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# MOLECULAR INTERACTIONS BETWEEN MYCOTOXINS AND LIVER ENZYMES INVOLVED IN DRUG METABOLISM IN RODENTS AND FARM ANIMALS

# MOLEKULARNE INTERAKCIJE MYCOTOXINA SA JETRENIM ENZIMIMA KOJI SUDJELUJU U METABOLIZMU MEDIKAMENATA U GLODAVCA I DOMAĆIH ŽIVOTINJA

**P. Galtier, G. Meissonnier, Joëlle Laffitte, Isabelle P. Oswald, N. Loiseau**

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## SUMMARY

Mycotoxins are well known for underging liver biotransformation in humans and animal species. Metabolites correspond to either oxydative derivatives such as hydroxymetabolites of aflatoxin $B_1$ or ochratoxin A or hydrolytic derivatives in case of trichothecenes. In some cases, highly reactive epoxides represent the first step in the formation of carcinogenic intermediates like *exo*-epoxides of aflatoxins. Hepatic phase II enzymes including transferases and hydrolases are involved in the conjugation of such oxidative metabolites. In this respect, they are generally considered as detoxifying enzymes: glucuronidation of deacetylated trichothecenes or hydroxy-aflatoxins, or glutathione conjugation of epoxides. The major metabolism of zearalenone consists of reduction leading to estrogenic zearalenols which is characterized by large interspecies differences. Concerning fumonisin $B_1$, this toxin would be poorly absorbed from the gastrointestinal tract and metabolised into hydrolytic products with lower toxic effect as apoptotic compounds.

Interactions between mycotoxins and liver drug metabolizing are crucial in terms of detoxication or bioactivation of these toxins in the organism of the human or animal consumers. Most of these interactions are consequences of the metabolic processes occurring in the liver. They result generally from the activity of cytochromes P450 and transferases. In relation to their hepatotoxicity, several studies demonstrate the inhibitory effects of mycotoxins on certain hepatic biotransformation enzymes, as recently demonstrated in pigs exposed to low doses of aflatoxin B1 or T-2 toxin. In other cases, specific cytochromes P450 or glutathione transferases are significantly increased in terms of both activity and protein expression, namely by aflatoxins, deoxynivalenol or fumonisins. Such results have been obtained in rodents and in farm animals like pigs, rabbits or poultry. The data strengthen the hypothesis that the normal metabolism of endobiotes or xenobiotics by the liver could be altered during chronic exposure to mycotoxins, particularly in farm animals or in humans exposed to aflatoxin B1, ochratoxin A, T-2 toxin, deoxynivalenol or fumonisin $B_1$.

Key words: mycotoxins, liver enzimes, drug metabolism, rodents, farm animals

Pierre Galtier, Guylaine Meissonnier, Joëlle Laffitte, Isabelle P. Oswald, Nicolas Loiseau, Laboratoire de Pharmacologie-Toxicologie UR66 INRA, 180 Chemin de Tournefeuille, 31931 Toulouse, France. e-mail: Pierre.Galtier@toulouse.inra.fr.

## 1. INTRODUCTION

Mycotoxins are a group of secondary fungal metabolites, exhibiting a wide range of chemical structures and a variety of toxicities in various target organs in animals and humans. The toxic effects of mycotoxins can be divided into those requiring metabolic activation in the exposed individuals, and those which do not. The enzymatic biotransformation changes undergone by a toxin in the body usually result in its detoxication consisting of a loss of toxicological activity. However, this is not the only possibility since a toxicologically active metabolite may be formed by bioactivation from a less active parent toxin. The main organ concerned with biotransformation is the liver and many toxins are substrates of hepatic drug metabolizing enzyme systems.

Even though mycotoxins are natural products, the biological fate of certain major toxins like aflatoxin B1 (AFB1), ochratoxin A (OTA), trichothecenes, zearalenone (ZEA) or fumonisin B1 (FB1) have been described. The reactions occurring in xenobiotic metabolism have been divided into two phases, whatever the animal species may be. Phase I reactions are those in which there is a change in the parent molecule by oxidation, reduction or hydrolysis. Microsomal cytochromes P450, flavin-containing monooxygenases, prostaglandin synthases, amine oxydases and dehydrogenases are the major enzymes involved in oxidations whereas reductive metabolism is mainly governed by epoxide hydrolases and reductases. Phase II reactions are those in which a conjugate is formed with a toxin or a toxin metabolite produced in a phase I reaction. The major conjugating enzymes are known to be UDP-glucuronosyltransferases (UGT), sulfotransferases, methyltransferases, *N*-acetyltransferases, glutathione *S*-transferases (GST) and aminoacyltransferases. Several data have been provided to better understand the biotransformation pathways of mycotoxins and to appreciate their consequences in terms of metabolite formation. From these data, it is clear that certain enzymes like cytochromes P450 are able to bioactivate mycotoxins in deleterious intermediates. In other cases, reductases or hydrolases would convert parent toxins into toxicologically active metabolites such as zearalenols or HT-2 toxin. By contrast, conjugation to glucuronic acid, sulfuric acid or glutathione can be considered as detoxifying enzymes leading to conjugates with lesser toxicological properties than the parent mycotoxin.

The aim of the present paper is to review, in rodents and in breeding animal species, the major contributions on the characteristics of hepatic biotransformation pathways undergone by the major mycotoxins in relation with their toxicity and to describe the effects of these toxins on the activity or expression of liver drug metabolizing enzymes.

## 2. BIOTRANSFORMATIONS OF MYCOTOXINS BY THE LIVER

### 2.1. Aflatoxins

In case of aflatoxins, biotransformations play a major role in the disposition and the toxicological activity of these toxins. So, bioactivation has been demonstrated as a prerequisite in most of the toxic and carcinogenic effects of aflatoxins (Eaton and Groopman, 1994). The former metabolism of AFB1 would involve reductions, hydroxylations, epoxidation and *O*-dealkylation. The ketone reduction of AFB1 into aflatoxicol would be associated with the activity of NADPH reductase activity while the other reactions are primarily carried out by the cytochrome P450 enzyme superfamily (P450). Epoxidation of AFB1 into a 8,9 *exo*-epoxide would be the origin of the mutagenicity and carcinogenicity of this mycotoxin. The major DNA adduct formed through *in vivo* or *in vitro* experiments is 8,9 dihydro 8-(N7guanyl)-9 hydroxyAFB1, whereas the extent of *in vivo* covalent binding of radiolabeled AFB1 to DNA in hepatic systems has been correlated with the incidence of cancer.

In addition to epoxidation, P450-dependent monooxygenases oxidize AFB1 to hydroxylated or dealkylated products including aflatoxins P1 (AFP1), Q1 (AFQ1), B2a (AFB2a) and M1 (AFM1). In all cases, efforts have been done to identify which cytochrome P450 isoforms are specifically involved in these various oxidations. In complementary DNA-expressed cytochromes P450, P4501A2 has been shown as the principal P450 enzyme responsible for the activation of AFB1 to its 8,9 epoxide at substrate levels more reflective of dietary exposure, whereas P4503A4 would contribute to epoxidation at relatively high substrate concentrations (Gallagher et al., 1994). On the other hand, P4501A2 would be

involved in the oxidation to AFM1 and P4503A4 responsible for the conversion of AFB1 to AFQ1 in liver microsomes. Additional data revealed AFB1 activation via lipid hydroperoxide-dependent mechanisms consisting of electrophilic DNA binding epoxide catalysed by microsomal prostaglandin H synthase and by cytosolic lipoxygenases (Liu and Massey, 1992).

GST-catalysed conjugation of activated AFB1 is thought to be the most important detoxification system and to play a key role in the protection of tissues from AFB1 toxicity. Actually, both α and μ classes of GST were able to conjugate $AFB_1$-8,9 epoxide (Gopalan et al., 1992) as a function of animal species differences in microsomal activation resulting in different ratios of *exo*- and *endo*-epoxides. In case of humans, Liu et al (1991) demonstrated that incubation of liver preparations from patients expressing high hepatic GSTM1 activity, inhibited the formation of AFB1-DNA adducts more than those from individuals with lower corresponding activity. The hydroxylated or *O*-dealkylated metabolites like AFM1, AFP1, or AFQ1 are readily conjugated to glucuronic acid or sulphate and further excreted in bile or urine of several animal species. The toxicological significance of glucuronidation and sulfation would probably be minimal since the carcinogenic potency of the parent AFB1 is 15 times that of AFM1 (Campbell and Hates, 1976).

### 2.2. Ochratoxin A

In case of ochratoxin A, 4-hydroxyochratoxin-A (4OH-OTA) is the major hepatic metabolite obtained after incubating ochratoxin A with rat liver microsomes and NADPH. Since this metabolism was inhibited by carbon monoxide and metyrapone, this suggests the involvement of cytochromes P450 in the 4-hydroxylation of ochratoxin A by liver microsomes. When ochratoxin A was incubated with pig or human liver microsomes, two epimeric hydroxylated metabolites (4R) and (4S)-OH-OTA were formed in approximately equal amounts. The formation of another metabolite, identified as 10-OH-OTA has been described after incubation of ochratoxin A with rabbit liver microsomes, although this compound has not yet been described *in vivo* (Stormer et al., 19983).

Possible polymorphism in 4-hydroxylation of the mycotoxin was investigated and gave further support to a possible cosegregation of the genes that regulate ochratoxin A and debrisoquine 4-hydroxylations (Castegnaro et al., 1989). Regarding 4-OH-OTA toxicity, this metabolite was determined as effective as ochratoxin A in terms of immunosuppression (Creppy et al., 1983).

### 2.3. Trichothecenes

Regarding trichothecenes, the metabolism of T-2 toxin (T-2) has only been described (Yagen and Bialer, 1993). Briefly, this toxin is known to be widely metabolized in liver. In the first step, it is mainly deacetylated at the C-4 position producing HT-2 toxin (HT-2), and to a small degree at the C-15 position giving T-2 triol. In the second step, these metabolites are conjugated with glucuronic acid and excreted in bile. Two other hydroxy-metabolites were also produced in small amounts: 3'OH-HT-2 and 3',7diOH-HT-2. These metabolic pathways were confirmed in most of *in vivo* investigations using various animal species.

A large variety of investigations demonstrated therefore the extreme complexity of T-2 metabolism in biological matrices. In breeding animal species, Bauer (1995) identified HT-2, 3'OH-T-2, 3'OH-HT-2, neosolaniol, 4-deacetylneosolaniol, T-2triol, T-2tetraol and deepoxyT-2tetraol as the major metabolites of T-2 in pigs. Concerning the toxicological significance of T-2 metabolites, it is now well established that HT-2 toxicity can be considered as similar to that of the parent T-2 whereas neosolaniol and deepoxy-T-2 would be 10 and 400 times less toxic than T-2.

### 2.4. Zearalenone

The estrogenic mycotoxin zearalenone has been described to undergo both reductions into α and β zearalenols and further conjugations by using *in vitro* models such as rat liver homogenate. Recently, it has been demonstrated that zearalenone is predominantly reduced by hepatic 3α- and 3β- hydroxysteroid dehydrogenases in mammals (Malekinejad et al., 2006).

Interspecies differences in the rate of absolute and relative metabolite productions in different subcellular fractions were identified. The highest amounts of α zearalenol were produced by pig hepatic microsomes whereas chicken microsomes produced the highest amounts of β zearalenol.

Studies on the conjugation of zearalenone with glucuronic acid indicated significant interspecies differences in the rate of glucuronidation, suggesting differences in the affinity of the individual substrate or the presence of different isoforms of glucuronosyltransferases. In terms of toxicity, the estrogenic activity of α zearalenol would be about 10 and 100 times greater than that of zearalenone and β zearalenol. So, interspecies differences in α reduction and conjugation of zearalenone could explain the large differences in estrogenic properties developed by this mycotoxin among animal species, particularly between pigs and poultry (Pompa et al., 1986).

### 2.5. Fumonisin B1

In case of fumonisin B1, only limited data are available regarding its hepatic metabolism. *In vivo*, this mycotoxin is known to be poorly absorbed from gastrointestinal tract. A study using primary rat hepatocytes cultures (Cawood et al., 1994) indicated that no metabolites were detected in the fractionation of the culture medium or by using hepatic microsomal preparations efficient in terms of esterase or P450 monooxygenase activities.

## 3. EFFECTS OF MYCOTOXINS ON THE EXPRESSION AND ACTIVITY OF HEPATIC BIOTRANSFORMATION ENZYMES

### 3.1. Aflatoxins

Due to their hepatotoxic properties, several mycotoxins are known to affect liver drug metabolizing enzymes. So, AFB1 has been described to decrease hepatic cytochrome P450 monooxygenases in rats given daily oral doses of 1 mg/kg b.w.,for 10 days (Galtier et al., 1984). In rabbits, the effects of chronic administration of aflatoxin B1 (AFB1) on liver drug metabolism enzymes were measured in three groups of 5 animals, each receiving over 5 days either vehicle or AFB1 at a daily oral dose of 0.05 or 0.10 mg/kg b.w. (Guerre et al., 1996). These treatments did not lead to lethality in any of the treated groups, but the body weight gain was altered. Biochemical exploration of plasma components revealed a dose-dependent hepatotoxicity characterized by cytolysis and cholestasis. At 0.10 mg/kd/day of AFB1, significant decreases were observed in total liver microsomal cytochrome P450, several P450-dependent monooxygenase activities, all individual P450 isoenzymes levels analysed by Western-blotting and glutathione S-transferase activities. By contrast, at 0.05 mg/kg/day of AFB1, even though total cytochrome P450 was decreased by 30%, only P450 1A1 and 3A6 isoenzymes, and aniline hydroxylation, pentoxyresorufin O-depentylation, aminopyrine, erythromycin, ethylmorphine and dimethylnitrosamine N-demethylations were affected. These decreases could be related to an increased heme catabolism observed during aflatoxicosis. In the same animal group, the only glutathione S-transferase accepting CDNB (1-chloro-2,4-dinitrobenzene) as substrate was decreased by 22%. UDP-glucuronyltransferase accepting p-nitrophenol as substrate was increased by 33 to 62% in both groups of animals.

The influence of aflatoxin B1 (AFB1) on some cytochrome P450-dependent monooxygenases activities was also investigated *in vitro* with rabbit liver microsomes hepatocytes (Guerre et al., 1997). A strong competitive inhibition of the mycotoxin on aniline hydroxylation was observed. The concentration which provoked a 50% inhibition (IC50) was around 20 microM, whereas a Ki of 3 microM was determined. In contrast, only weak inhibitions of both pentoxyresorufin and ethoxyresorufin O-dealkylases (PROD and EROD) activities were obtained. They were characterized by respective IC50 of 200 and 260 microM. The inhibition was 'non competitive' for PROD activity and 'mixed' for EROD. The Ki of the reactions were 177 and 510 microM respectively. Considering the fact that AFB1 had been previously reported to decrease microsomal hepatic cytochrome P450 expression, the results obtained in this study strengthen the hypothesis that the normal metabolism of xenobiotics by the liver could be altered in AFB1 exposure. Additional studies (Guerre et al., 2000a) were conducted in primary cultures of rabbit hepatocytes exposed to 0.1 and 1 microM aflatoxin B1 (AFB1) incubated in the culture medium for 72 h. In order to confirm the effects of the mycotoxin, 30 microM beta-naphthoflavone or rifampicin were used as respective inductors of P450 1A1 and 1A2 or 3A6. Dose-dependent decreases of CYP mRNA expression were observed in all AFB1-treated cells; however, these decreases were not specific. Moreover, P450 expression and activity are less decreased by AFB1 treatment than their

corresponding mRNA. Taken together, these results suggested that the specific P450 decrease observed during aflatoxicosis was not consequence of a specific decrease of their mRNA expression.

More recently, consequences of subchronic exposure to aflatoxin B1 (AFB1) on liver monooxygenase and transferase enzymes were compared in control pigs and pigs given 385, 867 or 1807 µg AFB1/kg of feed for four weeks (Meissonnier et al., 2007). Animals exposed to the highest dose of toxin developed clinical signs of aflatoxicosis, like hepatic dysfunction and decrease in weight gain. This group had significantly lower levels of liver cytochrome P450, ethoxyresorufin *O*-deethylase (EROD) activity, testosterone metabolism, P450 1A and P450 3A protein expressions. By comparison, pigs exposed to 867 µg AFB1/ kg of feed were clinically normal and displayed a similar pattern of liver P450 enzymes activity without changes in P450 3A expression. Benzphetamine and aminopyrine *N*-demethylase activities were increased in pigs exposed to 867 µg or 1807 µg AFB1/kg of feed. Pigs exposed to 385 µg AFB1/kg of feed had low levels of EROD activity and all other biotransformation and clinical parameters remained at control levels. Aniline hydroxylase activity, P450 2C protein expression, UDP-glucuronosyl and glutathione transferase activities were unaffected at all doses of AFB1. In conclusion, P450 1A and P450 3A appear to be specific targets of AFB1 even though pigs did not display any clinical sign of liver toxicosis.

### 3.2. Ochratoxin A

The chronic administration of OTA in rats has also been described to inhibit certain hepatic P450 dependent monooxygenases (Galtier et al., 1984). So, in the liver of rats receiving a high dose of 1.5 mg/kg b.w. for 15 days, total microsomal P450 and oxidations of aminopyrine, aniline and ethoxycoumarin were significantly reduced without any change in phase II activities including conjugations to glutathione, glucuronic or acetic acids.

### 3.3. T-2 toxin and diacetoxyscirpenol

T-2 toxin, like diacetoxyscirpenol, has been described to decrease the activity of hepatic P450 monooxygenases in rats exposed for 4 to 8 days to oral doses of 1 mg/kg of each trichothecene (Galtier et al., 1989). T-2 toxin is also known to affect liver microsomal P450 monooxygenases in rabbits exposed to low doses of toxin for some days (Guerre et al., 2000b). Three doses of T-2 toxin were orally and daily administered to rabbits for five days. At 0.50 mg/kg b.w., three of the five animals died, whereas only a weak decrease in body weight gain and moderate signs of toxicity occurred in rabbits receiving 0.25 mg/kg/day, and the body weight increased without any sign of toxicity at 0.10 mg/kg/day. At 0.25 mg/kg/day, total liver microsomal P450 content, and the activities of aminopyrine and benzphetamine N-demethylases, pentoxyresorufin O-depentylase, glutathione S-transferases accepting 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene as substrates (DCNB), were decreased. By contrast, ethylmorphine and erythromycin N-demethylases, ethoxyresorufin and methoxyresorufin O-dealkylases, aniline hydroxylase, and UDP-glucuronyltransferase accepting p-nitrophenol as substrate, were unaffected. The expression of P450 1A1, 1A2, 2A1, and 2B4, but not P450 2C3 and 3A6, were also decreased, whereas oxidative damages were observed, as demonstrated by increases in microsomal conjugated dienes, fluorescent substances and malonedialdehyde contents. At 0.10 mg/kg/day, neither significant effects on drug metabolizing enzymes nor microsomal oxidative damages were obtained.

More recently, consequences of subchronic exposure to T-2 toxin on liver monooxygenase and transferase enzymes were compared in control pigs and pigs given 540, 1324 or 2102 µg pure T-2 toxin/kg feed for 28 days (Meissonnier et al., 2008). Liver drug metabolizing enzymes were explored at the end of the experiment. In liver, the toxin did not induce lipid peroxidation, nor alter total cytochrome P450, P4502B, 2C or 3A protein expression or transferase activities. However, P4501A related activities were reduced for all pigs given T-2 toxin with P4501A protein expression decreased in pigs fed the highest dose. T-2 toxin exposure also reduced several N-demethylase activities.

### 3.4. Deoxynivalenol and nivalenol

In case of deoxynivalenol, the only available data were obtained in rodents. This toxin incorporated into the diet of rats (20 ppm) for 90 days, provoked increase in liver cytosolic GST activity whereas the

level of microsomal P450 liver was not influenced when compared to pair-fed controls (Morrissey et al., 1985). More recently, the effects of a range of environmentally relevant doses of deoxynivalenol in mice were measured through in subacute toxicological assay in which animals received 3 days per week for 4 weeks, 0.014, 0.071, 0.355 or 1.774 mg/kg b.w. (Gouze et al., 2006). The administration of 0.071 or 0.355 mg/kg b.w. led to increased liver microsomal pentoxyresorufin depentylase and cytosolic GST activities. Examining protein modulation, western blot analyses of liver fractions from mice receiving these doses, revealed increased levels in both P450 2b, GST α and *π* isoenzymes without any change in P450 1a expression. A significant competitive inhibition of deoxynivalenol on CDNB conjugation *in vitro* would indicate that the toxin is a putative substrate for GST. These results suggest that a subacute exposure to low doses of deoxynivalenol causes changes in the normal liver metabolism of xenobiotics.

Induction of hepatic GST has also been shown in mice receiving diets containing the structurally related trichothecene nivalenol (6–12 ppm) for 2 or 4 weeks (Yabe et al., 1993). Nivalenol was investigated in mice exposed to low oral doses of this toxin (0.014, 0.071, 0.355, 1.774 or 8.87 mg/kg bw) three days a week for 4 weeks (Gouze et al., 2007). Regarding liver drug metabolizing enzymes, the only glutathione transferase activity accepting 1-chloro-2,4-dinitro-benzene as substrate was transiently increased in mice receiving low doses (0.071 and 0.355 mg/kg bw) of nivalenol. Regarding the cytochrome P450 monooxygenases, no significant change was observed in ethoxyresorufin *O*-deethylase activity whereas both methoxyresorufin and pentoxyresorufin *O*-dealkylase activities were decreased by 38-45 % for the highest dose (8.87 mg/kg bw) of nivalenol. However, when analysed by western blot analysis, the protein expression of mouse P450 1a, 2b, 2c, 3a and 4a subfamilies was unchanged in these animals.

### 3.5. Zearalenone

The effects of zearalenone on basal and TCDD(dioxin)-induced mRNA expression and enzyme activity of P450 1A1 and 1B1 were recently investigated in MCF-7 cells (Yu et al., 2004). 50 nM zearalenone significantly reduced both basal and TCDD-induced activity and mRNA expression in cells. The oestrogen receptor antagonist 4-hydroxytamoxifen could attenuate these inhibitive effects of zearalenone. Interestingly, zearalenone could promote basal and TCDD-induced CYP1B1 activity without any effect on CYP1B1 mRNA expression. These results suggest that the effect of zearalenone on both the TCDD-induced CYP1A1 activity and the gene expression, involved the oestrogen receptor pathway.

### 3.6. Fumonisin $B_1$

Regarding the effect of fumonisin $B_1$ (FB1) on drug-metabolizing enzymes, the repeated intraperitoneal administration of this toxin has been described to induce certain hepatic monooxygenases in rat, namely cytochrome P450 1A1 and 4A1 proteins (Martinez-Larranaga et al., 1996). The induction of various hepatic cytochrome P450-dependent monooxygenases was also observed in ducks receiving high oral daily doses of this mycotoxin (5 to 45 mg/kg b.w.) for 12 consecutive days (Raynal et al., 2001). If no lethality or sign of toxicosis occurred, the liver and kidney weights were increased. Although the total microsomal P450 content was unaffected, benzphetamine, ethylmorphine, erythromycin N-demethylase and ethoxyresorufin O-deethylase activities increased (by 114, 242, 57 and 27% with 5 mg/kg/day and by 1024, 969, 200 and 147% with 45 mg/kg/day). By contrast, aminopyrine and nitrosodimethylamine N-demethylases, methoxyresorufin and pentoxyresorufin O-dealkylases, and UDP-glucuronyltransferase activities were only increased by using 45 mg/kg/day, whereas glutathione S-transferases activities remained unaffected.

By contrast, in rats exposed to oral doses of fumonisin $B_1$ (3 mg/kg b.w.) for 9 days, selective inhibition of P450 2C11 and 1A2 were observed without any change in P450 2B, 3A or 4A subfamilies expression (Spotti et al., 2000).

These results are consonant with decreases in total cytochrome P450, P450 4A subfamily and ethylmorphine demethylation observed in piglets (Galtier et al., 2003). Three-week-old weaned piglets were divided into two groups of four animals exposed or not to a daily oral dose of 0.5 mg/kg b.w. for 7 days. Among the measured biochemical parameters, plasma urea nitrogen, creatinine and ASAT were increased in FB1-treated piglets. Concerning

liver drug metabolising enzymes, fumonisin $B_1$ treatment provoked significant decreases in total cytochrome P450, P450 4A subfamily and ethylmorphine demethylation whereas both P450 1A and ethoxyresorufin *O*-demethylation were significantly increased. There was no significant change in other liver or renal monooxygenase or transferase activities, even though certain activities or expressions are different by considering the liver lobe or the kidney. These results confirm that a short term exposure of piglets to a low dose of FB1 can lead to hepatic and renal damages. In liver, some monooxygenase activities are decreased with possible consequences for the normal oxidation of endogenous or exogenous compounds. Concerning renal biotransformations, their invariability could be explained by the low exposure of kidneys to the unchanged FB1 which is known to be poorly excreted in the urine of all investigated animal species.

*In vitro* spectral interaction of fumonisin $B_1$ with cytochromes P450 was studied on liver microsomal suspension from pig pre-treated or not with specific inducers of cytochrome P450 1A, 2B, 3A and 4A subfamilies (Marvasi et al., 2006). In potassium phosphate buffered solution, fumonisin $B_1$ interacts aspecifically with all isoforms according to a polar interaction. In the particular case of microsomes prepared from pig induced with the P450 4A inducer clofibrate, a type I hydrophobic interaction was observed, traducing a potential capability of metabolism of this mycotoxin. This result would confirm the existence of molecular interactions between fumonisin B1 and cytochrome P450 4A subfamily.

## 4. CONCLUSION

Interactions between mycotoxins and liver drug metabolizing are crucial in terms of detoxication or bioactivation of these toxins in the organism of the human or animal consumer. Most of these interactions are consequences of metabolic processes occurring in the liver resulting from the activity of monooxygenases or transferases. In relation to their hepatotoxicity, several studies demonstrate the inhibitory effects of the major mycotoxins on hepatic biotransformation enzymes. Such results strengthen the hypothesis that the normal metabolism of xenobiotics by the liver could be altered during chronic exposure to mycotoxins, particularly in humans or animals exposed to aflatoxin B1, T-2 toxin, déoxynivalénol, nivalenol or fumonisin B1.

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## SAŽETAK

Ulaskom u organizam ljudi i životinja mnogi mikotoksini podliježu biotransformaciji u jetri. Njihovi su metaboliti najčešće oksidativni derivati (hidroksimetaboliti aflatoksina $B_1$ i okratoksina A) ili hidrolitički derivati trihotecena. U nekim slučajevima visoko reaktivni epoksidi su prvi korak u stvaranju karcinogenih intermedijera kao što su *ekso*-epoksidi aflatoksina. Hepatički enzimi II faze (transferaze i hidrolaze) sudjeluju u konjugaciji tih oksidativnih metabolita tj. njihovoj detoksifikaciji: glukoronidaciji deacetiliranih trihotecena i hidroksi-aflatoksina ili konjugaciji epoksida s glutationom. Zearalenon se reducira u više različitih estrogenih zearalenola, što ovisi o vrsti organizma koji ga metabolizira. Fumonizin $B_1$ se slabo apsorbira iz probavnog sustava te se metabolizira u hidrolitičke produkte koji su manje toksični.

Interakcije mikotoksina i hepatičkog metabolizma ključni su koraci u njihovoj detoksifikaciji ili bioaktivaciji u organizmu ljudi i životinja. Većina tih interakcija posljedica su upravo metaboličkog proceasa u jetri, posebice aktivnosti citokroma P450 i transferaza. Nedavno je dokazan inhibitorni učinak aflatoksina $B_1$ i trihotecena na određene hepatičke biotransformacijske enzime u svinja. S druge strane, aktivnost i ekspresija proteina specifičnih citokroma P450 i glutation transferaze značajno su povećani nakon djelovanja aflatoksina, deoksinivalenola i fumonizina, što je zabilježeno u glodavaca te domaćih životinja (svinje, kunići i perad). Ova istraživanja pokazuju da normalni hepatički metabolizam endobiotika i ksenobiotika može biti narušen tijekom kroničnog unosa mikotoksina, posebice u domaćih životinja i ljudi izloženih aflatoksinu $B_1$, okratoksinu A, T-2 toksinu, deoksinivalenolu i fumonizinu $B_1$.

Ključne riječi: mikotoksini, jetreni enzimi, metabolizam medikamenata, glodavci, domaće životinje