3.3. Toxicological interactions between mycotoxins

I. Alassane-Kpembi[a,b] and I. P. Oswald[a,b]

[a] INRA, UMR1331, Toxalim, Research Centre in Food Toxicology, 180 chemin de Tournefeuille, F- 31027 Toulouse cedex 3, France; [b] Université de Toulouse, INP, UMR1331, Toxalim, F- 31000 Toulouse, France

ioswald@toulouse.inra.fr

Abstract

Humans and animals are exposed to several toxins at the same time. The toxicity of combinations of mycotoxins cannot always be predicted based upon their individual toxicities. Interactions between concomitantly occurring mycotoxins can be antagonistic, additive, or synergistic. Several approaches have been used to determine the interaction between mycotoxins. The theoretical biology-based models of additivity, especially the Chou-Talalay method, are the most advanced. Using this latter model in several cellular systems and in an ex vivo intestinal explants system, we have observed a synergistic toxicity for trichothecenes, especially at low concentrations. The synergistic effects observed after exposure to a mixture of low concentrations of mycotoxins could pose a significant threat to public health.

Introduction

Mycotoxins are toxic secondary fungal metabolites produced under specific environmental conditions by a variety of food commodity spoiling molds, mainly *Aspergillus*, *Penicillium* and Fusarium. Global surveys indicate that 72% of the samples of feed and feed raw materials are positive for at least one mycotoxin [1]. Human and animals are simultaneously exposed to several mycotoxins and there is a need for an update of the traditional single mycotoxin risk assessment approach [2]. Indeed, simultaneous exposure to different toxins could result in antagonistic, additive or synergistic effects. Therefore, an increasing number of mycotoxin studies are devoted to their combined toxicity, especially the exploration of the type of toxicological interactions.

The toxicity of a mixture is complex. Testing for a possible interaction in mixture toxicity requires a comparison of the actual experimentally determined effects of the mixture to theoretically expected no interaction effects, the so-called additive effects. This prediction of no interaction is made based on the toxicity of the individual compounds. Stronger-than-

expected effects indicate synergism whereas lower-than-expected effects indicate antagonism. Several methods have been proposed but a generally agreed definition of zero interaction does not yet exist [3].

As for other food contaminants, the gastro-intestinal tract can be considered the first target for mycotoxin toxicity, and gut damages caused by these contaminants may lead to poor intestinal health [4-5]. The possible overlapping intestinal residency times of the numerous contaminants that can be carried by food could also make the gastro-intestinal tract one of the most exposed organs to mixture toxicity. We present here the analytical approaches used in mycotoxin toxicological interaction studies and the preliminary lessons we learnt from the combined toxicity of *Fusarium* mycotoxins towards the intestine.

Experimental approaches to assess mycotoxin toxicological interactions

The arithmetic definition of additivity. Some mycotoxin combination studies considered the additive effect to equal the arithmetic sum of the sizes of the effects for individual mycotoxins when tested separately. So the expected (additive) size for the cytotoxic effect of a mixture was defined as the sum of the cytotoxic effects induced by each mycotoxin alone in mono-exposure experiments [6].

Cytotoxic effect (mycotoxin 1 + mycotoxin 2) = Cytotoxic effect (mycotoxin 1) + Cytotoxic effect (mycotoxin 2)

Although intuitively plausible and very easy to handle, most researchers in the biomedical area seem to agree that combined effects do not simply equal the sum of single effects [7].

Factorial design experiments. The general assumption for mycotoxin studies using factorial design experiments is that when testing the effects of mixtures by different patterns of combination on the one hand, and the effects of each individual compound on the other, the effect of any compound could be predicted by subtracting the mean of the groups not containing the compound from the mean of the other groups [3].

Despite the fact that interaction is definitely revealed by such statistical methods, the nature of interaction with regard to additivity, synergism or antagonism is not clearly explored and has to be inferred indirectly [8].

The theoretical biology models-based definitions of additivity. The most commonly used theoretical biology models-based definitions of zero interaction are the Bliss independence criterion also known as Response Addition, the Loewe additivity model also named Concentration or Dose Addition [9] and the Median Effect Principle of the Mass action law [10].

The Bliss independence criterion applies for combinations of mycotoxins exerting toxicity via different modes and possibly sites of action. Conversely, the Loewe additivity model studies lie on the assumption that the mycotoxins act on the same biological sites, by the same mechanisms of action and differ only in their potency. Relatively simple Loewe additivity model extensions are the isobolographic method and its algebraic variant, the Interaction index.

Another concept that is independent of the mode of action and just considers both the potency (EC50) and the shape of the dose-effect curve for each mycotoxin and their mixture has been proposed. In this new approach, a computerized simulation of the individual dose-effect curves and the additive response from the combined effect of several mycotoxins is obtained using the Median-Effect Equation of the Mass action Law [10].Then interactions can be analyzed by a Combination-index – isobologram method. Besides indicating the type of interaction (additivity, synergy or antagonism), this index allows a quantitative assessment of the magnitude of the interaction.

As of 2015, 82 publications described mycotoxin *in vitro* interactions. Most of these publications (54) described experiments lacking dose-response considerations and assuming arithmetic additivity; a few publications (7) were factorial design experiments; the theoretical biology model-based experiments are gaining increased attention (21 publications).

Analysis of mycotoxins' combined toxicity

The studies concerning the in vitro interactions between mycotoxins mainly concern the regulated mycotoxins, especially aflatoxins, ochratoxins, fumonisins, zearalenone and trichothecenes, a few studies also concern the "emerging" toxins such as beauvericin and enniatins.

Interaction between Ochratoxins and other toxins. Among the 82 publications, 21 concerned the nephrotoxic ochratoxins. Of course, most of these studies involved renal cell lines or renal primary cells cultures with cytotoxicity as the main endpoint. However, mycotoxins associations including ochratoxins have also been screened for genotoxicity via DNA damages, clastogenic effects and mutagenic activity.

Interaction between Aflatoxins and other toxins. 24 papers questioned the in vitro genotoxic and cell viability effects of the hepatocarcinogenic aflatoxins in association with other mycotoxins. Aflatoxins combinations have been assessed for their cytotoxic and genotoxic effects using mainly human or animal primary hepatocytes or transformed cell lines, while papers addressing specifically the mutagenic activity referred to the Ames test using *Salmonella* Typhimurium strains.

Interaction between Fusariotoxins. Papers analyzing the combined toxicity of Fusarium group mycotoxins were the most abundant (37/82). The *Fusarium* mycotoxin combinations studies can be grouped in 3 groups involved (i) the major *Fusarium* toxins (i.e. Deoxynivalenol, Zearalenone, Fumonisin B1) studies, (ii) the trichothecenes group mycotoxin association and (iii) other studies involving the emerging *Fusarium* toxins (beauvericin and enniatins group). Our team gathered evidences for low dose synergistic intestinal toxicity for type B trichothecenes and antagonistic interaction for the most prevalent emerging *Fusarium* toxin enniatin B1 and the type A trichothecene T-2 toxin using the combination index-isobologramm method [11-13]. Proliferating human colorectal adenocarcinoma Caco-2 cells exposed to binary or ternary mixtures of type B trichothecenes (Deoxynivalenol, Nivalenol, and their acetylated derivatives) demonstrated mainly synergistic cytotoxicity at low mycotoxin concentrations (cytotoxic effect between 10 and 30-40 %). At higher concentrations (cytotoxic effect around 50 %), the combinations had an additive or nearly additive effect. This synergistic intestinal cytotoxicity of type B trichothecenes was confirmed on non-transformed porcine intestinal epithelial IPEC cells, with magnitude of synergy for 10% cytotoxicity ranging evaluated to range from 2 to 7. Conversely, porcine jejunal explants culture and IPEC cell culture exposed to enniatin B_1 and T2-toxin mixture exhibited concordant antagonistic toxicity. Altogether, these results indicate that the simultaneous presence of mycotoxins in food commodities and diet may be more toxic than predicted from the mycotoxins alone. Moreover synergistic toxicity may result from co-exposure to trichothecenes. This synergy should be taken into account considering the frequent co-occurrence of trichothecenes in the diet and the concentrations of toxins to which consumers are exposed.

Conclusion

For the main mycotoxins, reference doses for regulatory purpose already exist. Exposure below these levels is usually considered safe. Whether the consumer is also protected against combined exposure to mycotoxins if each component is present below its individual threshold dose is gaining increasing interest. A crucial issue for toxicodynamic interaction analysis is

the statement of the non-interaction response. Factorial designs allow a reliable detection of departure from the additive response, while the Chou-Talalay method makes it possible to determine the type of the interaction and to optionally quantify its magnitude.

Many studies, using different methodological approaches have been used to explore the interactions in mycotoxin combined toxicity. The main conclusions from all these studies are that (i) very few studies used a robust methodological approach for the analysis of the combined effect of mycotoxins, and (ii) the type of interaction in terms of additivity, synergy or antagonism varies accordingly with the mycotoxin combinations, and even with the concentrations tested. More studies employing the isobologram approach are needed to feed a reliable database for the interactions between mycotoxins.

Experiencing the latest approach, our lab demonstrated that trichothecenes mycotoxins exert synergistic low dose intestinal toxicity. These in vitro synergistic interactions deserve to be confirmed *in vivo*.

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