Effect of Ochratoxin A and Aristolochic Acid on an Epithelial Intestinal Cell Line

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Abstract

OchratoxinA (OTA) and aristolochid acid (AA) effects on cell viability and cytokine synthesis was analysed in an intestinal epithelial porcine cell line. The highest doses of AA and OTA (>1µg/mL) induce a significant (P<0.05) decrease of the cell viability. OTA and AA has little or no effect on IL-8 synthesis were induced a decrease of the synthesis of TNF alpha and IL-1 beta.

Keywords: *aristolochic acid, epithelial intestinal cells, ochratoxin A, swine*

Introduction. OchratoxinA (OTA) and aristolochic acid (AA) were associated with the Balkan endemic nephropathy (BEN), a chronic nephropathy described in several areas from the Balkan Peninsula and Romania. Even the effect at the renal level was well documented the effect of these toxins at intestinal level was no investigated.

Aims and objectives. We have investigated the effect of OTA and AA on a pig epithelial cell line: IPEC-1, in term of cell viability, and synthesis of some inflammatory cytokines: TNF alpha, IL-1 beta and IL-8.

Materials and methods. For the viability assays, IPEC-1 cells were cultivated in 96 wells plates and treated or not with 1-500 µg/mL OTA and AA. The cytokine synthesis was assessed in the supernatant of the cell culture by ELISA using commercial kits.

Results and discussion. With the exception of the low dose of 1µg/mL dose, that induce only a slight decrease of cell viability, the higher doses of AA and OTA induce a significant (P<0.05) decrease of the cell viability. For example the highest dose (500 µg/mL) induced a decrease of cell viability with 89% and 97%, for AA and respectively OTA. AA induced a progressive decrease of cell viability; by contrast OTA induced a failure of cell viability starting with the low doses (5 µg/mL). Cytokine synthesis, as investigated in the cell supernatant after 3h, 6h and 24h of exposure revealed that OTA and AA have different effects on the secretion of cytokines involved in inflammation. Thus, OTA and AA has little or no effect on IL-8 synthesis, while TNF alpha significantly decrease after 3h (P<0.05) exposure to 1 and 5 µg/mL OTA and 5 µg/mL AA; at 24h of exposure to the both toxins, the TNF-alpha synthesis was reduced as compared with 3h. At 24h, 5 µg/mL OTA induce a suppression of the cytokine synthesis. Similarly, in human peripheral blood mononuclear cells, concentrations of OTA between 5 and 7.5 µg/mL induced a complete

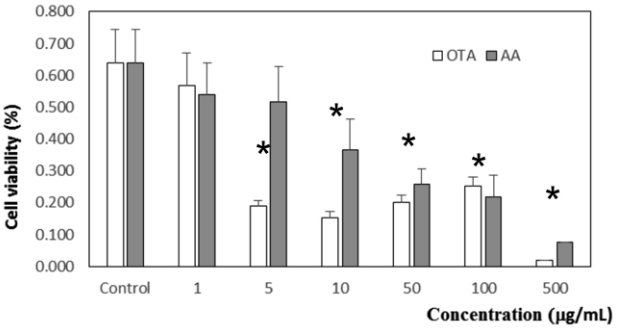

Fig. 1. Cell viability. IPEC cells were exposed to various concentrations of toxins and the viability of the cells was assessed by a MTT test. * indicate significant differences (P<0.05)

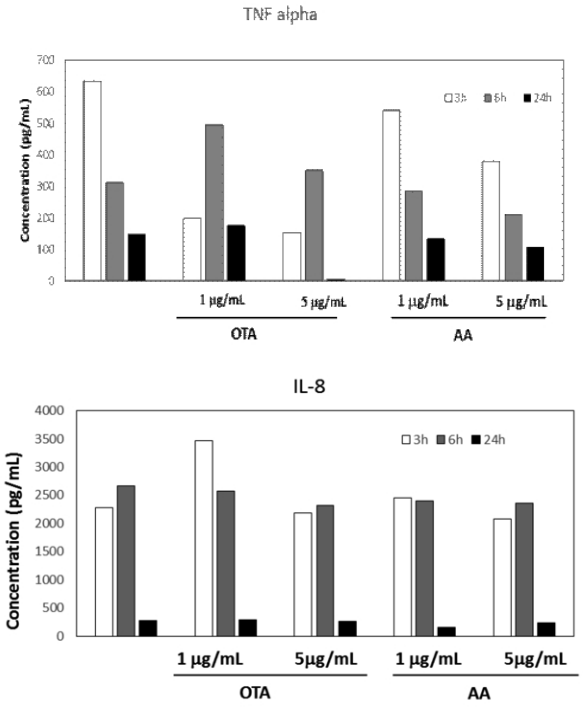

Fig. 2. Cytokine synthesis. IPEC cells were exposed to different toxins concentrations and the cytokine synthesis was determined by ELISA (a) TNF alpha synthesis (b) IL-8 synthesis

inhibition in TNF-alpha production after 24 h of exposure (Mechoud *et al.,* 2012). At 6h, OTA induced an increase of the TNF-alpha synthesis for both concentrations. Rat liver sinusoidal endothelial cells, were also able to synthesize TNF-alpha in the presence of OTA, but only after LPS exposure (Al-Anati *et al.,* 2010).

As compared with the control, AA induced provoked a slight decrease of TNF alpha synthesis. Wu *et al.* (2011) shown that AA, inhibits in human monocyte the TNF-α-induced MMP-9 cells, possibly through the NF-κB signal pathway. A significant decrease of IL-1 beta was observed after 3h, 6h or 24h ($P<0.05$) exposure to AA and OTA; the IL-1 beta was not detected in the cell supernatant after 24h of exposure (data not shown).

Conclusion. Taken together these results suggest that OTA and AA have different effects on the different immune parameters at the intestinal level, with important implications for the gut health.

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