A Narrative Review on Etiology and Mechanism of Aflatoxin B1 in the induction of Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma is one of the primary liver cancer. Even though the incidence of HCC differs among various geographic regions, ethnicity, gender and age, it is one of the most common malignancies among Asian and African populations. It is well represented as the fourth most common cancer in the world. It is estimated that more than 80% of HCC cases are attributed due to viruses including hepatitis B and C infections. However it is also quite interesting that consumption of hazardous alcohol, dietary exposure of aflatoxin and hemochromatosis are also highly associated with the initiation and development of HCC. Risk factors such as AFB1 could alter the DNA upon exposure, modify the proteins and induce oxidative stress and further leading to malignant transformation of the hepatocytes. In this review we narrated the basics of the etiology and molecular mechanisms behind the involvement of AFB1 in the initiation and development of hepatocellular carcinoma.

Keywords: Aflatoxin B1; Hepatocellular Carcinoma; AFB1-macromolecular adducts; AFB1-8, 9-epoxide.

INTRODUCTION

Cancer is one of the most dreaded diseases of the 20th century and spreading further with continuance and increasing incidence in 21st century. In world, cancer is the second most common cause for death of human beings, next to cardiac failure. In spite of several attempts to cure cancer, it is difficult to avoid patient dying. Destruction of cells due to cancer can occur at different parts of a body. Based on this there are different types of cancer namely, lung cancer, colon cancer of esophagus, breast cancer, liver cancer, etc. The frequency of different kinds of cancer varies enormously in different human populations. The incidence of breast and colon cancer is much higher in the United States than in Japan. The liver cancer rate is highest in third world countries and in these cases, diet is most likely the culprit.

Cancers most commonly associated with diet include esophagus, stomach, colon, liver and the prostate. Overall, it is clear that much of the variation in cancer incidence is environmental rather than genetic. The conviction is growing that the carcinogenic agents in the environment are active as cancer causing agents because they produce mutations. The hunt for carcinogens has been facilitated by Bruce Ames, who developed a test for carcinogens based on the mutagenic action of a compound on bacteria. Ames tests have shown that many carcinogens originate from food and chemical pollutants. Dietary factors continue to play a complex and multifactorial role in the etiology of cancer.

1.1. Etiology

Cancer is ultimately the result of interplay between environmental (exogenous) and genetic (host) factors. It is caused generally by the agents external to the body, though it is difficult to determine the exact nature of the agent.
Statistically it has been shown that 80% of human cancers are caused by environmental factors (1). These factors principally can be divided into three main broad groups such as,

a. **Physical** : Ionizing and non-ionizing radiations.

b. **Biological** : DNA/RNA containing oncogenic viruses.

c. **Chemical** : Variety of chemical compounds, viz.

- **Life style** : Cigarette smoking, tobacco chewing.
- **Dietary** : Groundnuts and other foodstuffs infected with fungus like Aspergillus flavus produce Aflatoxin B1 (AFB1), Ochratoxin A (OTA) and Fumonisins produced by Fusarium verticilliodes.

- **Occupational** : Asbestos, benzene, naphthylamines, beryllium, etc.

- **Iatrogenic** : Certain therapeutic drugs

Under the variety of chemicals, aflatoxin B1 is mainly responsible in causing liver cancer. It comes under one of the classes of chemical liver carcinogens, namely naturally occurring compounds. In liver cancer, hepatocellular carcinoma (HCC) is a primary stage of carcinomatous changes occurring in the liver cells.

1.1.1. **Mechanism of carcinogenesis**

Generally cancer development has been considered to consist three major steps namely, initiation, promotion and progression (Figure 1.1)

**Fig. 1.1. An outline Mechanism of Carcinogenesis**

Initiation is an irreversible process, starts when normal cells are exposed to carcinogenic substances and their DNA undergo damage that remains unrepaired or misread. In chemical carcinogenesis, initiation involves the uptake of a given carcinogen, which is subsequently distributed to organs for metabolism. Metabolic activation leads to reactive (electrophilic) species, which can bind to DNA rather than excretory carrier molecules.
The binding can then cause coding errors at the time of replication leading to mutation. The somatic mutation in a damaged cell can then be reproduced during mitosis to produce clones of mutated cells. The next stage in the carcinogenesis process is promotion, i.e., the expansion of the damaged cells to form an actively proliferating multi-cellular premalignant tumour cell population. The last stage known as progression, which produces new clones of tumour cells with increased proliferative capacity, invasiveness and metastasis (Plate 1).

1.1.2. Classification of primary liver tumours

There are three major types of Primary Liver Tumours. Table 1.1 represents the WHO classification of Primary HCC (2).

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Type</th>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Epithelial tumours</td>
<td>Liver cell adenoma, Bile duct adenoma and Bile duct cyst adenoma.</td>
<td>HCC (Liver cell) Cholangiocarcinoma, Bile duct cyst adenocarcinoma and Hepatoblastomas.</td>
</tr>
<tr>
<td>3</td>
<td>Tumor-like lesions</td>
<td>Cysts, Mesenchymal, Hemertona, Focal nodular, Hyperplasia, Peliosis, Teratoma.</td>
<td></td>
</tr>
</tbody>
</table>

1.1.3. Epidemiology of hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is one of the most common malignancies in Asian (3) and African populations (4,5) and may well represented as the fourth most common cancer in the world (6). National Cancer Office of the Ministry of Public Health (NCOMPH), P.R.C. (7) reported that, this malignancy is the third leading cause of cancer mortality in males, following cancer of the esophagus and stomach. HCC is a major problem not only in developed countries but also in most of the developing countries, where toxic industrial chemicals, air and water pollutants are sources of hepatocarcinogenicity in the former, food additives and fungal toxins are the major sources in the latter. Since liver is the major site in the body that metabolizes ingested materials, it is more susceptible to carcinogenic insult.

Cirrhosis is also a risk factor for HCC. The annual risk of developing HCC among persons with cirrhosis is between 1% and 6% (8). Cirrhosis of the liver has long been implicated in the development of primary liver carcinoma (9). It has been postulated that regenerative nodules in post-necrotic cirrhosis may be the precursor of liver cancer. Shih-Chien Sun et al., (10) reported that regenerative liver cells in cirrhotic nodules are more susceptible to environmental carcinogens such as aflatoxins.

1.1.4. Causative factors

Chronic liver diseases

A variety of metabolic, alcoholic, viral and idiopathic chronic liver diseases can lead to HCC. Hemochromatosis has the highest risk of malignant diseases/degeneration (11).

Infectious hepatitis
Chronic infection by Hepatitis B virus (HBV) is associated with high incidence of HCC. Among HBV gene product, Hepatitis B virus protein (HBx) is likely to play a causative role in the development of HCC. Höhne et al., 1990, showed that introduction of HBx gene into rodent cells causes cellular transformation (12). Kim et al., (1991) confirmed that HCC with the transformation of HBx gene in transgenic mice(13). Haviv et al., (1998) showed that HBx directly interacts with transcription machinery such as RNA Polymerase (14), TATA binding Protein and Transcription Factor II B (TFIIB) and other cellular proteins such as P53, a tumour suppressor protein. The pleiotropic activity of HBx may contribute to the modulation of gene expression, which finally leads to the formation of liver cancer (15).

A wide variety of synthetic and naturally occurring organic compounds identified as constituents of human foodstuffs, drugs and inorganic chemicals has been proven for their mutagenic/carcinogenic effects. Table 1.2 shows the list of organic and inorganic compounds that have been proven for their mutagenic / carcinogenic potential.

Chemical liver carcinogens

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Class</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Polycyclic Aromatic Hydrocarbons</td>
<td>Benzo(a)pyrene, Dimethyl benzanthracene</td>
</tr>
<tr>
<td>2</td>
<td>Azo Dyes (Aromatic Amines)</td>
<td>β-naphthylamine, N-methyl-4-amino azo benzene, 2-acetyl amino fluorine.</td>
</tr>
<tr>
<td>3</td>
<td>Nitrosamines and Amides</td>
<td>Dimethyl nitrosamine, Diethyl nitrosamine.</td>
</tr>
<tr>
<td>4</td>
<td>Naturally occurring compounds</td>
<td>Aflatoxin B1 (Food mycotoxin).</td>
</tr>
<tr>
<td>5</td>
<td>Various drugs and Miscellaneous agents</td>
<td>Cyclophosphamide, Bisulphan diethyl stilbesterol, Estrogen, Nitrogen mustard, β-propiolactone, Beryllium, Cadmium, Nickel, Chromium, Arsenic, Vinyl chloride, Asbestos, Saccharine, and Cyclamates.</td>
</tr>
</tbody>
</table>

The discovery of aflatoxins, a principal member of the mycotoxin family in the early 1960s has not been implicated in any health problems in animals or people, but the occurrence in the food supply cause concern, because many are potent toxic agents under experimental conditions. Groupman et al., (1995), reviewed that, since the discovery of aflatoxins 30 years ago, many epidemiological studies have been conducted to explore the link of aflatoxins with liver cancer (16). Most of these investigations have suffered from a lack of good data on aflatoxin exposure and/or poor information on cancer incidence. Recently Aflatoxin B1 (AFB1) has been classified as a known human carcinogen by the International Agency for Research on Cancer (17). After the epidemiological and biomarker studies that were used to relate dietary aflatoxin exposure with human cancer, it was confirmed as a group I human carcinogen (17).

1.2. Aflatoxins

Aflatoxins are a group of potent hepatocarcinogens produced as a secondary metabolite by the moulds Aspergillus flavus and Aspergillus parasiticus. These fungal moulds are widely encountered as a contaminant of cereal crops and nuts in humid areas of the world including India.

1.2.1. Epidemiology of Aflatoxin contamination

Aspergillus flavus contaminated regions include Eastern Asia and Sub-Saharan Africa, though occasionally AFB1 has been found to contaminate maize crops in the USA and barley crops in UK (18,19). The fact that hepatocellular carcinoma is a
leading cause of mortality in regions of the world where environmental levels of AFB1 are high and it has provided strong circumstantial evidence that this mycotoxin is a principal etiological factor in human liver cancer in Asia and Africa (20-22).

1.2.2. Tolerance limit

Action level and tolerance represent limits below which food/feeds contaminated with a toxin is allowed to be marketed by United States Food and Dairy Administration (USFDA) and by other countries. The recommendation made by USFDA as allowable aflatoxin level in animals feed is given in table 1.3.

It has been shown that AFB1 exposure occurs through the consumption of mold-contaminated groundnuts, grains, and animal feed (23). The aflatoxin tolerance varies with age, sex and health of the animal. Younger animals are most susceptible to aflatoxin poisoning. Pregnant and growing animals have more resistance than young animals, but less resistance than mature animals.

Table 1.3.

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Action level (ppb)</th>
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<tbody>
<tr>
<td>(i) Corn and peanut products</td>
<td>300</td>
</tr>
<tr>
<td>(ii) Cotton seed meal intended for beef, cattle, swine or poultry</td>
<td>300</td>
</tr>
<tr>
<td>(iii) Corn and peanut products intended for breeding beef, cattle,</td>
<td>100</td>
</tr>
<tr>
<td>breeding swine or mature poultry</td>
<td></td>
</tr>
<tr>
<td>(iv) Corn, peanut products and other animal feed and feed ingredients but</td>
<td>20</td>
</tr>
<tr>
<td>excluding cotton seed meal intended for immature animals and lactating</td>
<td></td>
</tr>
<tr>
<td>dairy animals</td>
<td></td>
</tr>
<tr>
<td>(v) Brazil nuts</td>
<td>20</td>
</tr>
<tr>
<td>(vi) Foods</td>
<td>20</td>
</tr>
<tr>
<td>(vii) Milk</td>
<td>0.5 (AFM1)</td>
</tr>
<tr>
<td>(viii) Peanut and peanut products</td>
<td>20</td>
</tr>
<tr>
<td>(ix) Pistachio nuts</td>
<td>20</td>
</tr>
</tbody>
</table>

1.2.3. AFB1 as a potent carcinogen

Aflatoxin B1, a metabolite of A. flavus is considered to be the most potent carcinogen and have been implicated as a causative agent in human liver cancer (23, 24). Chemically, AFB1 is named as 6-methoxy-2,3,4,5-tetrahydrocyclopenta-furo 8a,8b,8c,8d furo-10,11-chromen-2,3-dione, with the molecular formula of C17 H12 O6 and molecular weight of 312.276.
It is reported that AFB1 is possible to produce HCC by unit weight itself (25). Among various metabolites of fungal moulds (AFB1, AFM1, AFQ1, and AFB2α) AFB1 is believed to be a highly reactive carcinogen by producing the AFB1-8, 9-epoxide by the microsomal mixed function oxidases.

1.2.4. Metabolism of AFB1

AFB1 provides an interesting case history in the issues involved in the metabolism and reactivity of a pro-carcinogen. AFB1 is metabolically converted by Cyt-P450 enzymes, which lead to postulation of the epoxide (AFB1-8, 9-epoxide) as the electrophilic product involved in reactions with macromolecules. An important observation was the exo-isomer of the epoxide was at least 103 times more genotoxic than the endo-form (26), as a result of the important stereo chemical differences seen with PAH diol epoxides (27).

AFB1 by microsomal xenobiotic metabolizing enzymes, both in vivo and in vitro, results in the formation of AFB1-8, 9-epoxide, which binds to cellular macromolecules such as DNA, RNA, or proteins (28, 29) and exhibits mutagenic and carcinogenic activities.

1.2.5. Formation of AFB1-macromolecular adducts

The AFB1-8, 9-epoxide reacts covalently with the DNA to form adducts that presumably account for the biological effects. Of various adducts formed, the most quantitatively abundant both in vitro (30-33) and in vivo (31; 34-36) is 8,9-dihydro-8 (N7-guanyl)-9-hydroxy aflatoxin B1 (AFB1-N7-Gua). The AFB1-N7-Gua adduct has mutagenic properties that correlate with those of the biologically relevant DNA lesion(s) of AFB1. The other two abundant lesions formed by AFB1, specifically AFB1-FAPY and the AP site, have not been studied in sufficient detail (37).

Conclusion:

Covalent binding of AFB1 to adenosine and cytosine in DNA in vitro has also been reported. Common oxidative DNA damage, including formation of 8-oxodeoxyguanosine (8-oxodG) was observed in rat hepatic DNA following exposure to AFB1. A time-dependent and dose-dependent increase in hepatic levels of 8-oxodG residues in liver DNA treated with AFB1 has been reported. Thus the toxic effects of AFB1 can increase common endogenous DNA adducts. AFB1 induced lipid peroxidation is reported to be one of the main manifestations of oxidative cellular damage, in particular, DNA damage and formation of AFB1–DNA adducts, which may be the critical step in tumourigenesis. AFB1 mediated cell injury may be due to release of free radicals in vitro and also in vivo and these radicals initiate lipid peroxidation, a damaging process in biological systems and further predispose the normal hepatocytes to malignant cells.
REFERENCES


