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FIELD OF VISION

Mycotoxins are conventional and novel risk biomarkers for hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is a common malignant disease with poor prognosis. To improve the clinical outcome, early diagnosis of HCC arising from nonviral agents and hepatitis virus is important. Among several etiological factors, mycotoxins defined as carcinogens by the International Agency for Research in Cancer (IARC) might be one of the critical risk factors for nonviral HCC. Aflatoxin B1 is the most well-known carcinogenic mycotoxin for HCC, but the role of the other types of mycotoxin remains unclear. Several studies have reported that a chromatographic separation technique based on high-performance liquid chromatography can successfully detect the concentration of mycotoxins in plasma. Recently, serum level of ochratoxin A (OTA), a widely distributed mycotoxin classified as Group 2B by IARC, was evaluated in HCC patients in Egypt. The results suggested that serum OTA levels might be a good biomarker for HCC. In this article, we review recent studies of OTA, and discuss its possible significance as a biomarker of HCC.

Key words: Hepatocellular carcinoma; Mycotoxin; Aflatoxin; Ochratoxin; High-performance liquid chromatography

Core tip: Mycotoxins are one of the possible important carcinogens of hepatocellular carcinoma (HCC). Recently, a chromatographic separation technique based on high-performance liquid chromatography (HPLC) has been recognized as a useful method for the quantitative analyses of mycotoxins in the sera of individuals. Using this technique, the serum levels of ochratoxin A, a type of food mycotoxin widely spread in cereals, has been recently reported to be increased in the sera of HCC patients in Egypt. HPLC-based analysis of mycotoxins in the clinical samples would provide some new epidemiological information about non-viral HCC.

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COMMENTARY ON HOT TOPICS

Hepatocellular carcinoma (HCC) is a common malignancy, with a high prevalence worldwide[1,2]. The prognosis of HCC has remained poor, because many of the patients also have chronic liver diseases and are not suitable for radical surgical treatment[1-3]. Another obstacle to HCC treatment is that hepatoma cells display strong

resistance against standard chemotherapeutic drugs[2,3]. To enable early diagnosis and treatment, understanding the etiological risk factors of HCC is desirable. Currently, approximately half of all HCC patients suffer from chronic hepatitis B and C virus (HBV and HCV) infection, and remaining cases are affected by various types of etiological factors including alcohol abuse, cigarette smoking, mycotoxins, obesity, and oral contraceptive drugs[4]. Intriguingly, growing evidence has suggested that the types of etiological risk factors for HCC might differ between geographic areas. For example, obesity-associated HCC has become one of the most important medical issues in developed countries[5], while food contamination with mycotoxins remains a critical risk factor for HCC in developing countries, including South and East Africa, India and China[6].

Mycotoxins as a major dietary risk factor for liver cancer

In recent years, the relationship between cancer risk and mycotoxin has been universally publicized by global surveillance of food contamination. Mycotoxins are secondary metabolites produced by fungi and are present in various types of stored grains, and most of them are resistant against cooking, freezing and digestion after intake of contaminated food. Several food-contaminating mycotoxins have been defined as harmful carcinogens by the International Agency for Research in Cancer (IARC) (Table 1), that is, deoxynivalenol/nivalenol, zearalenone, ochratoxin, fumonisins and aflatoxins. Of these, aflatoxin B1 (AFB1) is the most well-known bioaccumulative toxin involved in the development of HCC[7]. AFB1 is produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, and mainly contaminates improperly stored cereals and peanuts. When individuals are exposed to AFB1 for a long time, mono-oxygenases produce reactive epoxide in the liver, leading to formation of toxic derivatives with nucleic acids and proteins[8]. AFB1 has a strong mutagenic effect, and induces G to T transversion within codon 249 of the tumor suppressor *p53* gene. Point mutation of p53 has been observed in approximately half of all patients with AFB1-associated HCC[9-11]. It has also been reported that geographical distribution of aflatoxin exposure and HBV infection overlap, leading to a synergistic effect on the genetic mutation of *p53*[10,11]. AFB1 is now regarded as the representative of orally ingested carcinogens, and has been classified as a Group 1 carcinogen by IARC (IARC 7th Annual Report on Carcinogens, 1987).

Ochratoxin A: A possible diagnostic marker of HCC?

Based on the clinical evidence of AFB1 in HCC, several researchers have turned their attention to the other type of mycotoxins. Ochratoxin A (OTA), which has been classified as a possible human carcinogen (Group 2B) by the IARC, is a secondary metabolite of *Aspergillus* and *Penicillium* fungi. OTA is widely spread in cereals such as barley, wheat, coffee and bread[12], and is well known for its possible contribution to nephritic diseases. Although the evidence is still open to debate, several studies have reported that OTA might be a causative agent of Balkan endemic nephropathy[13]. Moreover, OTA is increased to high levels in the plasma of patients with nephropathy in specific regions such as Tunisia[14], suggesting that OTA plays a critical role in the development of nephritic diseases.

Table 1 Classification of food mycotoxins as human carcinogens or potential human carcinogens

Group	Classification of food mycotoxins
1	Aflatoxin B1, B2, G1, G2
2A	-
2B	Aflatoxin M1, ochratoxin A, sterigmatocystin
3	Citrinin, patulin, luteoskyrin, cyclochlorotine, deoxynivalenol
4	-

Group 1: Carcinogenic to humans; Group 2A: Probably carcinogenic to humans; Group 2B: Possibly carcinogenic to humans; Group 3: Not classifiable as to its carcinogenicity to humans; Group 4: Probably not carcinogenic to humans (classified by the International Agency for Research in Cancer).

Unfortunately, the role of OTA in hepatocarcinogenesis remains unclear. Although the results of relevant studies are controversial, several have suggested that the carcinogenic effect of OTA is due to increased hepatotoxicity and DNA damage. Ehrlich *et al*[15] have tested the genotoxic effect of OTA in human hepatoma HepG2 cells using both micronucleus and single-cell gel electrophoresis assays, and have found that it causes pronounced dose-dependent effects on DNA damage. Renzulli *et al*[16] have reported that OTA induces DNA damage through oxidative stress, and this could be prevented by rosmarinic acid, a natural phenolic compound contained in many Lamiaceae herbs. Bouaziz *et al*[17] have reported that OTA triggers a p53- and caspase-dependent mitochondrial apoptotic pathway in HepG2 cells. In contrast, El Golli Bennour *et al*[18] have reported that OTA does not induce significant reactive oxygen species generation in cultured HepG2 cells, but induces mitochondrial and caspase-dependent apoptotic cell death mediated by p53 transcription-independent activities. The aforementioned different and controversial studies suggest that etiological analysis of patients with HCC is indispensable for assessing the relationship between OTA and HCC.

Until recent decades, meta-analysis of etiological risk factors for carcinogenesis has been complicated because traditional methods for assessing toxin exposure were mainly performed by questionnaires or standard enzyme linked immunosorbent assay and these cannot assess minute quantities of environmental toxins. During the last decades, however, a chromatographic separation technique based on high-performance liquid chromatography (HPLC) has enabled us to assess the concentration of mycotoxins in clinical samples such as urine and plasma[19]. HPLC enables us to detect OTA at a low level of 0.005 ng/mL in plasma[20], which is often less than the mean level in healthy individuals; therefore, this analytical method is preferable for evaluation of the

etiological significance of OTA. For example, Grosso *et al*[21] and Aslam *et al*[22] have examined the levels of OTA in the serum of bladder cancer patients by HPLC, and have reported that OTA is unlikely to be a risk factor for bladder cancer in Tunisia and Karachi. di Giuseppe *et al*[23] have examined serum OTA in patients in the Molise region in Italy, and have reported that the levels of OTA are significantly associated with C-reactive protein and cardiovascular risk score in men.

Until recently, there have been no studies investigating OTA in HCC patients using HPLC methods. Very recently, however, Ibrahim *et al* performed a case-control study of HCC patients from 2010 to 2012 in Egypt, and found that OTA was frequently increased in the serum. They measured serum OTA in 39 HCC patients using HPLC and compared it with the level in healthy individuals in Egypt. The highest incidence of OTA was observed in the HCC group. The mean level of serum OTA in the HCC and normal groups was 1.11 ± 0.3 ng/mL (0.129-10.93 ng/mL) and 0.201 ± 0.02 ng/mL (0.005-0.50 ng/mL), respectively (P = 0.0002). Multivariate analysis showed that serum OTA was an independent risk factor for HCC. The authors also found that OTA was well correlated with the levels of α-fetoprotein, which is a major tumor marker for HCC. To date, to the best of our knowledge, there have been no studies regarding the etiological relationship between OTA and HCC. Further studies on a large scale should be performed in different countries, and they might support the idea that mycotoxins, including OTA, are useful biomarkers of HCC.

Summary

Recently, progress in the diagnosis and treatment of hepatitis virus infection has significantly improved the outcome of patients with HBV- and HCV-associated HCC. Early diagnosis of HCC arising in patients with nonviral disease, however, has been remained difficult. HPLC-based analysis of clinical samples might offer a useful tool for detecting minute concentrations of environmental toxins that have accumulated in the host. Although food contamination with mycotoxins has so far been recorded in specific geographic regions, it is conceivable that current advances in the transportation system could cause unexpected food contamination in a wide area. Therefore, to address whether OTA would be a real causal agent for HCC, assessment of the level of mycotoxins, including OTA, in clinical samples would be of value.

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