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CLINICAL STUDY

Results of the first studies of occurence of ochratoxin A in human milk in Slovakia

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Abstract: Authors bring the work the purpose of which is to offer information about quality of human milk from the viewpoint of possible contamination with mycotoxin Ochratoxin A from the fist analyses in Slovakia. One of its main toxic effect is nephrotoxicity, immunotoxicity, neurotoxicity, teratogenity and carcinogenity.
Material and procedure: 76 samples of human milk from locality Martin and the corresponding zone were analysed by technique of High Performance Liquid Chromatography (HPLC) in conditions as mentioned below.
Results: Ochratoxin A was proved in 23 samples, 9 samples of the number with values from 2.3±0.99 ng/l to 60.3±25.93 ng/l, its occurence was at the level of quantification limit in the other 14 samples and it was undetectable in 55 samples of human milk.
Conclusion: Up to now findings of our study have pointed out that Ochratoxin A can occur in human milk also in our conditions and determined values range between limits like those in Germany and Switzerland *(Tab. 3, Fig. 1, Ref. 15).* Full Text (Free, PDF) *www.bmj.sk.*
Key words: human milk, ochratoxin A, high performance liquid, chromatography HPLC.

Ochratoxin A (Fig. 1) is ubiquitous mycotoxin produced by Aspergillus and Penicillium species. Vegetal and animal food is a hospitable substrate for growth and expansion of molds and genesis of the toxin. Several commodities provide particularly favourable conditions. They are cereals and their products.

Ochratoxin A occures significantly also in fruit juice, coffee and cocoa, raisins, dried fruit, nuts, pork and products of pork liver; exception is beef that is not dangerous from the point of view for contamination of nursing mother's milk ((1, 2, 4, 7, 12, 14).

Ochratoxin A as a natural toxin is a significant health problem because of its special effect in human organism. Its participation in irreversible up to fatal illness of kidney is proved and it is related to Balcan endemic nephropathy (1, 2, 4, 6, 8, 10).

Its other effects are immunotoxic, nephrogenic, teratogenic and mutagenic activities and according to IARC WHO it is classified as a potential carcinogenic agent of 2B category (1, 6, 8, 11).

The Nordic Working Group on Food Toxicology suggested a maximum TDI (tolerable daily intake) of Ochratoxin A 5 ng/kg of body weight (4, 6, 12).

High prevalence of contamination with Ochratoxin A found in samples of blood plasma in European countries and overseas is an indicator of continuous and widespread exposition of people.

Occurence of Ochratoxin A in human milk can be the reason of its toxicity, or exposition of newborn and breasted infants.

Purpose of the work

The work inteded to examine the occurence of Ochratoxin A in human milk, determine the level of contamination and evaluate the potential risk caused by the xenobiotic agent in breasted infants

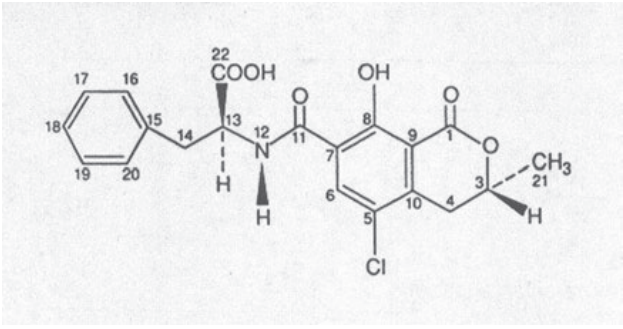

Fig. 1. Structural formula of Ochratoxin A.

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Observed file

We assembled the samples of human milk fom nursing mothers which were hospitalized with their children at The Clinic of Children and Adolescents in Martin within the period from March 2007 to August 2007. Age of the children was from 0 to 6 months. The patients were from the locality of Martin and the corresponding zone, Liptovský Mikuláš, Kysucké Nové Mesto, Trstená, Čadca, Žilina, Prievidza, Handlová. 51 mothers were from town agglomeration and 25 were from villages. 76 samples in volume of 25 ml minimally were totally collected.

We do not introduce data from the initiative analytical study.

Method of mycotoxin determination

We analysed samples of human milk by using the technique of High Performance Liquid Chromatography (HPLC). The chromatografic system Merck Hitachi was used.

We carried out the determination according to EN ISO 56 0511 and application methods of R-Biopharm Rhône Ltd., Neogen Europe Ltd. (1, 5, 9, 13).

Procedure and conditions

We extracted a test portion of human milk sample by n-hexan to remove the lipid fraction. The water fraction where Ochratoxin A is supposed we extracted with 1 % $NaHCO_3$ as an elution solution. After consequential filtration and mixing with buffered saline we concentrated the toxin with the use of immunoaffinity columns for Ochratoxin A.

Ochratoxin A was separated from the cleaned-up and concentrated extract by means of HPLC system with reverse phase separating column with following operating conditions. We used analytical separating column LiChrospher RP-18**,** temperature in column oven 35 °C, mobile phase acetonitrile : water : acetic acid with parts per volume 51:47:2, flow rate of mobile phase 1 ml/min. Detection: Fluorescence detector (excitation wavelenth 333 nm, emission wavelenth 433 nm). Dosage: Injection volume of 100 µl**.**

Results

With the use of HPLC method's detection limit of 4.8 ng/l we did not prove the presence of Ochratoxin A in 53 samples from the total number of 76 analysed samples.

Tab. 1. Numerical values of content of Ochratoxin A in human milk.

Samples of human milk contaminated by Ochratoxin A ng/l		
1 *2.30±0.99	4 *9.70±4.17	7 16.80±7.22
2 *4.97±2.14	5 14.60±6.29	8 47.60±20.47
3 5.42±2.33	6 16.40±7.05	9 60.30±25.93

From the number of 23 positive samples (30.2 %) there were 14 samples (18.4 % of the total number of 76 analysed samples) within the interval values from 4.8 to 14.4 ng/l which is the limit of quantification (LOQ) for the method used. It is impossible to perform quantitative interpretation within the interval.

We proved and determined Ochratoxin A within the interval from 2.3 ng/l to 60.3 ng/l in 9 samples of human milk (11.8 %) with volume of 25 ml; 4 values (in Tab. 1 marked *) were taken with use of multiple volume of human milk.

Analyses of small quantity of substance always goes together with the measurement uncertainty. The measurement uncertainty for HPLC method used is 43 % within concentration interval 14.4–100 ng/l.

The uncertainty within concentration interval 100–200 ng/l is 39 % (Tabs 2 and 3).

Discussion

In the pilot study, we bring the results of occurence of Ochratoxin A in human milk in Central Slovak region. We proved the presence of the toxin in 23 samples from the total number of 76 which means 30.2 %. The values were within the interval from 2.3 % to 60.3 ng/l (0.0023–0.0603 ng/ml). Calculation of the values in brackets is mentioned in order to make it possible to compare with values from mainly foreign reference.

17 samples of 80 samples of human milk were contaminated with Ochratoxin A within the interval 0.01–0.182 ng/ml in the Norwegian study, data from Sweden mention 23 contaminated samples of 40 samples 0.01–0.04 ng/ml. Kovacs et al (1995) in Hungary analysed 92 samples of which 38 contaminated were within the interval 0.2–7.3 ng/ml.

Several studies done in Italy came up with values: 9 samples of 50 analysed (1.7–6.6 ng/ml), in 22 of 111 (0.1–12.0 ng/ml) and Turconi et al (2004) mention 85.7 % contaminated samples with mean value 6.01±8.31 ng/l (0.00601±0.00831 ng/ml). De-

Tab. 2. Comment notes to term used in chemical analyses.

ND (4.8 ng/l)	Not detectable by the method used	For sample volume of 25 ml and volume of analysed filtrate of 25 ml, final volume 1500 µl, dosage 100 µl
<14.4 ng/l	Less than LOQ but higher than LOD	For sample volume of 25 ml and volume of analysed filtrate of 25 ml, final volume 1500 µl, dosage 100 µl
LOD	Limit of detection of the method	LOD is 4.8 ng/l for the method
LOQ	Limit of quantification of the method	LOQ is 14.4 ng/l for the method
Recovery	Recovery test	85.8 %

Tab. 3. Results of Ochratoxin A analyses in samples for quality assurance.

Quality assurance				
Test material	Assigned value of Ochratoxin A µg/kg	Satisfactory range of Ochratoxin A µg/kg	Measured value of Ochratoxin A µg/kg	Recovery %
1 T1742 Baby Food (FAPAS^{R})	0.60	0.34–0.87	0.49 0.54	81.7 90.0
2 Range of recovery values of Ochratoxinu A in every series tested samples: 79–105 %				

tectable l?? values were determined in Switzerland 5–14 ng/l (0.005–0.014 ng/ml) and in Germany 17–30 ng/l (0.017–0.030 ng/ml) (3, 10–12, 14).

When the values from European countries are compared with our results, the latter ones are similar to those obtained in Germany and Switzerland. The differences probably relate not only to alimentation patterns and geographical differences, i.e. we cannot exclude other unrecognized reasons.

Studies from Sierra Leone where high mortality in children probably relates with Ochratoxin A confirmed high incidence of the toxin and degree of contamination 200 up to 337000 ng/l (0.2–337 ng/ml) (4, 11, 14).

Among values above the limit of quantification of the method used LOQ 14.4 ng/l (0.0144 ng/ml) we determined maximal value 60.3 ng/l (0.0603 ng/ml). Based on the latter we can estimate the risk of exposure of a child eating human milk containing the value of Ochratoxin A.

The TDI value of 20 ng/4 kg of body weight is for a child with body mass of 4 kg and total daily intake of 630 ml of human milk. If our maximum value 60.3 ng/l was calculated, the child would take in 38 ng/630 ml, i.e. in excess of 18 ng in intaken volume of milk (1, 6, 8, 11).

Conclusion

Ochratoxin A is a toxin naturally occuring in human food and animal feed. Man is exposed to it via conaminated food. Mycotoxins and their metabolites are excreted from body by biological fluid. One of the way of elimination is human milk as well.

Numerous world studies are dedicated to the occurence of Ochratoxin A in human milk. For the first time Ochratoxin A was discovered in Germany (Garies et al, 1988) (4, 6, 10). The contamination of it is a world-wide problem affected by geographical, climatic, cultural, social and economical differences in every individual locality.

Our pilot study pointed out the fact the Ochratoxin can occur in human milk also in our conditions. In order to take measures against human milk contamination level and consequently risk of possible exposure for breastfed infant, it is essential to make a state-wide study comparing the degrees in particular regions of Slovakia.

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