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OCHRATOXIN A AND AFLATOXIN B1 AS FACTORS OF BONE DAMAGE AND NEURODEGENERATION THROUGH THE INFLUENCE ON THE IMMUNOMODULATION PROCESSES OF TNF-A AND IL-6 CONCENTRATIONS

Agnieszka Radzka-Pogoda[1,2)], Radosław Piotr Radzki[3)], Marek Bieńko[3)], Jarosław Szponar[4)], Barbara Sokołowska[5)], Anna Kulik[6)], Małgorzata Lewicka[7)], Andrzej Borzęcki[1)]

[1)] Department of Hygiene and Epidemiology, Medical University of Lublin, Poland
[2)] Doctoral School, Medical University of Lublin, Poland
[3)] Department of Animal Physiology, Faculty of Veterinary Medicine, University of Life Sciences in Lublin, Poland
[4)] Toxicology Clinic, Clinical Department of Toxicology and Cardiology, Stefan Wyszyński Regional Specialist Hospital, Medical University of Lublin, Poland
[5)] John Paul II University in Biała Podlaska, Poland
[6)] Józef Pilsudski University of Physical Education in Warsaw, Faculty of Physical Education in Biała Podlaska, Poland
[7)] Department of Epidemiology and Public Health, Medical University, Łódź, Poland

ABSTRACT

The wide distribution of mycotoxins, including aflatoxin B1 and ochratoxin A, in the environment and their influence on living organisms make them an interesting research problem. Numerous complications of intoxication with these substances are known, however, particular attention is paid to the effects on the skeletal and nervous systems. The inflammatory effect, presented by the increase in the concentration of cytokines - IL-6 and TNF-α may influence the immune dysregulation present in bone metabolism disorders, as well as in neurodegeneration. Mycotoxins also contribute to osteodegeneration by modifying vitamin D metabolism. Interestingly, and still unexplored, is the mechanism of intrauterine influence on bone metabolism and neurodegeneration processes. Understanding the above mechanisms may help in monitoring the toxic effects of intoxication with these toxins. It can also help develop methods of therapy for poisoning with this compound, in animals and humans.
Keywords: bone metabolism; bone development; bone remodelling; mycotoxin; aflatoxin B1; ochratoxin A; neurodegeneration.

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INTRODUCTION

Mycotoxins, including aflatoxin B1 and ochratoxin A, are widespread compounds in the natural environment of humans, which results in a high probability of intoxication with these substances, therefore many scientists undertake research to define the mechanisms of the toxic effects of aflatoxin B1 and ochratoxin A and discover methods to neutralize them. The inflammatory effect of these mycotoxins may influence the immune dysregulation present in neurodegenerative diseases and bone metabolism disorders, as well as in a number of other pathologies. The use of IL-6 and TNF-α as prognostic factors is currently the subject of numerous studies. Mycotoxins are substances with a toxic effect which are formed as a secondary product of the metabolism of mold fungi commonly found in the natural environment [1]. This group of compounds includes aflatoxins and ochratoxin A [2]. Aflatoxins were discovered in 1960, when in England more than 100,000 turkeys died from poisoning, as it turned out three years later, with aflatoxins, which were in the feed with groundnut grits [3]. The first cases of poisoning with ochratoxin in poultry were described in the United States by Hamilton and colleagues in the 1980s and concerned turkeys, laying hens and broiler chickens [4-6]. Aflatoxins are a group of compounds with specific chemical properties, produced by the genus Aspergillus, among which two species are abundant: Aspergillus flavus and Aspergillus parasiticus [7]. They are saprotrophs that develop rapidly in tropical and subtropical climate conditions, that is, at high temperature (optimally at 27°C) and high humidity. They thrive in soil and in damaged or decaying plants [7-10]. The spores are transmitted by insects (eg. crop pests), such as the European corn borer (Ostrinia nubilalis), but also by wind currents [7,11]. Ochratoxin A is produced by eleven species of the genus Aspergillus and ten species of the fungus Penicillium. The main species of fungi that produce ochratoxin A are P.verrucosum, A.ochraceus and A.carbonarium [12].

The growth of these fungi is not synonymous with the production of a large amount of ochratoxin, which depends on a number of internal factors such as water activity, pH and redox potential, as well as external factors such as relative humidity, temperature, oxygen availability and the quality and composition of the substrate [13]. It has been proven that the production of ochratoxin A is greater in illuminated places than in dark places, as well as in the presence of iron, copper and zinc ions and at pH-5.5. The higher pH, makes the slower the synthesis of ochratoxin [14]. Cereal grains are most often contaminated, which results from improper storage and transport conditions, for example, trade in food products between countries favors the spread of these fungi [8,15-17]. Other sources are nuts, dried fruit, vegetable oils, medicinal plants stored in the form of dried herbs, herbal spices, corn, rice, as well as meat and dairy products of animals consuming feed contaminated with these compounds [9,15,18-21].

Animals through their feed or the environment in which they live may be exposed to mycotoxins, which may accumulate and enter tissues intended for consumption by the consumer, such as milk or meat. This transition is referred to as carry-over [22]. The most common and also the most toxic is aflatoxin B1. It owes its toxicity to the presence of a lactone ring and two furan rings (one of them is located at the extreme end and has a double bond, which determines such a significant toxicity and is responsible for the possibility of tight binding with a protein or DNA molecule, as well as for disrupting the cell's operation) [23]. Aflatoxin B1 metabolism takes place in the liver with the participation of cytochrome P450, in the course of numerous reactions, such as hydroxylations, demethylations, epoxidations, substances excreted with urine and bile from the body, but also adducts (such as the covalent bond AFB1-8,9-) exo-epoxide with nitrogen N-7 of guanine), which accumulate in the microsomal fraction by binding with nucleic acids and liver protein. These compounds are mutagenic and contribute to the development of neoplasms [24-27]. The International Agency for Research on Cancer (IARC) classified aflatoxin B1 (AFB1) in Group 1 as "carcinogenic to humans" in 1993 [28]. They have an immunotoxic effect [29].

There is no safe concentration of mycotoxins in the consumed food [30]. Mycotoxins, both aflatoxins and ochratoxin A, are dangerous to humans, which is why their effect on animal organisms is so interesting. Food poisoning with aflatoxins may be acute or chronic, depending on the dose and time of exposure. Acute poisoning is characterized by pulmonary edema, abdominal pain, nausea, vomiting, jaundice, fever, internal organ haemorrhage, convulsions and even coma. Chronic poisoning is characterized by damage to the liver, kidneys and the central nervous system. There may be symptoms of liver cirrhosis, edema of the lower limbs, skin and respiratory allergies, impaired growth and development, mental disorders, increased susceptibility to infections, lack of appetite, malaise, etc. Aflatoxin is important in the spread of HIV infection through its immunotoxic effects to a CD4 + cell mediated immune response. (CD - cluster of differentiation) [31-35]. Ochratoxin A is also associated with the induction of many negative effects in animal and human body. It causes a dangerous disease called Balkan endemic nephropathy (BEN) [36-41].

Ochratoxin A is three times more toxic to poultry than aflatoxin, therefore ochratoxin poisoning is the most frequently observed in this group of animals [42]. Poisoning was observed in broilers receiving a single feed containing 16 mg/kg ochratoxin A, they showed signs of acute ochratoxicosis manifested by apathy, diarrhea, impaired motor coordination, exhaustion and death of chickens within 22-25 hours from the administration of the toxin [43]. Chronic OTA poisoning is associated with decreased feed consumption, increased thirst and kidney changes [44,45]. In contrast, chronic poisoning with ochratoxin A in poultry is characterized by disorders of: growth, blood coagulation, phagocytosis, bone tissue integrity and intestinal epithelium [46-50]. In the European Union countries, the maximum permissible doses of ochratoxin A are established to prevent intoxication with human food, which are, for example, 5 μg / kg in cereals (rice, barley) or 3 μg / kg for cereal products and cereal grains intended directly for human consumption [51]. Ochratoxin A has been recognized as a carcinogen by the International Agency for Research on Cancer (IARC) and has been classified as carcinogen group 2B [51,52].

Aflatoxin B1 is known mainly as hepatotoxin, and ochratoxin A as nephrotoxin, however, the destructive effect of ochratoxin A and aflatoxin B on bone tissue and neurodegeneration processes is extremely interesting. Mycotoxins, including aflatoxin B1 and ochratoxin A, are widespread compounds in the natural environment of humans, as mentioned above, which results in a high probability of intoxication with this substance, and therefore many scientists undertake research to define the mechanisms of their toxic action and to discover ways to neutralize them. Abnormalities in cytokine concentrations and disturbances in enzyme systems induced by these mycotoxins can lead to cell dysfunction. The pro-inflammatory effects of these substances may contribute to the loss of bone mass as well as the immune dysregulation present in neurodegenerative diseases. The use of IL-6 and TNF-α as prognostic factors is currently the subject of numerous studies.

Interleukin 6 (IL-6) and TNF-α are pro-inflammatory cytokines with multidirectional action (Fig. 1). The first of them is produced by monocytes and macrophages. Takes part in the immune response, hematopoiesis and carcinogenesis. It facilitates tumor growth by inhibiting apoptosis of neoplastic cells and inducing angiogenesis within it. It stimulates inflammatory processes by stimulating the differentiation of B lymphocytes into plasma cells, together with IL-1 it activates T lymphocytes, induces the production of acute phase proteins, and at the same time inhibits the production of TNF-α [53-55]. The second one, TNF-α, is mainly produced by monocytes and macrophages. It stimulates the production of acute phase proteins, phagocytosis, the formation and differentiation of B, T and NK lymphocytes. It induces an increase in the concentration of free radicals inside cancer cells, thus leading to apoptosis [56,57].

THE INFLUENCE OF MYCOTOXINS ON THE BONE METABOLISM

In a number of studies, the toxic effect on bone and nervous tissue has been proven, an example is study presented by Rouibah in doctoral thesis [52]. The author noted that the measurements of the diameter of the tibia and the degree of its porosity clearly proved the inhibition of bone tissue synthesis after 10 days the administration of ochratoxin. In the following days, bone synthesis resumed with increasing bone mass, there was apparent increase bone mineral density (BMD), manifested with a lower degree of porosity. Therefore, despite the resumption of bone tissue synthesis, it was not able to support the weight of the bird and, as a consequence, the bones were deformed. Huff, et al., [58] verified the effects of graded levels of both aflatoxin and ochratoxin on bone tissue using young chickens as animal model. During the examination the breaking strength, displacement before failure, and diameter of their tibias were determined. The detrimental effect of mycotoxins on bone tissue was manifested by the deceased mechanical resistance of bone and increased its flexibility. What is more, the mechanical weakening of bone was involved by the lower dose of aflatoxin (2.5 μg/g) and ochratoxin (2 μg/g), while the higher doses (5.0 and 4.0 μg/g, respectively) affected bone growth by decreasing of the bone central diameter [58]. The decease of mechanical properties of tibia after intoxication with ochratoxin A was reported by Duff et al. [49]. The authors observed generalized osteopenia of the skeleton with disturbances endochondral and intramembranous formation of bone tissue. In conclusion, the authors postulated that ochratoxin directly influence on osteoblast metabolism and in consequece cause the development of osteoporosis [49]. Interestingly, this opinion is the first one that indicates the direct influence of mycotoxins on metabolic disorders of the skeletal system.

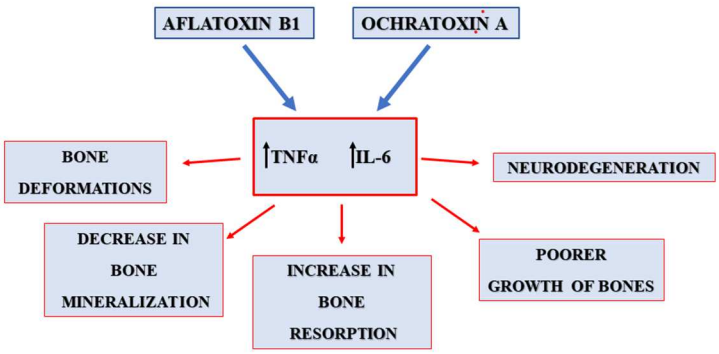

Fig. 1 The biological effects of Aflatoxin B1 and Ochratoxin A.

Despite the existence of a direct relationship between mycotoxins and bone tissue metabolism, the indirect effect of these toxins on bone is also important. The relationship of IL-6 and TNF-α and bone metabolism and osteoimmunology is extremely interesting, as evidenced by one of the publications from 2020 conducting a meta-analysis exploring the above topic [59]. TNF-α and IL-6 regulate immunity through various mechanisms. TNF-α inhibits the activity of osteoblasts at some stages of differentiation, and in addition stimulates the proliferation and differentiation of osteoclasts (Fig. 1). IL-6 acts in a complex way by affecting osteoblasts and osteoclasts, resulting in double effects. It can inhibit the action but also stimulate the action of osteoblasts. In in-vitro studies, an increase in the number of IL-6 receptors on the surface of osteoblasts was observed, a decrease in preosteoblast proliferation and differentiation, and the finally induction of apoptosis of mature cells. At the same

time, there are different reports on the inhibition of osteoblast apoptosis by IL-6 and the intensification of their differentiation through the p21 gene. Futhermore, both TNF-α and IL-6 can mediate the activity of osteocytes [59,60].

Ochratoxin A intoxication is associated with an increase in the expression of IL-6 and TNF-α, as is the exposure to aflatoxin B1, which suggests their indirect influence on bone destructive processes [29,61]. Weidenbach et al. [62] found that Ochratoxin A induces TNF-α release from the isolated and bloodfree perfused rat liver. Wang et al. [61] described an increase of IL-6 level in the histological analysis of liver tissue after OTA treatment. Mehrz et al. [63] documented that aflatoxin B1 treatment induces pro-inflammatory response in murine CNS-derived cells by the increase of IL-6 and TNF-α. The increase in TNF-α and IL-6 levels after expression into aflatoxin B1 was also assessed by Meissonnier et al. [29] by real time PCR in spleen of pigs.

THE EFFECTS ON THE VITAMIN D METABOLISM

Furthermore, there is another indirect mechanism of the influence of mycotoxins on bone metabolism and it depends from vitamin D metabolism. This relationship was reported by Sergeev et al. [64,65]. The authors described the effect of aflatoxin B1 and T-2 on Ca metabolism and the hormonal system of vitamin D in young rats. Administration of mycotoxins involved hypocalcemia and decreased calcium absorption. What is more, they noted the decreased the activity of alkaline phosphatase in the mucosa of the small intestine, and disturbances in trabecular bones compartment, leading to osteopetrosis. The authors observed also lowering activity of 25-hydroxylase in liver by 58% and decreased serum level of 25(OH)D3 (28%) as a consequence of intoxication with T2 toxin, while the aflatoxin B1 cause the reduction by 33% and 34%, respectively. Next, the activity of 25(OH)D3-1-hydroxylase in kidney was unchanged while the 24-hydroxylase activity tended to decrease. Additionally, the expression of nuclear 1,25(OH)2D3 receptors in the small intestine mucosa decreased, while the cytoplasmic receptors increased 2.5-fold, which indicates a decrease in the internalization of the receptors Sergeev et al. [64]. Aflatoxin B1 may interfere with various vitamin D-induced molecular pathways [66]. In conclusion, disturbances of calcium metabolism due to the influence of mycotoxins may be related to vitamin D3 deficiency Sergeev et al. [65].

BONE DEFECTS RELATED TO INTRAUTERINE EXPOSURE TO MYCOTOXINS

The effect of mycotoxin on the fetus is multidirectional and the osteotropic effects often depend on the dose burdening the fetus. Nevertheless, regardless of the dose to which the fetuses are exposed, the effect of mycotoxins determines disturbances in ossification processes [67]. This relationship is also common to different animal species. This is proved by the studies of Abdulrazzaq et al. [68], El-Nahla et al. [69] and Fetaih et al. [70]. These authors, in studies with mice, rabbits and rats, respectively, using different doses, exposure times and routes of administration, showed disturbances in ossification of the axial skeleton. Despite this common dependence of the cited studies, differences can also be noticed. Abdulrazzaq et al. [68] while administering aflatoxin B1 intraperitonealy in a single dose of 20 mg/kg to mice on the 7th or 13th day of fetal life, he also noted abnormalities of ossification of the supercatal bone, pelvic and thoracic limbs, as well as metacarpal/metatarsal phalanges. Rabbits that were an animal model in the studies of El-Nahla et al. [69] treated with aflatoxin B1 in the dose of 0.05 mg/kg by gavage were characterized by malformation of ribs and sternales and shorter pelvic limb bones. Fetaih et al. [70] using rats and the aflatoxin B1 in the dose of 1 mg/kg applied by gavage between 6th and 15th days noted additionally disorder in ossification of thoracic and pelvic limb bones. Wangikar et al. [71,72] used ochratoxin or aflatoxin b1 exclusively and in combination in rats between days 5 and 15 of gestation. The authors showed abnormal ossification of the skull manifested by its reduced thickness, leading to encephalopathy following the administration of ochratoxin. It should be assumed that the underlying causes of these changes are the direct influence of mycotoxins on osteoblasts and osteoclasts, causing disturbances in osteoid mineralization, which has a direct impact on the periosteal new bone formation [50].

INFLUENCE ON THE NEURODEGENERATION PROCESSES

In 2017, Mehrzad et al. [63] Described the pro-inflammatory effect of aflatoxin B1 on CNS-derived cells. The effects of aflatoxin B1 on cells and nervous systems are poorly understood, and mouse pure primary astrocytes, subventricular neural precursor cells (NPC) and microglial cell lines (BV2) were used to evaluate it. Cells were exposed separately to the appropriate level (20 ng/ml) of AFB1 for 1, 2, 3, 6, 12, 24 and 48 hours in culture.

At each time point of the study, the following were determined: total free radical production, production of cytokines IL-1β, IL-10 and the IL-6, TNF-α of interest, also a set of genes involved in the immediate response to danger, such as TLR2, TLR4 and iNOS, etc. There was a significant increase in the number of free radicals in microglial cells after 24 hours of the test, their slight increase in the remaining tested cells. The toxin also induced an increase in pro-inflammatory cytokines, in microglia cells the increase in TNF-α was dominant, and in astrocytes IL-6. An increase in TLR2, TLR4, MyD88 and NF-κB mRNA expression was also observed. The above results may suggest an effect of an appropriate dose of AFB1 on the immune disorders observed in neurodegenerative diseases [63].

Another study assessing the effect of mycotoxin on the process of neurodegeneration, this time ochratoxin A, is a study by Chansawhang et al. [73]. Researchers assessed the effect of this toxin on microglia activation in the central nervous system, and also investigated the effect of corticosteroids on the above process. Murine microglial cells (BV-2) were stimulated with OTA, then the severity of OTA-induced inflammation was determined by pretreatment with corticosterone. The expression of proinflammatory mediators including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and inducible nitric oxide synthase (iNOS) was determined. Phosphorylation of mitogen activated protein kinases (MAPKs) was analyzed by western blotting. A significant increase in the expression

of all of the above pro-inflammatory markers was observed.

Pre-treatment with corticosterone was performed, enhancing the neuroinflammatory response to ochratoxin via a mineralocorticoid receptor (MR) dependent mechanism associated with increases in extracellular signal regulated kinase (ERK) and p38 MAPK. The results of this study suggest a direct effect of ochratoxin A on microglia activation and indicate the enhancement of neurodegeneration by low levels of corticosterone [73].

CONCLUSIONS

The wide distribution of mycotoxins in the environment and their influence on living organisms make them an interesting research problem. Numerous complications of intoxication with these substances are known, however, particular attention is paid to the effects on the skeletal and nervous systems. Periodic inhibition of the proliferation of osteogenic cells by mycotoxins, both aflatoxin B1 and ochratoxin A, a decrease in bone mineralization and the influence on lower collagen synthesis lead to poorer growth, less stiffness and bone deformation. One of the mechanisms of action of these mycotoxins is the increase in the concentration of cytokines - IL-6 and TNF-α, which have a destructive effect on bone tissue. Mycotoxins also contribute to osteodegeneration by modifying the vitamin D metabolism. Interesting, and still unexplored, is the mechanism of intrauterine influence on bone metabolism and neurodegeneration processes. By increasing the concentration of cytokines IL-6 and TNF-α, these mycotoxins also influence the processes of neurodegeneration. Understanding the above mechanisms may help in monitoring the toxic effects of intoxication with these toxins. It can also be helpful in developing methods of therapy for poisoning with this compound, in animals and humans.

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Radosław Piotr Radzki
Szkoła Doktorska, Uniwersytet Medyczny w Lublinie
radoslaw.radzki@up.lublin.pl