Artykuł przeglądowy *Review*

Review of the most important mycotoxins

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Summary

This study briefly describes the history of mycotoxins and gives a general overview of the most frequently detected mycotoxins. The discovery, chemical structure, producers, as well as the occurrence of mycotoxins have been discussed.

Keywords: history of mycotoxins

History of mycotoxins

Mycotoxins are substances produced by microscopic fungi of non-protein nature. They are toxic for humans and animals. First verify records about toxicity of molded food are from the end of last century from Japan (43). Some of them are connected to traditional experience of people from East Asia. Primarily, experience of people with yellow rice area described. Yellow rice dried (in thin layer) on direct sun lost its toxicity (photolysis of mycotoxin citreviridin). In thirties years, samples of cereals contaminated by molds of strain *Fusarium* were examined in USSR. Those molds and their extracts caused toxic effects in examined animals. Toxic effects were related with disease called as alimentary toxic aleukia (ATA). Groups of scientists, which solved this problem were broken in forties years, because they were not able to eliminate outbreaks of ATA in Soviet Union during Second World War (17). In this period more than 10 000 people died, because of ATA (officially was declared 17 000) (48). Model example of identification of *Fusarium* mycotoxins as a promoter of ATA was done by Uen in 70 years (43). In 40 years was placed on the market penicillin called as „miracle produced by molds". Discovery of penicillin, its isolation, and effectiveness in treatment of diseases brought for Flemming – Florey – Chain Nobel price. However, discovery of penicillin by Flemming was done randomly. In the end of twenties years, he carried out experiment in bacteriological laboratory with various strains of staphylococci. Some plates were stored on laboratory table and occasionally controlled him. During control, plates were contaminated by micro flora in air. Contamination caused growth of mold colonies and colonies of staphylococci around them become transparent. Dissolving of colonies was related to the presence of molds on the plates. Molds were recultivated, and pure culture was isolated. Determination of their properties followed after isolation. Ten years after discovery of penicillin prof. Florey started experimental study on isolation of pure penicillin and resolving its chemical structure. He was successful and industrial production of penicillin become reality. Chain prepared first synthetic penicillin when benzyl side chain attached to molecule of 6 – aminopenicillin acid substituted with dimethoxybenzyl. According to (39) Alexander Flemming had luck, that isolated strain of mold did not produce also mycotoxins. Strains with combination of these properties are very rare.

Czechoslovak scientists in the end of Second World War also isolated original strain producing penicillin. Preparation MYKOIN B was prepared in illegal conditions as original Czechoslovak penicillin. Interest in toxic substances produced by molds appeared suddenly. In 1916 approximately 10 000 turkeys died in English farms. Disease was named as Turkey – X. Analytical chemistry at this time was advanced in comparison with 30 years, when soviet scientists were able to identify only toxic extracts and their knowledge about toxin properties were insufficient mycotoxins were bound with fatty acids (40). Identification of other mycotoxins followed and they were isolated from peanuts included in feed mixtures intended for turkeys. Production strain of mycotoxins was identified as *Aspergillus flavus* and toxins were named as aflatoxins. For closer identification of mycotoxins combination of letters B (blue), G (green) and numbers is used. Letters are related to colors produced under ultraviolet radiation (365 nm). Number index was established according to sequence from thin layer chromatography. Aflatoxin M was discovered in milk, and in liver (5, 48).

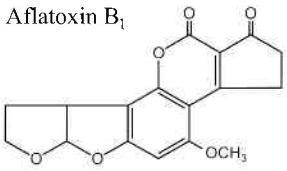
Mycotoxins become object of study also in Czechoslovakia. Research started in Medicine Faculty in Brno in relation to mold contamination of tomatoes in Fruta Company. Mold contamination caused significant change of sensory properties of final products. Employees of hygiene service, responsible for sensory evaluation of products, become ill with symptoms typical for damage of liver. Doc. RNDr. Miroslav Polster, CSc. was autho-

rized to solve this problem. He studied chemistry, but for a long time worked also as a microbiologist. In his person were connected properties, knowledge in chemistry and experience in microbiology, required for scientific work in this field. Research of mycotoxins in Slovakia was concentrated in Research Institute of Preventive Medicine in Bratislava. MUDr. Zdenka Jesenská was concerned with study of micromycets. The field of study was extended on examination of foodstuff and food, water and working space of humans. Important practical knowledge about mycotoxins was collected. Toxic metabolites of microscopic filamental fungi and micromycets become bio indicators of environmental pollution. In seventies years, research and routine examinations of mycotoxins were spread in numerous laboratories.

The most important mycotoxins

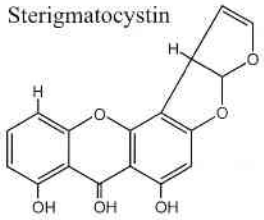
Aflatoxins. Aflatoxins present group of related substances and discovery of them was beginning of new age boom in mycotoxin research. Aflatoxin B_1 is known as strongest natural cancer promoter. The basic aflatoxins are: B_1, B_2, G_1 and G_2. Important is also derivate of aflatoxins aflatoxikol, which is bound to blood albumins. Some natural aflatoxins are transformed to aflatoxikol through various biochemical processes. Aflatoxikol is able to change itself on Aflatoxin B 12,3 – epoxid, which is bound on DNA and RNA. This process is biochemical principle of his mechanism of action in proteosynthesis and finally in transfer of genetic information.

Aflatoxin B_1



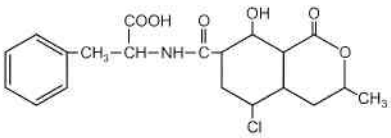
However, research of aflatoxins is difficult; they belong to the group of well-studied carcinogens. Aflatoxins are also used as a model example of cancer promoter. Aflatoxins are produced exclusively by *Aspergillus flavus* and *Aspergillus parasiticus* (8). Aflatoxin production depends on temperature, humidity, and access of air, structure and chemical content of matrix. Influence of presented microflora is also important (inhibition of aflatoxins production is depressed by pressure of *Aspergillus niger*). Substances, which are able to block biosynthesis of aflatoxins are known (for instance coffein, 23). In opposite some substances (microelements) increase their production (8, 10). Generally, at the present time level of knowledge about importance of mycotoxin production and mechanism of their effects are not sufficient. Aflatoxins can cause Rey syndrome, hepatitis and depression of immunity in humans. The hazards related to aflatoxins are presented through intake of food and they are more important in some professional branches. Laboratories routinely testing presence of aflatoxins in food and all commodities have to use just small volumes of dissol-ved solutions and protective clothes are required. The problem is feed production, storage and manipulation. Aflatoxins are frequently detected in animals contaminated via feed intake, dust inhalation or absorption through skin. Dust is more important source of aflatoxins (higher concentration) than original substrate. Presence of aflatoxins in blood and urine of employees from feed production factories was recorded (15, 16, 34). Parallel occurrence of aflatoxins in dust was confirmed. At the end must be visible situation when most of the problems are related imported food, feed, and foodstuff. Precious control on the border, proper storage of potentially dangerous foodstuff, can eliminate problem with aflatoxins.

Sterigmatocystin



Sterigmatocystin. Sterigmatocystin is, on the basis of chemical structure, related to aflatoxins. Broad spectrum of strains *Aspergillus*, *Chaetomium*, and *Emericella*, *Bipolaris nodulosa*, *Farrovia malagensis*, and *Monicillium nordinii* produce it. Sterigmatocystin has hepatotoxic effect and cancerogenity is predicted. In Slovak Republic, sterigmatocystin was detected in cheeses and other substrates (43). It seems that biochemical processes important for mycotoxin production are the same for sterigmatocystin and aflatoxins. Sterigmatocystin fluorescents in UV spectrum (365 nm) in slightly bricking color, and at in short wave UV spectrum intensively yellow. After spraying with $AlCl_3$ fluorescence become intensively yellow also at UV spectrum below 360 nm (12).

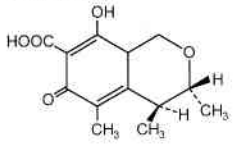
Ochratoxin A



Ochratoxin A. Ochratoxin A is the most important and the most toxic mycotoxin from group of ochratoxins. Fungi from strains *Aspergillus* and *Penicillium* produce it. Mechanism of toxicity is based on principle, that fenylalanin part of his molecule is substituted by t-RNA with fenylalanin. Fenylalanin in ochratoxin A molecule is bound on kumarin sequence, which interfere its connection to protein chain. Consequence of this process is stopping of proteosynthesis. Fenylalanin can be used as an antidote, because of competitive inhibition with ochratoxin A during binding on t-RNA. Preparations in which fenylalanin was substituted with other aminoacid were prepared by 18, 32 and 43. Dominant effect of ochratoxin A is depression of immunity and damage of kidney. The main source of ochratoxin A is cereals. Cereals produced in Slovak republic are also contaminated by ochratoxin A, but detected concentrations are bellowing MRL (14). The source of ochratoxin is also meat products. Production of ochratoxins by culture molds used for processing of smoked salami was described (1). The important source can be also coffee. This information is related to toxicological significant findings of ochratoxin A in human blood (blood cans from Germany, Austria and Switzerland). As a source of ochratoxin A occurrence in blood was identified coffee. Raw coffee in Slovak Republic is divided to groups according to quality (I, II, III). Group I does not contain ochratoxin A, group II rarely under limited concentrations, and group III regularly and very frequent is over limited presence of ochratoxin A. Another source of ochratoxins is blood of pigs, where ochratoxin is bound to albumins (home made products, 3).

Citrinin. Hetherington and Raistrick discovered citrinin as s metabolit of *Penicillium citrinum*. It is produced also by other molds from strains of *Penicillium* and *Aspergillus*. Its structure was described by Cogne (4). Originally was citrinin used as an antibiotic, but because of toxicity (nephrotoxicity) was later refused. Citrinin occurrence was recorded in yellow rice, but in our conditions dominant source of citrinin are cereals (45).

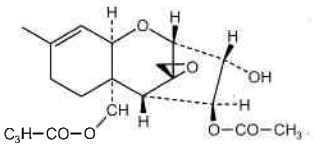
Citrinin



Citrinin belongs among acid mycotoxins, in basic conditions it is destroyed. Citrinin is easily detected, because of color and intensive yellow fluorescence under ultraviolet radiation (365 nm). Antibacterial effect can be used for its microbiological examination (39).

Trichocethens. Trichocethens are typical, because of presence of trichocethens nucleus. This nucleus contains epoxid group, which is used for their detection. They were known before period of mycotoxin boom. Trichocethens cause ATA, cardiomyopatia of cobalt beer and pellagra (20, 27, 36, 43-45, 48). Specific group of trichocethens is macrocyclic trichocethens. Typical member of this group is satratoxins, substances produced by *Stachobotrys alba* and toxins of *Dendrodochium toxicum*. These toxins were systematically studied in USA and Hungary (13, 48). Baccharinoides are mycotoxins produced by plants from strain *Baccharis*. They transform trichocethens produced by soil microscopic fungi. The plants growth around Orinocco river and they cause toxicities of cattle (21, 33, 35), which do not recognize them as a dangerous. Chemistry, biosynthesis, biological effects, spreading in environment and other aspects of trichocethens are studied intensively (4, 6, 39). More than 150 trichocethens were identified, but information are concentrated on T-2 toxin, nivalenol (NIV), moniliformin (MO), diacetoxyscirpenol – anguidin (DAS), and deoxinivalenol – vomitoxin (DON) (28, 41). On the basis of toxicity trichothecens are divided ontoxins of gastrointestinal tract, dermatotoxins, immunotoxins, hematotoxins, and genotoxins (24).

Diacetoxyscirpenol



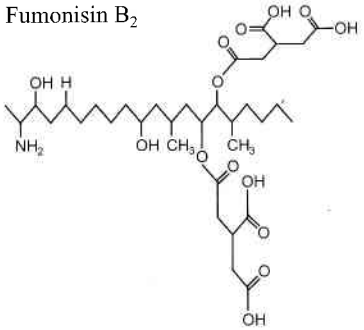
Total biological effects of trichocethens were described in ATA, some of them cause also vomitus. Phytopathology must be also considered in relation to strain *Fusarium* that contaminates culture plants (13, 45).

Slovak Republic is located in area without termophilic and psychrophylic *Fusarium* and we have problems only during very hot summer.

During the wars in Southeast Asia and Afghanistan, Soviet army as a biological weapon used yellow rain. Probably, extract of cultures of *Fusarium* and maybe also other products of trichocethens were applied. These substances have similar effect as yperit and they cause immune depression and problems in hematopoesis. Trichocethens can be used as a radiomimetic substances (they enhance influence of ionization). Radioprotection of soldiers and using of radiomimetics have a significant tactic and strategic influence on effectiveness of atomic weapons. All of these information areas are discussed and their credibility is doubted. In 1996 Academia published (19) a book contradictory to previous opinions. On the other hand, trichocethens were detected by prof. Mirocha (22) and also in other laboratories (33). Probably, yellow rain contained also other toxic substances included macro cyclic (6) trichocethens. Cultures of toxic microscopic fungi can be easily spread for a long distances. Production of biological extracts, contrary to classical and chemical weapons, can be hidden in enemy territory. Food – supply of army and strategy of storage of food is usually a target of biological attacks and this eventuality must be calculated.

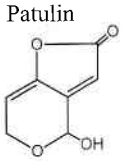
Fumonisins. Fumonisins are substances (fumonisin A_1, A_2, B_1-B_4, the most frequent is B_1) derived from unsaturated fatty acids. Microscopic fungi of strain *Fusarium* produce them. They cause diseases of animals, lekoencephalomalatia of horses, pulmonal edema of pigs, nephrotoxicity, hepatotoxicity and hepatocancerogenity of laboratory animals (31). Biochemical principle of mechanism is interaction with metabolism of sphingolipids and their membrane structures (47). Contrary of mycotoxins they are easily soluble in water and less soluble in polar dissolvent. Experimentally was confirmed, that they induce malignant form of tumors in animals. In humans is epidemiological relation to occurrence of cancer of esophagus (46). In our conditions the main source of fumonisins is maize (24).

Fumonisin B_2



Patulin. Patulin was discovered in forties years as an antibiotic active metabolite of *Penicillium patulum*. Before, patulin was isolated by Chain et al. (4) as an antibiotic was used for a short time. Then cancerogenic properties in animals were detected and using of patulin for a treatment was forbidden. It is produced by strains of *Aspergillus*, *Byssochlamys* and *Penicillium*. In nature is wide spread and important source are fruits, frequently in apples. Toxicities of cattle after consumption of decomposed silage (40) are known. In fruits are presented protective substances (vitamin C), which defend destruction of patulin during thermal treatment of compote, and cider (pure patulin is destroyed at 80°C). Significant is contamination of food intended for children nutrition. Maximum residue limit of patulin was established on the basis of cancerogenity (it is cancerogenic for some laboratory animals; Ames test of mutagenicity was negative). Acute forms of toxicity were described in cattle, primarily damage of lungs in combination with edema. Patulin is one from the small group of mycotoxins, which can be destroyed by antidote. The principle of detoxication is reaction of patulin with –SH groups. Decomposition of patulin was also described during alcohol fermentation (30).

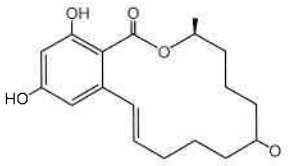
Patulin



Penicil acid. Penicil acid a substance very similar to patulin and also biological effects (includes cancerogenity) are comparable. It occurs at the same conditions as patulin but generally its importance is not so significant (45).

Zearalenon. Zearalenon is estrogenic mycotoxin (26) produced by some molds from strain *Fusarium*. Other producers of zearalenon are: *Fusarium sporotrichoides*, *Fusarium oxysporum*, *Fusarium moniliforme*, and *Fusarium crookwellence* (11, 29). Estrogen, antiestrogen and anabolic effects are recognized. Zearalenon is activated in organisms of live animals, urine and the rest eliminate 5% by feces. During lactation approximately 40% of zearalenon is eliminated by milk (30).

Zearalenon



Zearalenon can cause hyperestrogenismus (43) in humans and animals. The most significant problems can arise in farmers, which produce contaminated cereals and consume them. Problems can become serious also during germination, when growth of fusarium molds is rapid and mycotoxin production is huge. Risk group of consumers is vegetarians, which consume higher portion of food of plant origin. A lot of documents were collected to verify presence of statistical correlation among occurrence of zearalenon and trichocethens in nutrition of mothers during pregnancy and occurrence of some failures of nervous system in their offspring. Similar relation is considered in occurrence of male homosexuality. Experiments on laboratory animals were done to explain these effects (37). Some derivates of zearalenon are without toxic effects besides anabolic and they are used for feeding of cattle. The last news in this field is implantants, saturated by hormones, which offer better protection of consumers (42).

Cyclochlorotin. Cyclochlorotin was discovered by Maromo and originally was called islandiotoxin. Later, in dependence on structure of its molecule, was renamed by Yoshioka et al on cyclochlorotin (4). It is considered as a hepatotoxin and it causes carcinoma of liver, very frequently in countries with high level of rice consumption. In Slovakia it growths also on rice substrate and strain 137 was cultivated (30).

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