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 B₁ (AFB₁) are the two major risk factors in multifactorial 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 AFB₁ to the mutagenic AFB₁-8,9-epoxide as well as the generation of reactive oxygen species by HBV and AFB₁ sensitising the cells to AFB₁-induced p53 249Ser 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 B₁, chemoprevention.

INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for 5.5% of all cancer cases worldwide (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 B₁ (AFB₁) has been observed in both animals...
exposure to AFB metabolism of AFB acute hepatic injury (Sudakin, 2003). The liver is the primary site of biotransformation of ingested AFB occurring and also the most potent. Acute dietary predominant human CYP450 isoforms involved in human environmental condition, poor processing and lack of AFLATOXIN B1: TOXICOLOGY AND METABOLISM
Of the aflatoxins, AFB is the most prevalent, the most occurring and also the most potent. Acute dietary exposure to AFB has been implicated in epidemics of acute hepatic injury (Sudakin, 2003). The liver is the primary site of biotransformation of ingested AFB. The predominant human CYP450 isoforms involved in human metabolism of AFB are CYP 3A4 and CYP 1A2 (Figure 1). Both enzymes catalyze the biotransformation of AFB to the highly reactive exo-8,9-epoxide of AFB (Guengerich et al., 1998). CYP 1A2 is also capable of catalyzing the epoxidation of AFB to yield a high proportion of endo epoxide and hydroxylation of AFB to form aflatoxin M1 (AFM1), which is a poor substrate for epoxidation (Guengerich et al., 1998), less potent than AFB (Wild and Hall, 2000) and generally considered detoxification metabolite while CYP 3A4 can also form AFQ1 a less toxic detoxification metabolite. CYP 3A5 metabolizes AFB mainly to the exo epoxide and some AFQ1 (Wang et al., 1998). Polymorphism studies with CYP 3A5 reveal that this isoenzyme 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 to the 8,9-epoxide (Kitada et al., 1989).
Epoxidation of AFB 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 aflatoxin-N7-guanine (Guengerich, 2001). The aflatoxin-N7-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 (Johnson et al., 1997; Farombi et al., 2005 a). The AFB, 8,9 exo and endo epoxides can be conjugated with glutathione resulting in the formation of AFB-mercapturates catalyzed by glutathione S-transferase (GST) (Johnson et al., 1997). The exo and endo epoxide can also be converted non-enzymatically to AFB-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 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
significant proportion of these toxins, particularly AFB₁. 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 AFB₁ 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 AFB₁ contents of 33, 13, 65, and 7 µg/kg, respectively, and maximum AFB₁ contents of 245, 79, 480, and 160 µg/kg, respectively (Dawlatana et al., 2002). AFB₁ 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, AFB₁ 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.

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

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 Mabekoji, 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. AFB1 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 a 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 AFB1 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 AFB1 and HBV (Peers et al., 1987; Yeh et al., 1989). In animal model, interaction of AFB1 and HBV has also been demonstrated. In a transgenic mice over expressing the large envelope polypeptide of HBV and fed with AFB1 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 AFB1 and HBV came from the studies of interaction between AFB2 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 AFB1 to the reactive AFB1-8,9-epoxide. Furthermore using urinary AFB1 metabolite and aflatoxin-albumin adducts as biomarkers, studies in Shanghai, China and Taiwan showed a synergistic interaction between exposure to AFB1 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 (HbsAg) status and AFB1-albumin supporting the synergistic interaction between HBV and AFB1. 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 AFB1. GST M1 and T1 phase II detoxification genes involved in the detoxification of AFB1-8,9-epoxide has been identified. In a cohort study in Taiwan, Sun et al. (2001), found a statistically significant relationship between detectable levels of AFB1-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 AFB1-albumin adduct level and GSTT1 genotype was also significant.

The tumor suppressor gene p53 has been implicated in the synergistic interaction between AFB1 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 (249ser) is commonly found in HCC from patients in regions with dietary aflatoxin exposure. *In vitro* studies have supported this finding demonstrating that AFB1 can induce this mutation (Puisieux et al., 1991; Aguilar et al., 1993). In a study in China, all the patients with 249ser mutations showed evidence of chronic HBV infection (Ming et al., 2002). Similarly in Taiwanese patients with HCC, all the 249ser 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 249ser mutation or any type of p53 mutation.

**MOLECULAR MECHANISMS OF HCC**

**Role of transcription factor nuclear factor-kB (NF-kB) 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-kB and hepatocarcinogenesis via transcriptional regulation of genes involved in cellular transformation, proliferation, survival, invasion and metastasis has been defined by several investigations. NF-kB belongs to a family of dimeric transcription factors composed of p50 (NF-kB1) and p65 (RelA) subunit (Arsura and Cavin, 2005). In addition, c-rel, relB and p52 (NF-kB) subunits have also been identified (Baldwin, 1996). In most cells there is a preponderance of p50/p65 heterodimer NF-kB over others. In resting state, NF-kB is sequestered in the cytoplasm by the chaperon family of specific inhibitory proteins termed IxBs. Several members of the IxB regulatory family has been characterized, including IxB-α, IxB-β and IxB-γ. IxB molecules are mostly cytosolic. IxB-α has been implicated in the regulation of NF-kB 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 IxB which leads to liberation of NF-kB
Figure 2. The schematic representation of signaling cascades involving NF-κB and Nrf2. The activation of NF-κB 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-κB activation via the IκB kinase (IKK) multiprotein complex. IKK promotes the phosphorylation of IκB with further ubiquitination and subsequent degradation by 26S proteasome. NF-κB 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 dimer 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 hepatocytes 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.,
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 mitochondria 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 and 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-dependent 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 AFB1-mediated HCC**

AFB1, 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 AFB1 with HBV.

It has been proposed that HBV infection directly or indirectly may induce the specific CYP that metabolise AFB1 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 AFB1 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 AFB1 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 AFB1, 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 AFB1 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 AFB1 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 chemotheraphy for HCC. Alternative treatments such as transcatheter arterial chemoembolization, percutaneous intratumoral ethanol injec-
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 HEPATO-PROTECTIVE 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-N7-guanine adducts by enhancing the conjugation of AFB1-8,9-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 decrease 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-family 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).

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 AFB1-induced liver tumors (Lambert and Yang, 2003). Quin et al. (2000) investigated the chemoprevention of hepatocarcinogenesis by green tea in rats treated with AFB1 and CCl4 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 AFB1 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).

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 hepa-
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 EGCG and epicatechin (EC) levels, components of green tea displayed significant and dose-dependent increases in urine of individuals administrated 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 EGCG 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 an 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 biflavonoid 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, 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 phase I 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 blotting 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 scavenger 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 Cu²⁺-induced oxidation of rat serum lipoprotein in a concentration-dependent manner and elicited significant chelating effect on Fe²⁺ (Farombi and Nwokekeafor, 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 AFB₁ 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 AFB₁ 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 AFB₁ 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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