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Review

The importance of accounting for sex in the search of proteomic signatures of mycotoxin exposure

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ABSTRACT

Mycotoxins are natural food and feed contaminants that are toxic to human and animals. Proteomics is an adequate toolbox to investigate the mode of action and the effects of mycotoxins, as these toxicants often alter protein synthesis and degradation, as well as induce changes of important post-translational modifications. For instance, the contaminant deoxynivalenol induces a severe ribosomal stress that affects protein production, whereas the toxin Fumonisin B1 can alter the phosphorylation of a large number of proteins, and patulin is a potent proteotoxic molecule. The response to most mycotoxins is sex-dependent, males being generally more sensitive than females. In addition, for some toxins, the toxic effects observed were different for each sex. Nevertheless, the importance of accounting for a sex-dependent response is often overlooked in toxicology studies involving mycotoxins. Here we review the information that proteomics has provided in pre-clinical studies of mycotoxin exposure as well as the differential response of males and females to these molecules to highlight the need of including male and female individuals when evaluating the impact of mycotoxins in the cell proteome.

Significance: The current trend in mycotoxicology is the combination of several -omics techniques in order to understand the mechanism of action and effects of these toxic natural food contaminants. One of the goals of these experiments is to determine "potential biomarkers" of mycotoxicoses. Nevertheless, the strategy followed in biomarker research must take into account as many possible factors as possible in order to find robust biomarkers for differential diagnosis. Among the factors that can have an influence in the response to mycotoxins, one of the most important is sex. Traditionally, males are preferentially used in research, as they are more sensitive to mycotoxins and their response is not dependent on hormonal levels, thus less variable. However the intrinsic and hormonal differences between sexes makes that results obtained in males are often not directly transferrable to females. In this review, we want to highlight (1) that proteomics has a great potential on mycotoxin research, and (2) the need in taking into account sex differences in proteomic studies, mostly when the discovery of robust biomarkers of mycotoxins response is desired.

1. What are mycotoxins and why are they so important?

Many fungal species from the *Alternaria*, *Aspergillus*, *Claviceps*, *Penicillium* and *Fusarium* genera frequently colonize agricultural products such as grains, cereals, fruits, spices and animal forages. In certain conditions of temperature and humidity, the growth of these filamentous fungi results in the synthesis of secondary metabolites known as mycotoxins that will accumulate in the substrate where the fungi grow [1–3]. Mycotoxins are small molecules of diverse chemical nature that are toxic to human and animals at low doses. The production of these metabolites can take place before crops harvest, and/or during storage. Mycotoxins are, therefore, natural food and feed contaminants and so their presence in the food chain is unavoidable nowadays [1–3]. Actually, in spite of the growing efforts in improving pre-and post-harvesting agricultural practices, recent surveys indicate that 70% of raw materials are contaminated with these toxins [4].

Mycotoxins represent a major issue in food safety and public health, as they are related with the development, exacerbation and/or aggravation of very distinct syndromes, diseases and dysfunctions in mammals [5,6]. In order to reduce dietary exposure, many countries have developed regulations and recommendations for the presence of some mycotoxins. For instance, the EU has set maximum levels for zearalenone, deoxynivalenol, aflatoxins, fumonisins, Ochratoxin A and patulin in human food [7]. Generalities on the structure, major producing fungi, adverse effects and mode of action of these toxins are summarized in Table 1.

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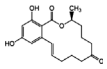
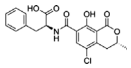
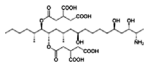
Table 1
Major mycotoxins: formula, producing species, affected crops and adverse effects.
Adapted from [137].

Mycotoxin	Chemical structure	Major producing fungi	Main contaminated crops	Main adverse effects & mode of action
Deoxynivalenol (DON)		*Fusarium graminearum*, *F. culmorum*	Wheat, maize, barley, oats, rye	Feed refusal, emesis Reduction of growth Immunotoxicity Not classifiable as to carcinogenic to humans Binds to ribosomes and activates MAP kinases
Zearalenone (ZEN)		*F. graminearum*, *F. culmorum*	Wheat, maize, barley, oats, rye	Endocrine disruptor (interaction with estrogen-receptors)
Ochratoxin A (OTA)		*Aspergillus* section *Circumdati* or section *Nigri*, *Penicillium verrucosum*, *P. nordicum*	Cereals, nuts, dried fruits, coffee, cocoa	Nephrotoxic (renal tumors) Carcinogenic to animals and possibly to humans
Fumonisin B1 (FB1)		*Fusarium* section *Liseola*, *Aspergillus niger*	Maize (*Fusarium* spp.) Grapes (*A. niger*)	Inhibition of sphingolipid biosynthesis Induction of apoptosis in liver Tumorigenic in rodents Possibly carcinogenic to humans
Aflatoxin B1 (AFB1)		*Aspergillus* section *Flavi*	Maize, peanuts, nuts, pistachios, other dried fruits	Genotoxic carcinogen Carcinogenic to humans
Patulin (PAT)		*Byssochlamys nivea*, *Penicillium expansum*, *Aspergillus* section *Clavati*	Fruits especially apples, silage	Gastrointestinal ulceration Immunotoxicity Neurotoxicity

2. What are the main challenges we face in mycotoxicology research nowadays?

Outlining the real danger associated with the presence of mycotoxins in food is not a simple task, as this danger is defined by three main factors that can interplay, and that are not all well understood nowadays [8]. The first factor is the presence of the so-known "emerging mycotoxins" for which not much information on their toxicity is available. In fact, the advancement of new screening and more sensitive detection techniques keeps identifying new candidates to enter the mycotoxin family [9], as well as is giving precise information of their occurrence. However, the real danger associated with the presence of these "emerging mycotoxins" is yet to be defined though toxicological studies [10]. A second factor is that mycotoxins can also be present in different modified forms from the "original" or "parent" mycotoxin [11,12]. The modified forms can be more toxic than the parent compound, and are produced either in the plant or in the human/animal organism in an effort in reducing biologically active fungal metabolites or during food/feed processing [13]. The third factor is the combination of mycotoxins and their interaction. Actually, different mycotoxins are simultaneously present in variable proportions in food and feed. This is because fungi infecting crops can produce more than one mycotoxin, and/or more than one fungal species can contaminate the same commodity [14,15]. Furthermore, different commodities containing different contaminants are associated in the same meal. As simultaneous exposure to different toxins can result in antagonistic, additive or synergistic effects, the presence of mycotoxin mixtures can lead to toxic effects unpredicted by the simple combination of the effect of each toxin alone [14,15]. This means that even if two or more toxins are present in concentrations below those considered safe, the exposure to the mixture can result in a toxic effect [1,16]. The pathology associated with the exposure to mycotoxins is called mycotoxicosis [2]. The symptoms of a mycotoxicosis can be very variable and depend mainly on the type of mycotoxin (some of them summarized in Table 1), but also on its concentration and the duration of the exposure [3]. The combination of concentration and time of exposure defines if the mycotoxicosis is acute (short-term exposure to a high dose of toxin) or chronic (long-term exposure to low doses). Whereas acute toxicity has a rapid onset and a typical symptomatology, the chronic exposure to low doses of mycotoxins has a more insidious effect, often resulting in unspecific syndromes such as reduced feed intake and slow growth in farm animals, or in cancer or other severe syndromes in humans [2,17]. The presence of mycotoxins can also exacerbate pre-existing inflammatory processes like chronic intestinal inflammatory diseases [18,19] or increase susceptibility to certain infections such as coccidiosis in poultry, colibacillosis, salmonellosis in pig and mice, swine respiratory disease, and aspergillosis in poultry and rabbits, among others [5,20]. Additionally, some host-related factors can influence the in-vivo effect of mycotoxins. The sensitivity to mycotoxins depends in general on differences in the digestive physiology and anatomy, metabolism and excretion capabilities. For each mycotoxin, additional factors related with their specific mode of action/toxicity are important. As a result, the onset of an exposure to a given mycotoxin depends on factors like species, sex, age, nutritional status, pre-existing diseases and microbiota [17,21–26]. Sex has a great influence in the response to xenobiotics including mycotoxins. This is mainly due to differences in hormonal levels as well as differences in pharmacokinetics and pharmacodynamics, although other factors seem to be also involved, such as the influence of hormones in the expression of hepatic detoxifying enzymes or the intrinsic differences in cell composition and structure [27–31]. A sexual dimorphism has been described for the toxicity of many mycotoxins, which will be reviewed below.

3. The proteomic toolbox in mycotoxicology

The result of the consumption of food contaminated with mycotoxins depends on many elements such as the presence of modified forms, the combined presence of different toxins, their interaction, and several factors related with the host. To analyze all the possible effects of mycotoxin exposure in this complex context we need to privilege unsupervised, -omics techniques like proteomics instead of the traditional reductionist, supervised technical approaches that often explore only one specific question. However, if we perform a quick bibliographic search, we can observe that the use of proteomics in mycotoxicology has been mainly restricted to the study of the pathways involved in mycotoxin synthesis in the corresponding fungal species, and it is still giving its first steps in studying the host response to the presence of mycotoxins. Actually, PubMed searches using the words "proteomics" and "mycotoxin" retrieved 112 studies, from which only 19 were oriented at evaluating the toxic effects of mycotoxins in the