Letter to the Editor

Gisela H. Degen

Comments on an MNF Review about Ochratoxin A

We have read with great interest the comprehensive review on the toxicity and carcinogenicity of ochratoxin A (OTA) recently published in this journal [1]. This is a careful assessment of various key aspects that have been studied for this important mycotoxin, such as its occurrence, diseases associated with OTA exposure, toxicokinetics, genotoxicity, and mechanism of action.

In such a detailed review (citing over 500 papers) it is not unusual that some errors, *e.g.* incorrect references, are detected after it appears in print (see Erratum on page 1192, in this issue). This may not justify a letter asking for minor corrections, but, a major correction is required as our data on the modulation of OTA genotoxicity by indomethacin (quoted reference [371]) are misrepresented in the text (p.77), and thus do not support some conclusions drawn by the authors.

Quote from page 77:
"The implication of the LOX pathways in OTA genotoxicity was also reinforced by the absence of OTA-DNA adduct formation when cells were pretreated with NDGA and a higher dose of indomethacin (10 μM), which inhibits all the AA biotransformation pathways. These findings correlate with a report by Degen et al. [371] in which the addition of 10 μM indomethacin to 16 μM OTA inhibited OTA-mediated micronuclei formation in ovine cells."

However, OTA-mediated micronucleus formation in ovine cells was not "inhibited" by indomethacin, instead it was clearly increased (!) in the presence of indomethacin (Fig. 2 in [2]). This observation – presented and discussed also at two meetings [3, 4] – clearly contradicts the interpretation by Pfohl-Leszkowicz and Manderville that the genotoxicity of OTA (induction of micronuclei) depends upon its metabolic conversion by prostaglandin H synthase.

With respect to bioactivation of OTA, we have also studied the proposed peroxidase-mediated metabolism of tritiated OTA in vitro by both, highly active microsomal prostaglandin H synthase (PGHS) preparations and by horseradish peroxidase, but we did not detect an enzyme-mediated increase in covalently bound OTA radioactivity [5]. This reinforces our original conclusion that "metabolic activation by PGHS seems not to be required for mycotoxin genotoxicity" [2].

Secondly, caution is indicated when arguing on the basis of modulatory effects on the toxicity of OTA observed either *in vivo* or *in vitro* as interactions may be rather complex and involve factors other than enzyme inhibition: In particular, as both OTA and indomethacin are known to bind with high affinity to serum proteins (references cited in [1] and [2]), they can compete for protein binding. Indeed, in buffer with fetal calf serum (that is present in many cell culture media) addition of indomethacin has been found to increase the fraction of free OTA [5]. As a result the *in vitro* toxicity of OTA was increased in the presence of indomethacin as the free OTA readily penetrates the cell membrane. On the other hand, when indomethacin (or similar enzyme "inhibitors") increase the fraction of free OTA in blood this can result in a shortening of the enterohepatic recirculation of OTA and/or an enhanced elimination thereby lowering the *in vivo* toxicity of OTA.

Being aware of the controversy surrounding the issue of bioactivation of OTA we feel it is important to share this information with the readers of this review.

Gisela H. Degen, Maria M. Gerber, Stephanie Stock

References

[1] Pfohl-Leszkowicz, A., Manderville, R.A., Ochratoxin A: An overview on toxicity and carcinogenicity in animals and humans, *Mol. Nutr. Food Res.* 2007, *51*, 61–99.

[2] Degen, G. H., Gerber, M. M., Obrecht-Pflumio, S., Dirheimer, G., Induction of micro-nuclei with ochratoxin A in ovine seminal vesicle cells, *Arch. Toxicol.* 1997, *71*, 365–371. (quoted as [371] in [ref 1]).

[3] Degen, G., Gerber, M.M., Stock S., Modulation of ochratoxin A induced genotoxicity in ovine seminal vesicle cell cultures by serum and by indomethacin. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1996, *353 (Suppl. 4)*, R127 (Abstract).

[4] Stock, S. Tinel H., Gerber M., Degen G.H., Increased genotoxicity of ochratoxin A in ovine seminal vesicle cell cultures in the presence of indomethacin, an inhibitor of prostaglandin H synthase. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1997, *355 (Suppl.4)*, R165 (Abstract).

[5] Stock, Stephanie, Das Mykotoxin Ochratoxin A: Untersuchungen zu in vitro Genotoxizität und Metabolismus. Dissertation (Thesis) 2004, Justus-Liebig-Universität Giessen, Germany. http://geb.uni-giessen.de/geb/volltexte/2005/2188/

Correspondence: Professor Gisela H. Degen, Institut für Arbeitsphysiologie an der Universität Dortmund, Ardeystr. 67, D-44139 Dortmund, Germany
E-mail: degen@ifado.de
Fax: +49-231-1084 403

