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Monitoring of Aflatoxins and Ochratoxin A in Czechoslovak Human Sera by Immunoassay

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Aflatoxins are toxic secondary metabolites of certain strains of Aspergillus flavus and A. parasiticus. These fungi grow under specific condition of temperature and humidity on a wide range of foodstuffs including nuts and grains. The majority of the above moulds produce mainly aflatoxin B_1 which is one of the most potent hepatotoxin and hepatocarcinogen known in experimental animals, including nonhuman primates. Epidemiological studies suggest that it may also be an important factor in the etiology of human liver cancer (Linsell and Peers 1977).

Another dangerous mycotoxin contaminating cereals is ochratoxin A produced by several species of Aspergillus and Penicillium. Experimentally, feeding of ochratoxin diets has a deleterious effect on a number of animal species (nephrotoxic, hepatotoxic, teratogenic, and immunosuppressive effects). Ochratoxin A is regarded as a causative agent for endemic Balkan nephropathy of man (Krogh et al. 1977). In addition, aflatoxin and ochratoxin can interact to produce synergistic toxicity. Incidents of acute mycotoxin poisoning in humans are rare, but prolonged exposure to subacute levels may be a serious problem.

Since a level of food contamination with aflatoxins and ochratoxin A has been found low in Czechoslovakia (Fukal et al. 1987; Fukal 1989), human exposure to these mycotoxins is supposed to be negligible. However, analysis of food samples provides only indirect evidence of mycotoxin ingestion and no evidence about mycotoxin absorption. Direct evidence can only be obtained by analysis of human body fluids. Therefore, we decided to carry out a monitoring of aflatoxin and ochratoxin A level in human sera. Such

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monitoring of mycotoxin concentrations in food or human sera requires the determination of trace amounts of mycotoxin.

In general, TLC and HPLC are most commonly used to analyze mycotoxins and its metabolites. The lengthy and complex procedures used to purify the mycotoxins prior to analysis have seriously limited the application of these approaches in large-scale epidemiologic studies. The recent development of immunochemical techniques opens the possibility of determining individual exposure in a relatively large human population. These assays have the advantage of high specificity and sensitivity. Sample through-put is high, and the methods are technically simple and can be performed at low cost (Pestka 1988).

MATERIALS AND METHODS

Aflatoxin B_1 (AFB_1) was purchased from Calbiochem (U.S.A.). The rabbit polyclonal antiserum and the radioligand for radioimmunoassay (RIA) of AFB_1 were prepared as described previously (Fukal et al. 1987). Kits for RIA of ochratoxin A were obtained from Institute of Nuclear Research (Košice, Czechoslovakia).

Human sera were obtained from two Bohemian hospitals. Samples were mixed in equal proportions with methanol and the precipitate produced was removed by centrifuging. In aliquots of the resulting supernatants, aflatoxins and ochratoxin A were determined directly using RIA procedures described previously (Fukal et al. 1988; Fukal 1989).

RIA of AFB_1 was equally specific for aflatoxins B_1, G_1, and Q_1, with cross-reactions of 24%, 15%, 0.4%, and 0% for aflatoxins B_2, G_2, M_1, and ochratoxin A, respectively. Thus the term aflatoxin is used in interpretation of results without further specification. RIA of ochratoxin A showed cross-reactions of 100%, 4%, 27%, 0%, 0%, and 0.4% for ochratoxins A, B, C, L-phenylalanine, D-phenylalanine, AFB_1, and citrinin, respectively.

RESULTS AND DISCUSSION

The detection limits of the RIA-methods were 1.5 pg per tube and 5 pg per tube giving assay sensitivities in serum 30 ng/L and 100 ng/L for AFB_1 and ochratoxin A, respectively.

Table 1 shows that aflatoxin was not detected in the majority of the human serum samples analyzed. Only 1.8% of samples possessed higher aflatoxin

concentration than the detection limit of the RIA applied. The highest level was 74 ng/L.

Table 1. Frequency of aflatoxin contamination of 227 human sera under specific concentration range

Aflatoxin concentration range (ng/L)	No. of samples in range	Mean ± S.D.	Percent of incidence
< 30	223	---	98.2
30-100	4	59.8 ± 7.3	1.8
>100	0	---	0

Table 2. Frequency of ochratoxin A contamination of 143 human sera under specific concentration range

Ochratoxin A concentration range (ng/L)	No. of samples in range	Mean ± S.D.	Percent of incidence
< 100	108	---	75.5
100-500	19	341.6 ± 38.1	13.3
500-1000	15	812.3 ± 46.4	10.5
> 1000	1	1260	0.7

A much higher frequency of an ochratoxin A occurence was indicated (Table 2). 24.5% of samples contained ochratoxin A at levels exceeding the detection level with the maximum concentration of 1260 ng/L.

In the U.K., Wilkinson et al. (1988) demonstrated that none of the 27 human sera analyzed, contained detectable aflatoxin at levels greater than 20 ng/L. Tsuboi et al. (1984) found 34% of serum samples, obtained from healthy Japanese males, in the range of aflatoxin concentration of 20-1169 ng/L. Studies of patients with Reye's syndrome and of healthy subjects from the southeastern United States and the Third World have indicated even higher levels of serum aflatoxin (Ryan et al. 1979; Nelson et al. 1980; Hendrickse et al. 1982; Denning et al. 1988). Gareis et al. (1988) found trace amounts of ochratoxin A in 4 of 36 randomly collected human milk samples in the F.R.G. . However, we are aware no study on monitoring ochratoxin A in human sera.

Human liver microsomes have the ability to metabolize AFB_1 at a high rate. There are generally at least five types of metabolic reactions characteristic of AFB_1: reduction, hydroxylation, hydration, O-demethylation, and epoxidation leading to the respective formation of aflatoxicol, aflatoxin M_1 and aflatoxin Q_1, aflatoxin B_{2a}, aflatoxin P_1, and aflatoxin B_1-8,9-oxide (Hsieh and Wong 1982). AFQ_1 appears to be the major detectable metabolite produced by human microsomal metabolism in contrast to many animal species in which AFB_1-8,9-dihydrodiol is the major soluble metabolite (Moss and Neal 1985). The antibody used in this study is specific to the furan ring end of the AFB_1 molecule. Thus it is detecting aflatoxins with high toxicity, including AFQ_1. Metabolism of ochratoxin A has not been studied in such details.

In Czechoslovakia, the tolerance limits for the presence of AFB_1 and ochratoxin A in human food are 5 and 20 ppb, respectively. In spite of this regulation the results presented here demonstrate an ingestion of considerable amounts of ochratoxin A by rather great portion of the subjects tested.

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