Primary prevention of hepatocellular carcinoma in developing countries

Christopher P. Wild a, *, Andrew J. Hall b

a Molecular Epidemiology Unit, Algernon Firth Building, School of Medicine, University of Leeds, Leeds LS2 9JT, UK
b Communicable Disease Epidemiology Unit, London School of Hygiene and Tropical Medicine, University of London, Keppel Street, London, UK

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Abstract

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world with 80% of cases occurring in developing countries. The cancer is rapidly fatal in almost all cases with survival generally less than 1 year from diagnosis. The major risk factors for this cancer have been identified as chronic infection with hepatitis B (HBV) and hepatitis C (HCV) viruses and dietary exposure to aflatoxins. There is a safe and effective vaccine to prevent chronic HBV infection. Given estimates that approximately 70% of HCC in developing countries is attributable to HBV then vaccination could prevent more than 250,000 cases per year in these areas of the world. A major challenge now is to ensure the availability of vaccine in countries with endemic infection. Development of a vaccine against HCV is more problematic due to the genetic heterogeneity of the virus. However, with 24% of HCC in developing countries attributable to HCV (approximately 93,000 cases per year) a vaccine would make a major contribution to cancer prevention. Aflatoxins contaminate dietary staple foods (groundnuts, maize), are potent animal hepatocarcinogens and are carcinogenic in humans with particularly high risks in individuals with a concomitant infection with HBV. 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. An alternative measure is to modulate the metabolism of aflatoxins once ingested using chemopreventive agents e.g., oltipraz. The resources available in countries with endemic hepatitis infection and fungal contamination of foods are often severely limited. Clearly HBV vaccination has to be the priority in the reducing the incidence of HCC. However, there are currently 360 million chronic HBV carriers worldwide and HBV vaccine is still not incorporated into many national immunisation programs. Thus measures to reduce food spoilage by fungi and the associated dietary exposure to aflatoxins is also a desirable public health goal. © 2000 Elsevier Science B.V. All rights reserved.

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1. General introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world with an estimated...
473,000 new cases annually. Most patients survive less than 1 year after diagnosis. Eighty percent of the cases occur in developing countries with age standardised incidence rates in males exceeding 20 per 100,000 per year in eastern Asia and sub-Saharan Africa [1]. In China alone 235,000 new cases of this tumour occur each year representing almost 50% of the world total. The cancer is more common in men with a male:female ratio exceeding three in high incidence areas. HCC accounts for almost 25% of all cancers in men in West Africa and up to 10% of all adult male mortality.

The major risk factors for HCC are chronic infection with hepatitis B virus or hepatitis C virus [2] and dietary exposures to the food contaminants, aflatoxins [3,4]. The prevalence of chronic HBV and HCV infection has been estimated based on surveys of carriage rates in different countries. HBV chronic carrier rates exceed 10% in Africa, China and Oceania (other than Australia and New Zealand) while the highest chronic HCV infection rates (>3%) are found in Africa and Japan. Based on these prevalence rates and a relative risk of 20 associated with either chronic infection, Parkin et al. have estimated that 52% and 25% of the world total of HCC are associated with HBV and HCV infection, respectively [5]. Some of these cases may be the result of joint infections although few studies to date have been large enough to evaluate the HCC risk in people with combined infections because of the rarity of this combination in control subjects.

Aflatoxin exposure is also prevalent in developing countries [6] and often the regions of highest exposure are the same as those with high HBV infection rates. The risk of HCC associated with ingestion of aflatoxin is most elevated in individuals with concomitant HBV infection [7]. The mechanisms of interaction between these two risk factors are still being elucidated and this information will contribute to the overall rationale for prevention of HCC [6,8]. To date there are no data on interactions between HCV infection and aflatoxin exposure.

The route to control of hepatitis infection is vaccination and this is discussed here for both HBV and HCV. The first results demonstrating an effect of HBV vaccination of children on HCC incidence have already been reported from Taiwan [9]. There are currently 170 million carriers of HCV in the world but availability of a vaccine is still some way from development. Reduction of aflatoxin exposure is more complex and difficult to achieve in principle. However, given that there are currently 360 million HBV carriers worldwide, for whom the HBV vaccine will not offer protection then a large population will continue to be at increased risk of HCC due to aflatoxin. A variety of approaches to reducing exposure are presented here. We conclude by examining the priorities for HCC prevention in countries which have limited resources and infrastructure for cancer prevention in general.

2. Hepatitis B virus

2.1. Introduction

The association between HBV and HCC was first recognised because of the geographical relationship between persistent infection with the virus and the incidence of the tumour [2]. Subsequent studies, both case-control and cohort, confirmed the relationship in virtually all societies in which studies were undertaken. The single exception is Greenland where chronic HBV is common and HCC is rare. This may provide clues to effective means of prevention for carriers of the virus and deserves further investigation although at present there is no explanation for this observation. The answer is not simply some feature of Eskimo culture since the usual strong association has been observed amongst Eskimos in Alaska. The association between HBV and HCC has been of particular interest since it represented one of the earliest known viral cancers and the first cancer to be preventable by vaccination [2].

2.2. Hepatitis B “carriage”

The presence of the hepatitis B surface antigen in the bloodstream for a period of 6 months or more is the standard definition of “carriage”. It is this marker that has been shown to be associated with an approximately 50 fold increased risk of HCC. The antigen is produced by replication of the virus in the hepatocyte, and is produced during active replication in great excess. Ironically it is this great excess that was first utilised as a vaccine. Heat inactivated plasma
from carriers was found to be highly effective at preventing infection. However, the major objective of vaccination varies geographically. In low-incidence countries the target is acute hepatitis B, which is primarily a sexually transmitted infection of adolescents and young adults. In contrast in areas of high incidence of HCC it is carriage of the virus that is the target. The major determinant of carriage is age at infection with the virus [10]. Infection around the time of birth from an infectious mother (usually a carrier of the virus) results in infection of 90% or more of the infants. In around 90% of these children this infection results in carriage of the virus for many years. The mechanism of persistence of infection in this situation appears to be passage of hepatitis B antigen from the mother across the placenta in utero resulting in immunological tolerance of the virus by the infant.

During the first 5 years of life there is also an elevated risk of carriage, at around 25%, if the child is infected. This is thought to result from the immaturity of the immune system. This elevated risk is particularly important in areas such as sub-Saharan Africa where this is the principle age of infection with the virus [11]. The precise mechanism of transmission is not understood but is related to domestic crowding of young children and may involve either open skin sores or salivary exchange.

In contrast, past the age of 10 years the risk of becoming a carrier is only around 5% and may be considerably lower. Here the likely explanation of carriage is genetic susceptibility and the major routes of transmission are by sexual intercourse and contaminated needles.

2.3. Prevention

The way in which vaccine can be used to prevent the carrier state is defined by this close relationship between age at infection and carriage. It is clear that vaccine must be delivered as early in life as possible. Fortunately, if the vaccine is delivered within 48 h of birth in combination with hyperimmune globulin, carriage is reduced by more than 90% even from highly infectious carrier mothers. Vaccine alone in this situation reduces carriage by at least 70%. Some studies of recombinant vaccine suggest that this may reduce carriage by as much as 90% but the trials did not use concurrent controls (for obvious ethical reasons) and therefore may not be quantitatively accurate. Since hyperimmune globulin is expensive to manufacture the current public health programmes in Asia and Africa use vaccine alone. In China this has resulted in vaccine effectiveness of around 70% in some areas and as much as 90% in others [82]. This is despite the fact that some 40% of carriers in China result from perinatal transmission from the mother.

In Africa, introduction of the vaccine into the routine infant vaccination programme reduced carriage rates by 94% [12]. In the Gambia Hepatitis Intervention Study the most recent estimate shows that this level of protection is maintained up to the age of 9 years [13]. Since this is well past the ages at which the risk of becoming a carrier is high these children effectively now have life long protection against hepatitis B associated liver cancer.

This protection has recently been established in Taiwan where the temporal association between introduction of universal HBV vaccination and a decline in childhood liver cancer strongly suggests a direct causal effect [9].

2.4. Economics and size of effect

Estimates of the cost effectiveness of universal vaccination are highly dependent on both the local epidemiology and health care costs; the total cost of prevention includes the additional storage and delivery costs of incorporating the vaccine into existing immunisation programmes. In sub-Saharan Africa the current cost of vaccine for one individual, comprising three doses of vaccine, is approximately 2 US$ and therefore estimates indicate that prevention of a case of HCC will cost about 100 US$ [14]. This estimate assumes no discounting because the life gained is 20 to 40 years in the future. If discounting is applied, taking into account among other parameters future currency devaluations, the estimate rises some seven-fold. Even at this discounted price the effectiveness is comparable to that of a clean water supply in preventing a diarrhoeal death. If discounting is not used then this is one of the most cost-effective interventions available to endemic countries against any disease.

In Asia and Africa the major cause of HCC under the age of 50 years is HBV infection. HCV then
becomes of increasing importance with age. This reflects the differing ages at infection — HBV is a childhood infection and HCV is an adult infection — and the subsequent incubation periods to cancer of 20 to 30 years. Since across all ages HBV accounts for at least 70% of HCC the vaccine should prevent in excess of 60% of cases of this cancer. However, in those younger than 50 years this may be as high as 90%. Since this younger age group are the most economically active and have a large number of dependants the vaccine is likely to be highly cost beneficial as well as cost effective.

3. Hepatitis C virus

3.1. Introduction

HCV was only recognised in 1989. The use of sero-epidemiology since then has made it clear that this is second only to HBV as a cause of HCC. However, the epidemiological pattern of infection is as different from HBV as is the virus. This is an RNA virus that shows a high mutation rate within individuals and a wide geographical and temporal variation in genotype. This makes the development of a vaccine considerably more difficult than was the case for HBV. Unlike HBV the virus does not integrate into the host genome.

3.2. Epidemiology and carriage

As with HBV infection, persistent viral replication is the major risk factor for cancer with most cases occurring in the presence of cirrhosis. However in contrast to HBV, carriage is a much more common outcome of infection with probably around 80% of all infections resulting in carriage [15]. The determinants of this are not clear. Direct transmission by blood contamination, usually through a needle, is by far the most important mode of transmission [16]. Thus intravenous drug users have very high prevalences of infection — in excess of 80% in many countries. Geographical variation in prevalence is limited with most countries having rates around 1% although Japan, Spain and Italy have somewhat higher rates. Egypt is exceptional with prevalences as high as 20% in the general population. This is almost certainly due to transmission of the virus in the mass injection treatment of schistosomiasis in the past. Sexual transmission is possible but appears to be rare. Perinatal infection is also uncommon except where the mother has a dual infection with HIV. This pattern of transmission results in a predominantly adolescent and young adult age at infection with the subsequent cancer occurring in the middle aged and elderly [2].

3.3. Prevention

A vaccine against this virus is likely to be some way off. The virus exhibits a marked genetic heterogeneity and patients can be infected with a mix of genomes which can also vary over time making vaccine development problematic. Current approaches include development of vaccines against recombinant envelope proteins and the core protein. Although vaccine development is therefore a target for the future, screening for HCV in the blood transfusion service has already dramatically reduced infection rates where it has been introduced. The emphasis placed on clean, safe needle use by the HIV control programme has probably played an important role in reducing infection although this is difficult to quantify. WHO is about to launch a global programme for safe injections of all kinds that, if successful, will reduce HCV, HIV and HBV infections.

4. Aflatoxins

4.1. Introduction

4.1.1. Occurrence

Fungi can form an enjoyable part of the human diet in many societies. However, undesirable fungal contamination of foods also occurs and one consequence is human exposure to a broad class of toxic fungal metabolites, termed mycotoxins [17,18]. It has been estimated that mycotoxins contaminate up to 25% of the world’s food supply [19] and it is known that these toxins induce a range of both acute and chronic disease in humans [20]. One potent group of mycotoxins are the aflatoxins consisting of aflatoxin B1 (AFB1), G1, B2 and G2, which are secondary
metabolites of Aspergillus flavus and A. parasiticus. Aflatoxin contamination of foods occurs predominantly, although not exclusively, in developing countries with hot, humid climates and is found on a variety of oilseeds and cereal crops [17]. Exceptionally high levels (several hundred to thousands of ppb) can occur in groundnuts and maize, which are dietary staples in parts of the developing world. These levels exceed the regulatory limits for contamination of foods in developed countries [21] (which are set at 20 ppb or lower) by one to two orders of magnitude.

4.1.2. Human toxicity

Aflatoxins are among the most potent naturally occurring hepatocarcinogens known and induce tumours inprimates, rats, mice, hamsters, fish, toads, birds, etc. [3,4]. Epidemiological and other mechanistic data have established that aflatoxins are human hepatocarcinogens [3,4]. There appears to be a synergistic interaction between HBV and aflatoxin in HCC risk [7,22]. This hypothesis is supported by observations in animals infected with hepatitis viruses (see Refs. [6,8]). Aflatoxins are also hepatotoxic and have been associated with some outbreaks of acute toxicity in exposed populations [3]. Finally, aflatoxins are immunosuppressive in animals resulting, for example, in an increased susceptibility to infections (viral, bacterial) and a reduced response to vaccines [23]. The most consistent effects appear to be on the cell-mediated immune response [24]. The impact of aflatoxins on the immune system of exposed people is unknown. However, given the morbidity and mortality from infections in developing countries, if exposure does modulate susceptibility to infectious agents then the public health relevance of aflatoxins would be hard to ignore.

4.1.3. Biomarkers of exposure

It is difficult to accurately assess aflatoxin exposure at the individual level either by asking questions about consumption of foods suspected to be major sources of exposure or by direct analysis of foods. This is because diets can be extremely monotonous in composition and because obtaining representative food samples for analysis is problematic due to the heterogeneous nature of aflatoxin contamination. The earlier identification of aflatoxin metabolites in human body fluids [25] spurred efforts in the early 1980s to develop biomarkers of human exposure to these toxins [26]. The availability of specific antibodies formed the basis of immunoassay approaches for the detection of aflatoxin metabolites in human urine samples [27–30]. In order to integrate exposure over a longer time period the binding of aflatoxins to peripheral blood albumin was investigated in rodents [31,32] and was later demonstrated in exposed people [33–35]. These approaches have now been applied in many populations worldwide and have revealed an extent of exposure, both in duration and level, unexpected from food analyses alone [6,36]. The use of biomarkers permitted the prospective studies in Shanghai and Taiwan, which revealed increased risks of HCC in people exposed to both HBV and aflatoxin [7,22,37]. Of interest was the observation that conventional attempts to assess aflatoxin exposure by dietary questionnaire in one of these studies failed to reveal an association between aflatoxin exposure and HCC risk [7].

A further achievement in terms of biomarkers for aflatoxins was the identification of a specific mutation in the p53 tumour suppressor gene in HCC from regions of the world with high aflatoxin exposure [38,39]. These data were recently summarised in relation to the levels of aflatoxin-albumin adducts in people from these same areas [6]. An exciting development by Kirk et al. [40] has been the detection of the same mutation in plasma DNA in The Gambia collected from cases of HCC, an alteration infrequent in control subjects.

While the above biomarkers are valuable in assessing human exposure and in studying the link between that exposure and disease they also have potential as outcome measurements in intervention studies designed to reduce aflatoxin exposure [41]. This has been demonstrated in a rodent model where intervention with olitipraz reduced both aflatoxin-albumin adducts and liver tumour burden [42]. In the future as efforts are made to find the most effective approaches to reducing exposure these biomarkers, which reflect important events in the pathogenesis of the disease, will be useful intermediate endpoints for study of the efficacy of intervention procedures.

4.2. Prevention of aflatoxin-related disease

Interventions to reduce aflatoxin-related disease can be considered in terms of those which are appli-
The above approaches are considered briefly below. The purpose of this review is to focus on foods for human consumption and particularly measures applicable in rural communities of developing countries where there is a high level of subsistence farming. Industrial scale processes to control aflatoxin levels in commercial crops are not considered here.

**4.2.1. Community level intervention**

**4.2.1.1. Pre-harvest crop management.** Pre-harvest would be the most effective point of control because this is the point at which the crop is infected by the fungus. The infestation of crops by *Aspergillus* most readily occurs under conditions of stress involving drought, high temperatures, insect induced injury or other processes which lead to damage of the crop [43]. For example, in the case of groundnuts drought stress in the 4 to 6 weeks prior to harvest is reported to lead to a decrease in moisture content and an increase in soil temperature which permits the *A. flavus* in the soil to infect the groundnut kernels [44]. A number of cultural practices pre-harvest may limit fungal infection and aflatoxin contamination [45]. Irrigation can be an effective measure but in sub-Saharan Africa is often unavailable or not cost-effective. Similarly, fungicides and pesticides may not be an attractive option given the limited cost-effectiveness for subsistence farmers and the limited success in their application. As insect damage and wounding is often correlated with aflatoxin contamination of, for example maize, insecticides could also be consid-
er entered. However, in some instances fungicides and pesticides may actually result in higher levels of mycotoxin contamination and this should be a parameter considered in assessing the effectiveness of these compounds [46] in addition to the economic and environmental acceptability.

One method suggested to control aflatoxin contamination is the introduction of non-aflatoxigenic strains of *Aspergillus* to compete with the aflatoxin producing strains [47–49]. For example, the application of non-aflatoxigenic strains in maize plots led to reduced aflatoxin levels in years when weather conditions favoured contamination [49,50]. It was noted that different *Aspergillus* strains may be important for airborne crops such as maize and soilborne crops such as groundnuts. Inoculation of non-aflatoxigenic strains of *A. flavus* and *A. parasiticus* into soil in which peanuts were grown showed reductions in aflatoxin contamination between 74.3% and 99.9% [51]. The use of these strains would need careful validation in the environment for which they are intended and there is concern that the strains could revert to aflatoxin producers by anastomosis from toxigenic strains in the field. It worth noting that certain *Aspergillus* strains which are nonaflatoxicogenic can produce other toxins, e.g., cyclopiazonic acid the production of which is not always correlated with that of the aflatoxins themselves [52]. Finally, there is also the problem of food spoilage by the fungus which would not be solved by non-aflatoxicogenic strains.

Genetic engineering may offer novel ways of tackling the problems of pre-harvest contamination by mycotoxins. As has been argued recently, the use of biotechnology to counter famine and poverty in Africa is a valid one as long as potential problems of exploitation and adverse environmental impact are countered [53]. The author cited research underway to develop maize resistant to maize streak virus. A feature of this work is that it is performed in Africa with field trials conducted locally to test safety and efficiency in tropical conditions. Given the widespread fungal and mycotoxin contamination of maize and groundnuts a similar approach to address these latter problems has great potential.

Cloning of the genes involved in the biosynthesis of aflatoxins represents a pathway to engineering non-aflatoxicogenic strains of *Aspergillus* [54]. In addition, the genes coding for enzymes, which influence the ability of the aflatoxigenic fungus to colonize the host plant, are also being identified and could be used to create effective biocompetitive, non-aflatoxicogenic strains [55].

Rather than focus on the *Aspergillus*, an alternative approach has been to select for varieties of cereal grains and oilseeds resistant to aflatoxin. For maize and groundnuts there is known variation in resistance among genotypes [50,56]. Resistance in this sense may involve plant resistance to fungal infection or limitation of aflatoxin biosynthesis once infection has occurred. Initial efforts went into identifying naturally resistant crop varieties but only partial resistance to aflatoxin contamination is observed (see Ref. [57]). Nevertheless, these studies provided insights into understanding both the factors, which make the plant resistant to infection and the biosynthetic pathway for aflatoxins, thus providing a basis to potentially interfere with these processes by genetic engineering of resistant plants [50,55,58]. Some plants, e.g., groundnuts, produce antifungal compounds such as phytoalexins which can provide protection against the invading fungi (see Ref. [44]). Recently, kernel proteins of maize which contribute to resistance to aflatoxin production have been identified having an ability either to inhibit fungal growth per se or toxin formation [59–61]. These observations may permit identification of the genes involved and offer opportunities to insert these genes into the crop to provide resistant genotypes. The strategies may involve cloning and amplifying host plant antifungal genes or incorporation of new non-host inhibitor genes into those plants (see Ref. [54] for a review).

4.2.1.2. Post-harvest crop management. Aflatoxins can accumulate during food storage, particularly under hot, humid conditions where there is additional risk of rodent and insect damage. Food spoilage and contamination with mycotoxins can be significant problems in circumstances of traditional storage at the local farm level [44,62]. Considerable effort has been put into removing aflatoxins from foods post-harvest by (1) physical methods such as thermal inactivation, irradiation, sorting etc., (2) chemical means, including solvent extraction, (3) adsorption for example using minerals, e.g., phyllosilicate clays,

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A variety of chemical degradations (e.g., acids, alkalis, aldehydes, oxidising agents, ammoniation and sodium bisulphite) and (5) by biological decontamination [63,64]. The majority of these methods are aimed at commercial crops and are appropriate only for feeds as they alter the organoleptic properties of the foods making them unfit for human consumption.

The current review focuses only on methods which could be applicable at the local farm level and these ideally comprise a package of measures designed to inhibit further fungal growth and aflatoxin production in storage. The growth of Aspergillus post-harvest is influenced most critically by temperature, moisture content and storage time; at the level of subsistence farmers groundnuts may be stored upwards of 6 months and maize for longer periods. Insect infestation can increase both moisture content and temperature, act to spread spores more widely in the crop and cause physical damage which further promotes fungal infection [65]. The main approaches to contain contamination levels are those concerned with management of the crop in the field immediately after harvest, improved drying and storage to limit moisture content, and the possible use of pesticides and biological pest control. Many of the points discussed below have been highlighted more generally in relation to reducing losses of crops at the small farm level [62].

Extended time of leaving the harvested crop in the field prior to storage increases the risk of unseasonal rain damage and promotion of fungal growth prior to storage. If the crop is left in the field for some time then it should be raised on platforms rather than left on the ground. In the case of groundnuts stacking should be with the pods exposed to the air to improve ventilation.

Manual sorting of obviously damaged kernels or pods can reduce the quantity of aflatoxins [66]. Although there is not a direct relationship between aflatoxin level and visible fungal contamination of groundnuts there is a substantial reduction in contamination achieved by this sorting approach. Sun drying is most widely used method prior to storage and this can be effective in reducing moisture content and may lead to destruction of a proportion of the aflatoxins present [67]. In the case of groundnuts sun drying should be performed on a cloth rather than directly on the earth to avoid humidity from the ground and to permit rapid gathering in case of unseasonal rainfall.

Once in storage it is advisable to keep the crop from contact with the earth either by raising on wooden pallets or a concrete floor and ensuring adequate ventilation in the storage facility to prevent an increase in moisture content. Measures to control insect and rodent damage should also be employed.

4.2.2. Intervention at the individual level

4.2.2.1. Dietary change. The majority of aflatoxin in high exposure countries is from groundnuts and maize where these form the dietary staples of the populations concerned. One option therefore is to avoid consuming these foods so frequently by developing a more varied diet. It is reported that through mass health education programmes in parts of the People’s Republic of China (Qidong, Haimen and Fusui) individuals have changed from a maize-based to a rice-based diet to reduce aflatoxin exposure even though rice is a more expensive commodity [68]. Rice in these areas has been shown to be less frequently contaminated with aflatoxins than has maize [68] and interestingly we observed some years ago that aflatoxin–albumin adduct levels were lower in villages in Fusui having a rice compared to maize-based diet (Wild C.P., Chen J. and Montesano R, unpublished data). However, for many communities in developing countries a change in diet is simply not feasible. Cooking processes can reduce aflatoxins to a limited extent but these effects are variable and can produce other toxic metabolites (see Ref. [64]). It would be of interest to investigate in more detail the effects of traditional food preparation techniques on aflatoxin levels [69]. Nevertheless, some aflatoxin will be ingested even in situations where community level interventions are implemented.

4.2.2.2. Chemoprevention. The principle of chemoprevention is to limit the carcinogenic process subsequent to exposure such that cancer incidence is reduced. Since the discovery of aflatoxins in the early 1960s there has been tremendous progress in understanding the toxicology of these compounds [70] and this has provided a rationale both for developing biomarkers of exposure to aflatoxins and for modulating their metabolism in vivo. These two
areas of research have been integrated to permit the evaluation of chemopreventive strategies in humans exposed to dietary aflatoxins by examining the impact on aflatoxin biomarkers [41].

Different animals species show a marked interspecies sensitivity to aflatoxin–DNA and –protein adduct formation [71] and susceptibility to aflatoxin carcinogenesis [4,72]. Induction of glutathione-S-transferases (GST) and aflatoxin aldehyde reductase reduces aflatoxin-DNA and -protein adduct formation and blocks aflatoxin carcinogenicity in rats [73–75]. Therefore, a similar modulation of the balance between aflatoxin activation and detoxification in humans has been sought and the drug, which has been used, oltipraz, is one originally prescribed to treat schistosomiasis [41].

Kensler et al. [76–78] have demonstrated that when oltipraz is administered to Chinese people exposed environmentally to aflatoxin there is an increase in the level of GST conjugation of aflatoxin 8,9-epoxide but also an inhibition of cytochrome P450 1A2 activity which activates aflatoxin to this reactive epoxide. These effects were demonstrated by modulation of urinary AFM1 (a product of CYP1A2 metabolism of AFB1), aflatoxin–albumin adducts in peripheral blood and urinary aflatoxin-mercapturic acid in subjects receiving the drug under different treatment protocols. As the effects on enzyme induction are more prolonged than the half-life of the drug itself this has meant that transient, intermittent administration can affect both metabolite and adduct profile. Currently, a Phase 2b clinical trial with an intervention over 1 year administering 250 or 500 mg oltipraz weekly is underway in Qidong, People’s Republic of China [41].

The Phase 2b trial will permit selection of a safe and effective dose of oltipraz for a Phase III trial, specifically including an evaluation as to whether the minor side effects seen in the 8-week intervention of the Phase 2 trial are any more marked with long-term intervention. Specifically the adverse side effects included a syndrome involving numbness, tingling and sometimes pain, in the extremities [79]. The phase III trial should evaluate the chemopreventive action of oltipraz against aflatoxin carcinogenesis and this would normally require disease incidence as an outcome. Unless aflatoxin exerts a hepatocarcinogenic effect late in the natural history of the disease then a long follow-up would be required to see an effect on liver cancer. Alternatively if any of the biomarkers are demonstrated to be strong predictors of cancer risk then these could be used as surrogate measures of disease outcome. It is unlikely that the transient aflatoxin adducts (DNA or albumin) will fulfill this requirement at the individual level; this is suggested indirectly in rats where a correlation between adducts and liver cancer occurred at the group but not individual level [42]. There is some hope that the specific p53 codon 249 mutation may be more predictive of individual risk. In this respect the recent identification of this mutation in the plasma of Gambians with liver cancer or cirrhosis is encouraging [40].

Other enzyme inducers are likely to become available in the next few years and these may be more potent than oltipraz [41]. However, alternative rationales to chemoprevention may also be explored. The addition of mineral adsorbents (e.g., aluminosilicate clays) in animal feeds to bind aflatoxins and reduce uptake into the blood stream from the gastrointestinal (GI) tract is used in the USA and southeast Asia [64]. One such clay, hydrated sodium calcium aluminosilicate, binds to aflatoxins in the GI tract reducing bioavailability and toxicity [80]. Whether this type of compound will have the requisite lack of toxicological and nutritional effects to permit application to humans is still an open question. There may be other parallel approaches to prevent absorption of aflatoxins from the GI tract. For example, natural products such as chlorophyllin may be used to absorb aflatoxins and reduce the amount of toxin reaching the liver [81]. The utilisation of these compounds in humans would require careful evaluation including a consideration of the possible removal of essential nutrients from the diet.

5. Conclusions

The major causes of HCC, one of the most common cancers in the world, have been identified. HBV accounts for 70% of cases in developing countries and therefore the fact that there is now a safe and effective vaccine should result in a major reduction in the incidence of this tumour. The current challenge is to ensure the inclusion of the vaccine in national immunisation programmes and this must be
the first priority in prevention of HCC. HCV is associated with 24% of HCC in developing countries. Here development of a vaccine is more challenging and is probably still some years from introduction. However, as the principal modes of transmission are understood there is an opportunity to significantly reduce the prevalence of this infection. If aflatoxin poses it’s greatest carcinogenic risk in HBV carriers then HBV vaccination will also eventually contribute to reducing the risks associated with exposure to this dietary contaminant. However, the current large numbers of chronic HBV carriers and the incomplete access to the vaccine, for example only 1% of children in Africa currently have access, means that concurrent attempts to reduce aflatoxin exposure are also important. In this respect low technology efforts to reduce fungal contamination as well as the genetic engineering of resistant crops should be evaluated. As with the chemopreventive agent oltipraz, biomarkers of aflatoxin exposure will prove useful in evaluating the effectiveness of these approaches to reduce exposure.

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