwankwo JO, Tanhnteng JG, Emerole GO (2000). Inhibition of aflatoxin B_1 genotoxicity in human liver-derived HepG2 cells by kolaviron biflavonoids and molecular mechanisms of action. Eur. J. Cancer Prev. 9**:**351-361.

Omer RE, Bakker MI, van't Veer P, Hoogenboom RL, Polman TH, Alink GM, IdrisMO, Kadaru AM, Kok FJ (1998). Aflatoxin and liver cancer in Sudan. Nutr. Cancer 32:174-80.

Pang R, Tse E, Poon RT (2005). Molecular pathways in hepatocellular carcinoma. Cancer Lettl. Oct 16; [Epub ahead of print]

Peers F, Bosch X, Kaldor J, Linsell A, Pluijmen M (1987). Aflatoxin exposure, hepatitis B virus infection and liver cancer in Swaziland. Int. J. Cancer. 39:545-53.

Petzer JP, Navamal M, Johnson JK, Kwak MK, Kensler TW, Fishbein JC (2003). Phase 2 enzyme induction by the major metabolite of oltipraz. Chem. Res. Toxicol. 16:1463-9.

Poon RT, Fan ST, Tsang FH, Wong J (2002). Locoregional therapies for hepatocellular carcinoma: a critical review from the surgeon's perspective Ann. Surg. 235:466-86.

Puisieux A, Lim S, Groopman J, Ozturk M (1991). Selective targeting of p53 gene mutational hotspots in human cancers by etiologically defined carcinogens. Cancer Res. 51:6185-9

Qin G, Ning Y, Lotlikar PD (2000). Chemoprevention of aflatoxin B1-initiated and carbon tetrachloride-promoted hepatocarcinogenesis in the rat by green tea. Nutr. Cancer. 38:215-22.

Roebuck BD, Liu YL, Rogers AE, Groopman JD, Kensler TW (1991). Protection against aflatoxin B1-induced hepatocarcinogenesis in F344 rats by 5-(2-pyrazinyl)-4-methyl-1,2-dithiole-3-thione (oltipraz): predictive role for short-term molecular dosimetry. Cancer Res. 51:5501-6.

Ross RK, Yuan JM, Yu MC, Wogan GN, Qian GS, Tu JT, Groopman JD, Gao YT, Henderson BE (1992). Urinary aflatoxin biomarkers and risk of hepatocellular carcinoma. Lancet. 339:943-6.

Sabbioni G, Wild CP (1991). Identification of an aflatoxin G1-serum albumin adduct and its relevance to the measurement of human exposure to aflatoxins. Carcinogenesis 12:97-103.

Sales AC, Yoshizawa T (2005). Updated profile of aflatoxin and Aspergillus section Flavi contamination in rice and its byproducts from the Philippines. Food Addit. Contam. 22:429-36.

Sell S, Hunt JM, Dunsford HA, Chisari FV (1991). Synergy between hepatitis B virus expression and chemical hepatocarcinogens in transgenic mice. Cancer Res. 51:1278-85.

Sharma YP, Sumbali G (1999). Incidence of aflatoxin producing strains and aflatoxin contamination in dry fruit slices of quinces (Cydonia oblonga Mill.) from the Indian state of Jammu and Kashmir. Mycopathologia. 148:103-7.

Shen G, Xu C, Hu R, Jain MR, Nair S, Lin W, Yang CS, Chan JY, Kong AN (2005). Comparison of (-)-Epigallocatechin-3-Gallate Elicited Liver and Small Intestine Gene Expression Profiles between C57BL/6J Mice and C57BL/6J/Nrf2 (-/-) Mice. Pharm Res. 2005 Nov; 22(11):1805-20.

Shen HM, Shi CY, Shen Y, Ong CN (1996), Detection of elevated reactive oxygen species level in cultured rat hepatocytestreated with aflatoxin B1.Free Radic. Biol. Med. 21:139-46.

Shirakata Y, Kawada M, Fujiki Y, Sano H, Oda M, Yaginuma K, Kobayashi M, Koike K (1989). The X gene of hepatitis B virus induced growth stimulation and tumorigenic transformation of mouse NIH3T3 cells. Jpn J Cancer Res. Jul; 80(7):617-21.

Siame BA, Mpuchane SF, Gashe BA, Allotey J, Teffera G (1998). Occurrence of aflatoxins, fumonisin B1, and zearalenone in foods and feeds in Botswana. J. Food Prot. 61:1670-3.

Sofowora GG, Choo EF, Mayo G, Shyr Y, Wilkinson GR (2001). *In vivo* inhibition of human CYP1A2 activity by oltipraz. Cancer Chemother Pharmacol. 47:505-10.

Sporn MB, Roberts AB (1984). Role of retinoids in differentiation and carcinogenesis.J. Natl Cancer Inst. 73:1381-7.

Stern MC, Umbach DM, Yu MC, London SJ, Zhang ZQ, Taylor JA (2001). Hepatitis B, aflatoxin B(1), and p53 codon 249 mutation in hepatocellular carcinomas from Guangxi, People's Republic of China, and a meta-analysis of existing studies. Cancer Epidemiol. Biomarkers Prev. 10:617-25.

Sudakin DL (2003). Dietary aflatoxin exposure and chemoprevention of cancer: a clinical review. J. Toxicol. Clin. Toxicol. 41:195-204.

Sun CA, Wang LY, Chen CJ, Lu SN, You SL, Wang LW, Wang Q, Wu DM, Santella RM (2001). Genetic polymorphisms of glutathione S-transferases M1 and T1 associated with susceptibility to aflatoxin-related hepatocarcinogenesis among chronic hepatitis B carriers: a nested case-control study in Taiwan. Carcinogenesis 22:1289-94.

Tang ZY (2001). Hepatocellular carcinoma--cause, treatment and metastasis. World J. Gastroenterol. 7:445-54.

Terashima K, Takaya Y, Niwa M (2002). Powerful antioxidative agents based on garcinoic acid from Garcinia kola. Bioorg. Med. Chem. 10:1619-25

Turner PC, Mendy M, Whittle H, Fortuin M, Hall AJ, Wild CP (2000). Hepatitis B infection and aflatoxin biomarker levels in Gambian children. Trop. Med. Int. Health. 5:837-41

Twu JS, Lai MY, Chen DS, Robinson WS (1993). Activation of protooncogene c-jun by the X protein of hepatitis B virus. Virology 192:346-50.

Ueda H, Ullrich SJ, Gangemi JD, Kappel CA, Ngo L, Feitelson MA, Jay G (1995). Functional inactivation but not structural mutation of p53 causes liver cancer.Nat. Genet. 9:41-7.

Vargas EA, Preis RA, Castro L, Silva CM (2001). Co-occurrence of aflatoxins B1, B2, G1, G2, zearalenone and fumonisin B1 in Brazilian

corn. Food Addit. Contam. 18:981-6.
Vasanthi S, Bhat RV (1998). Mycotoxins in foods--occurrence, health & economic significance & food control measures. Indian J. Med. Res. 108:212-24.
Wang H, Dick R, Yin H, Licad-Coles E, Kroetz DL, Szklarz G, Harlow G, Halpert JR, Correia MA (1998). Structure-function relationships of human liver cytochromes P450 3A: aflatoxin B1 metabolism as a probe. Biochemistry 37:12536-45.
Wang JS, Huang T, Su J, Liang F, Wei Z, Liang Y, Luo H, Kuang SY, Qian GS, Sun G, He X, Kensler TW, Groopman JD (2001). Hepatocellular carcinoma and aflatoxin exposure in Zhuqing Village, Fusui County, People's Republic of China. Cancer Epidemiol. Biomarkers Prev. 10:143-6.
Wang JS, Qian GS, Zarba A, He X, Zhu YR, Zhang BC, Jacobson L, Gange SJ, Munoz A, Kensler TW (1996). Temporal patterns of aflatoxin-albumin adducts in hepatitis B surface antigen-positive and antigen-negative residents of Daxin, Qidong County, People's Republic of China. Cancer Epidemiol. Biomarkers Prev. 5:253-61.
Wang JS, Shen X, He X, Zhu YR, Zhang BC, Wang JB, Qian GS, Kuang SY, Zarba A, Egner PA, Jacobson LP, Munoz A, Helzlsouer KJ, Groopman JD, Kensler TW (1999). Protective alterations in phase 1 and 2 metabolism of aflatoxin B1 by oltipraz in residents of Qidong, People's Republic of China. J. Natl. Cancer Inst. 91:347-54.
Wang LY, Hatch M, Chen CJ, Levin B, You SL, Lu SN, Wu MH, Wu WP, Wang LW, Wang Q, Huang GT, Yang PM, Lee HS, Santella RM (1996). Aflatoxin exposure and risk of hepatocellular carcinoma in Taiwan. Int. J. Cancer. 67:620-5.
Waris G, Huh KW, Siddiqui A (2000). Mitochondrially associated hepatitis B virus X protein constitutively activates transcription factors STAT-3 and NF-kappa B via oxidative stress. Mol. Cell Biol. 21:7721-30.
Wattenberg LW (1985). Chemoprevention of cancer. Cancer Res. 45:1-8.
Weil R, Sirma H, Giannini C, Kremsdorf D, Bessia C, Dargemont C, Brechot C,Israel A (1999). Direct association and nuclear import of the hepatitis B virus X protein with the NF-kappaB inhibitor IkappaB alpha. Mol. Cell Biol. 19:6345-54.
Wild CP, Fortuin M, Donato F, Whittle HC, Hall AJ, Wolf CR, Montesano R (1993). Aflatoxin, liver enzymes, and hepatitis B virus infection in Gambian children.Cancer Epidemiol Biomarkers Prev. 2:555-61.
Wild CP, Hall AJ (2000). Primary prevention of hepatocellular carcinoma in developing countries. Mutat. Res. 462:381-93.
Wild CP, Turner PC (2002). The toxicology of aflatoxins as a basis for public health decisions. Mutagenesis 17:471-81.
Wild CP, Turner PC (2001). Exposure biomarkers in chemoprevention studies of liver cancer.IARC Sci Publ. 154:215-22.
Wild CP, Yin F, Turner PC, Chemin I, Chapot B, Mendy M, Whittle H, Kirk GD, Hall AJ (2000). Environmental and genetic determinants of aflatoxin-albumin adducts in the Gambia. Int. J. Cancer. 86:1-7.
Williams JH, Phillips TD, Jolly PE, Stiles JK, Jolly CM, Aggarwal D (2004). Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. Am. J. Clin. Nutr. 80:1106-22.
Xu C, Li CY, Kong AN (2005). Induction of phase I, II and III drug metabolism/transport by xenobiotics. Arch Pharm. Res. 28:249-68.
Yang CF, Liu J, Wasser S, Shen HM, Tan CE, Ong CN (2000). Inhibition of ebselen on aflatoxin B(1)-induced hepatocarcinogenesis in Fischer 344 rats. Carcinogenesis. 21:2237-43.
Yeh FS, Yu MC, Mo CC, Luo S, Tong MJ, Henderson BE (1989). Hepatitis B virus, aflatoxins, and hepatocellular carcinoma in southern Guangxi, China. Cancer Res. 49:2506-9.
Young RW, Beregi JS Jr (1980). Use of chlorophyllin in the care of geriatric patients. J Am Geriatr. Soc. 28:46-7.
Yu MW, Lien JP, Chiu YH, Santella RM, Liaw YF, Chen CJ (1997). Effect of aflatoxin metabolism and DNA adduct formation on hepatocellular carcinoma among chronic hepatitis B carriers in Taiwan. J. Hepatol. 27:320-30.