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Combined toxic effects of mycotoxins

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Abstract

It is known for many years that several food items, derived from plants infected by fungi in the field during growing of the plant or during harvest and storage of the food item, can contain concomitantly different mycotoxins. As these combined mycotoxins occur simultaneously in the food item, consumption of the food will lead to a combined intake depending on the absorption rates of the different mycotoxins. Therefore, the question is justified whether such a combined intake of mycotoxins would lead to a possible higher risk for adverse health effects than the intake of one of these mycotoxins alone. It will be dealt with on the basis of some practical cases of such combined intake of mycotoxins of which research data are available. This is the case for citrinin and ochratoxin A, but as the workshop focuses on trichotecenes and so this paper concentrates on these. When the mycotoxins are of similar structure and of the same species, or of the same families, it is likely to expect that the mode of action of the mycotoxins and or the toxicity profiles will be quite similar. This indicates that such related mycotoxins are likely to exert only additive effects, which is important to know. In terms of risk assessment, these mycotoxins could be dealt with by establishing a group daily tolerable intake (TDI) or a provisional tolerable weekly intake (PTWI). In terms of risk assessment those mycotoxins which interact in synergistic manner are of more concern. It is concluded that, at present tools are not fully developed to establish the type of interaction or whether there is any interaction at all.

Keywords: Addition; Antagonism; Synergism; Combination toxicology; Mycotoxins

1. Introduction

Several mycotoxins, either from the same or from different fungal species, occur simultaneously in plant products. However, its implication for food safety assessment is generally not known, as there is relatively little information on the interaction between concomitantly occurring mycotoxins and the consequence for the toxicity. There are several combinations of mycotoxins that frequently occur as established in analytical–chemical monitoring programs. These combinations are summarised in Table 1.

2. Mechanistic interactions of mycotoxins; theory

The data on combined toxic effects of mycotoxins are generally limited, particularly with respect to trichothecenes. Most data available are related to ochratoxin A (OTA), which will be discussed later. The available data show that adequate studies to

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Table 1
Frequently occurring combinations of mycotoxins in different plant products

Mycotoxins	References
Ochratoxin and Citrinin	Pohland et al., 1992; Vrabcheva et al., 2000
Ochratoxin and Zearalenone	Halabi et al., 1998
Ochratoxin and Penicillic acid	Stoev et al., 2001
Ochratoxin and Aflatoxin B1	Sedmikova et al., 2001
Patulin and Citrinin	Martins et al., 2002
Fumonisin B1 and Moniliformin	Gutema et al., 2000
Aflatoxin B1, Fumosin B1, Zearalenone, Deoxynivalenol, Nivalenol	Sardjono et al., 1998; Eskola et al., 2001; Gonzalez et al., 1999
Deoxynivalenol, Nivalenol, Diacetoxyscirpenol, T-2, HT-2, and other trichothecenes	Eskola et al., 2001; Pronk et al., 2002

establish antagonistic, additive or synergistic effects after combined exposure to mycotoxins are rare, and it is known that the issue of combined toxicity is very complicated. In general, a lot of such studies are difficult to interpret. When a special endpoint is studied and the experimental design and the statistical aspects are well addressed, conclusion on the type of interaction might be more straightforward. The best approach to start with is to try to understand how mycotoxins can interfere at cellular level and thus interact with the toxicity of another mycotoxins. Riley (1998) considered these aspects and stated that study of the mechanism of action of toxic compounds is founded in the belief that cells are molecular machines. To understand how mycotoxins interfere with the cellular machinery, it is only necessary to understand how toxic compounds alter behaviour of the molecules of life. In Table 2 examples are given of how some mycotoxins interact with cell functions and molecules of life and/or prevent their biosynthesis.

Fig. 1 indicates where in the cascade of actions a mycotoxin can interfere with cellular functions (Riley, 1998; Riley and Norred, 1996), and how exposure to multiple toxins can lead to extremely complicated biological responses within a relative simple system like a single cell. Mycotoxins with similar mode of action would be expected to have at least additive effects. Conversely, some interactions could have subtractive effects, for example, the ability of cyclopiazonic acid to prevent the lipid peroxidation induced by patulin or the ability of fungal serine palmitoyl transferase inhibitors, such as sphingofungins, to prevent the fumonisin-induced accumulation of free sphingoid bases. Unfortunately, mycotoxins as a group cannot be classified based upon their mechanism of action. This is not surprising as one considers the diversity of chemical structures that are encompassed by the group of secondary fungal metabolites called mycotoxins (Riley, 1998). Nevertheless, an understanding of the mode of action in simple *in vitro* stems can provide a rational basis for predicting interactions between mycotoxins. In this manner, it can also help setting priorities for specially designed studies to establish interactions, preferably those suspected to reveal synergistic actions.

Riley (1998) presented some examples of which only the interactions of Deoxynivalenol (DON), fumonisin, OTA with infectious agents are mentioned. In practice, the interaction of aflatoxin B1 and hepatitis B infections in man is well known; however, the ability of fumonisins or cyclopiazonic acid on hepatitis B infectivity and pathogenicity have not been studied (Riley, 1998). Similarly, the possible interaction between OTA and certain viruses, such as the hantane virus, which are both endemic in the Balkan area, have not been studied yet. Both this virus and ochratoxin are known to cause chronic kidney damage (Pfohl-leskowicz et al., 2002). The ability of DON (and other trichothecenes) to suppress immune response to pathogens in animals is well documented (Riley, 1998). Considering such information together could indicate possible combined toxic effects, which can lead to renal disease (Fig. 2).

The theoretical consideration based on the cellular mode of action what interaction between mycotoxins can be predicted is not a final answer. The toxicokinetic behaviour, metabolism and the toxicodynamic aspects are of influence on the final outcome when

Table 2
Probable primary biochemical lesions and the early cellular events in the cascade of cellular events leading to toxic cell injury or cellular deregulation of some selected mycotoxins

Mycotoxin	Initial lesion → cascade of events	References
Aflatoxin	Metabolic activation → DNA modification → cell deregulation → cell death/transformation	Riley and Norred, 1996; Eaton and Gallager, 1994
Citrinin	Loss of selective membrane permeability → cell disruption → cell death (apoptotic?)	Ansari et al., 1991
Deoxynivalenol	Inhibition of protein synthesis → disruption of cytokine regulation → altered cell proliferation → cell death apoptosis?	Rotter et al., 1996
Fumonisins	Sphinganine N-acyltransferase → disrupted lipid metabolism → cell deregulation → cell death/apoptosis	Riley et al., 1996
Moniliformin	Pyruvate and α-ketoglutarate decarboxylase → loss of respiration control → cell death	Thiel, 1978
Ochratoxin	Disruption of phenylalanine metabolism → reduced PEPCK → reduced glyconeogenesis → cell death (metabolic activation → inhibition of protein/DNA synthesis → apoptosis?) (Altered membrane permeability → disruption calcium transport → cell deregulation → cell death)	Creppy, 1995; Marquardt and Frohlich, 1992; Benford et al., 2001
Patulin	Nonprotein sulfhydryl depletion → altered ion permeability and/or altered communication → oxidative stress → cell death (inhibition of macromolecular biosynthesis → cell death)	Riley and Showker, 1991; Wouters and Speijers, 1996
T-2 toxin	Inhibition of protein synthesis → ? → cell death (apoptotic?) (transient Ca^{2+} elevation → endonuclease activation → apoptosis)	Ueno, 1991; Bunner and Morris, 1988
Zearalenone	Cytosolic estrogen receptor → estrogenic response → disruption of hormonal control → ?	Mclachlan, 1993; Canady et al., 2001

Adapted from Riley, (1998).

man or experimental animals are exposed to a mixture of some mycotoxins. What happens if different types of cells/tissue are involved, in other words, what is the result of the interaction between different tissues? Similarly, what will be the final result if more organ systems are affected by the mycotoxins? With respect to the toxicokinetic part the application of physiologically based pharmaco kinetic (PBPK) models could be useful.

In practice, the outcome of combined exposure to mycotoxins might either quantitatively or even qualitatively be different from what would be predicted. The result can also depend on the species or the type of endpoint studied (Pohland et al., 1992). Therefore, further specific experiments, which are complicated and include a lot of effort, are needed. These studies need carefully planned experimental designs for more accurate statistical analysis. At present, there is an increase in the successful application of such specially designed studies for chemical mixtures, including ray design, response-surface design and fractionated factorial design (Feron et al., 1998; Groten et al., 1998; Tajima et al., 2002). However, at present there is only one such study performed with mycotoxins, which will be discussed later.

3. Experimental studies on interaction between different mycotoxins

When the data available from toxicity studies in which animals were exposed to several mycotoxins concomitantly is reviewed, it is striking that there are not so many studies performed. Particularly, there is not much information on trichothecenes. Therefore, the example of the combination of OTA and citrinin is outlined here, of which there are relatively more studies. The outcome of these studies can also be

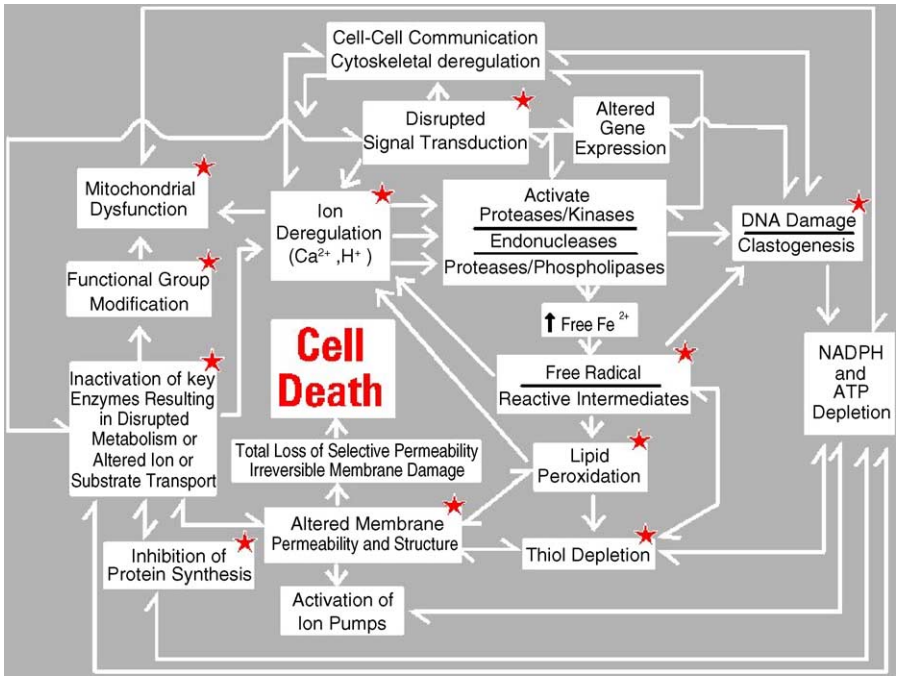

Fig. 1. A flow chart showing interrelationship of toxin initiated biochemical changes which might lead to cell death (Stars indicate interactions which could serve as initiating-events). Taken from: Riley (1998); Riley and Norred (1996).

compared with what would be predicted on the basis of the previously described theoretical consideration. The results of these studies are summarised in Table 3.

Results of the different studies are quite variable. However, both OTA and citrinin are nephrotoxic compounds; a number of studies have shown synergistic effects for endpoints related to the nephrotoxicity. This

Table 3
Interaction of ochratoxin A with citrinin

Test system/measurement	Effect	References
Hepatoma cells—RNA/DNA, protein synthesis	Synergistic	Creppy et al., 1980
Renal cortical cells—organic ion transport	Additive or slightly Synergistic	Berndt and Hayes, 1979
White leghorn pullets—kidney function	Antagonistic	Glahn et al., 1988
Layer chick—renal ultrastructure	Additive	Brown et al., 1986
Mouse to hepato—renal carcinogenesis	Synergistic (only for renal effect)	Kanisawa, 1984
Chick embryo—morphology	Additive	Vesela et al., 1983
Broiler chicks—growth depression, water consumption increase	Antagonistic	Huff et al., 1981
Fetal rat—malformation	Synergistic	Mayura et al., 1984
Rat—renal Na^+–K^+ and Mg ATPase	Synergistic (only for Na^+–K^+ ATPase)	Siray et al., 1981
Mouse—lethal effect	Synergistic	Sansing et al., 1976
Guinea pig—lethal effect	Synergistic (females), additive (males)	Thacker et al., 1972
Dog-nephrosis	Indication for synergitic	Kitchen et al., 1977
Miniature swine—renal–cortical cubes PAH ions and protein synthesis	Synergistic for protein synthesis; other effects additive	Braunberg et al., 1994

Modified from Pohland et al., 1992

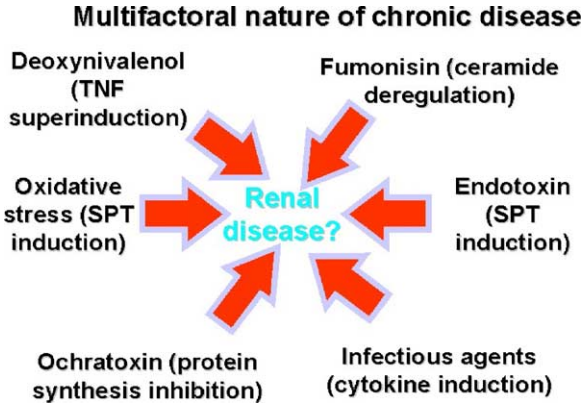

Fig. 2. A theoretical example of mycotoxins and an infectious agent acting in concert to initiate or exacerbate renal dysfunction. Taken from: Riley (1998).

observation is in line with what could be expected on the basis of theory considered. When also data on other mycotoxins are considered, it becomes clear that the animal species, the endpoint studied and the type of mycotoxins largely encompass the resulting toxicity and determine whether the effects of the combined mycotoxins will be antagonist, additive or synergistic.

4. Interaction studies involving trichothecenes

At present, there are a few studies in which a combination of trichothecenes and other mycotoxins have been investigated. Morris et al. (1999) performed feeding studies in young turkeys which were administered 20 mg DON or 100 mg moniliformin (M) alone or in combination. No toxic synergy was observed in this 21-day study. Furthermore, in weaner pigs, combined administration of fumonisin, DON, T-2 together with OTA, in quantities expected to be present in feeds of central European origin, resulted, as a rule, in changes identical to those observed after single administration of OTA (Müller et al., 1999). Only radical formation and antibody formation were suppressed after OTA was combined with fumonisin B1 (FB1) or DON, in contrast to the response to OTA administered alone. Müller et al. (1999) concluded that the data available so far from other literature and those obtained in their studies showed synergistic amplification of clinical and immunosuppressive changes due to the simultaneous action of relatively small quantities of the mycotoxins, OTA, FB1, DON and T-2, is unlikely.

Lusky et al. (2001a, b) investigated the effects of the administration of OTA, zearalenone (ZEA) and DON for 50–60 days to over 90 days, either alone or in combination on health and the residue levels in pigs. Because of the rapid metabolism, the administration of 1000 μg DON/kg feed and 250 mg ZEA/kg feed, either alone or in combination with other mycotoxins, did not lead to detectable residues in tissues and organs. In combination, these mycotoxins do probably represent an additional health risk to the consumer. There was an effect of simultaneous administration of ZEA and DON on the metabolism and secretion of OTA. It was concluded that a clear additive or synergistic effect was not observed.

Some recent studies addressed the combined administration of mycotoxins in in vitro test systems. Human lymphocytes cultures were used to study the individuals sensitivity among humans and the effect on in vitro immunoglobulin (Ig) production. Furthermore, proliferative responses of cells exposed to a combination of two of the four investigated toxins were studied. The four trichothecenes included in this study were T-2 toxin, Diacetoxyscirpenol (DAS), Nivalenol (NIV), and DON. All four of the tested trichothecenes effectively inhibited mitten-induced lymphocyte proliferation. There were no statistically significant differences in sensitivity to the toxins between lymphocytes from female and male blood donors. The effect of a mixture of trichothecenes did not cause a pronounced effect. Combinations of NIV with T-2, DAS or DON resulted in additive toxicity in the lymphocyte proliferation test, while combinations of DON with T-2 or DAS resulted in an inhibition that was slightly lower than that could have been expected from the inhibition produced by the individual toxins. The tested trichothecenes inhibited both proliferation and Ig productions in human lymphocytes in a dose-dependent manner with limited variation in sensitivity between individuals. Combined exposure to two of the trichothecenes resulted mainly in additive or antagonistic effects, although synergistic effects cannot be excluded and should be further investigated (Thuvander et al., 1999).

The interaction between five mycotoxins species (NIV, DON, T-2. ZEA and FB1) was studied using the DNA synthesis inhibition assay in L929

fibroblasts by Groten et al. (1998) and Tajima et al. (2000). First, a central composite design was applied to identify possible interactive effects between mycotoxins in the mixtures (27 combinations from 5^5 possible combinations). The two factor interactions of particular interest were further analysed by the use of a full factorial design (5×5 design) to characterise the nature of those interactions more precisely. Results showed that combined exposure to several classes of mycotoxins generally results in an additive effect with a few minor exceptions indicating synergistic interaction. In general, the nature of the interactions characterised in the full factorial design was similar to the nature of those observed in the central composite design. However, the magnitude of interaction was relatively small for NIV, DON, T-2, ZEA and FB1.The interaction between ZEA and FB1 could be interpreted as synergistic with respect to the inhibition of DNA-synthesis. The interaction between NIV and T-2 was characterised as synergistic because the effect of T-2 was potentiated in the presence of the high NIV level and the lack of parallelism which indicate additivity (Groten et al., 1998; Tajima et al., 2000).

5. Concluding remarks

A theoretical consideration on the basis of cellular modes of actions of the mycotoxins would be very useful as a starting point to screen certain combinations for their possible synergistic or additive effects. Subsequently, a higher priority for further special research should then be given to those combinations of mycotoxins that are expected to include synergistic effects. Additive effects, which seem to be the case for most combinations of trichothecenes, could be implemented within the hazard assessment by establishing a group tolerable daily intake (TDI). However, the number of adequate combination studies for trichothecenes are limited, and more studies are desirable. Future studies should take advantage from research projects, such as those carried out by Groten et al. (1998) and Tajima et al. (2000) which address the issue of interaction of chemical mixtures, and follow their recommended experimental design criteria. It is also important to consider the animal species and the type of endpoint to be used in future studies.

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