Letter to the Editor

Annie Pfohl-Leszkowicz

Answer to the Prof. Degen's Letter to the Editor

We thank Prof. Degen for her careful reading of our paper. We do not think that it is really a misunderstanding.

The sentence should not be taken out of context. Moreover we indicated that different data clearly imply a PEROXYDASIC pathway (not specifically prostaglandin synthase PGHS, also known as cyclooxygenase 1 COX1). We explained and demonstrated in our different papers that the two major pathways involved in the biotransformation leading to genotoxicity of OTA are: (i) co-oxidation *via* the lipoxygenase pathway, and (ii) co-oxidation *via* CYP epoxygenase. Perhaps the explanation for this conclusion was too brief and requires some clarification.

The results obtained by Degen *et al*. [1], who observed a proportional increase of micronuclei formation in ovine cells treated simultaneously with increasing amounts of indomethacin and OTA appear at first glance to be contradictory to our results. On the contrary, our results, in the same range of relative indomethacin/OTA concentrations, are in complete agreement and also confirm the results obtained *in vivo* [2]. In fact, in the presence of 10 μM indomethacin and 16 μM OTA, Degen *et al*. DID NOT OBSERVE micronuclei formation, which correlates well with the *in vivo* study (9 μM indomethacin and 15 μM OTA, [2]) and our experiment in which total inhibition of DNA adduct formation was obtained when cells were treated with 10 μM of each compound. Degen *et al*. [1] interpreted their results as a competition between indomethacin and OTA for plasma protein binding. In fact, this could be partially involved as more free OTA would be available. Nevertheless, this is not the sole event and could not explain the other results obtained with either low dose of indomethacin, NDGA or epinephrine [3]. It is noteworthy that micronuclei in ovine seminal vesicle cells are observed only when cells were treated with 33 μM OTA, a dose which causes high cytotoxicity and inhibition of repair enzyme. Another major discrepancy between our model and theirs is the presence of the Ah receptor in the ovine cells. The induction of this receptor led to expression of CYP 1A isoforms. We have shown that the CYP 1A family was induced by OTA and implicated in its genotoxicity [4, 5]. Thus, the results obtained in ovine cells could be due to an increase of toxifying CYP (1A), in conjunction with a decrease of PROTECTING enzymes such as PGHS. In our model, a close parallel between inhibition of lipoxygenases and/or CYP P450 epoxygenases pathways, and a decrease of OTA genotoxicity induced by pre-treatement with 10 μM indomethacin or NDGA were observed, whereas inhibition of some PGHS pathways is in relation with an increase of genotoxicity (0.1 μM epinephrin or indomethacin).

Interestingly, the OTA derivatives formed with pig microsomes are similar to those obtain under culture cell conditions where lipoxygenase and CYP P450 epoxygenase activities were predominant compared to PGHS (*i. e.* cells pre-treated with 0.1 μM indomethacin or epinephrine). The hypothesis of CYP P450 epoxygenase being implicated in the formation of these metabolites was confirmed by their disappearance when CYP P450 epoxygenase is inhibited (*e. g.* pre-treatment with NDGA or 10 μM indomethacin). These metabolites are particularly important because they were generated exclusively in BEAS-2B cells specifically expressing CYP 2C9 [6], a CYP highly involved in OTA genotoxicity and carcinogenicity in rats [5]. Thus, the pathway which seems to be implicated in their formation is CYP P450 epoxygenase. Two of these metabolites have been identified as a dechlorinated, decarboxylated OTA [7] and OTHQ (ochratoxin hydroxyl quinone), leading to genotoxicity [8].

One metabolite (called II in the paper by Pinelli *et al*., [3]) was formed in cells pre-treated with NDGA and 10 μM indomethacin, which both inhibited the lipoxygenase pathway. The retention time of this metabolite corresponded to that of a metabolite exclusively formed by *in vitro* incubation of OTA in the presence of microsomes from rabbits pre-treated with phenobarbital [6], and corresponds to 10-OH-OTA which is not genotoxic.

It has been demonstrated that lipoxygenase as well as prostaglandin synthase required peroxide to initiate enzymatic activity. Reactive oxidised species generated during peroxidase catalysis presumably initiate free radical chain reactions that lead to epoxidation or oxidation of xenobiotics.

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In conclusion, OTA is biotransformed into genotoxic metabolites *via* a lipoxygenase pathway and CYP P450 epoxygenase, whereas PGHS (COX1) decreases OTA genotoxicity. Thus there is no contradiction, but a confirmation of the original conclusion of Degen *et al*. that "metabolic activation by PGHS seems not to be required for genotoxicity". We can add that other peroxidasic pathways are important and explain the generation of quinone derivatives which are well known to mediate genotoxicity [9].

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