Effect of Cereal Contaminants on the Inflammation and Oxidative Stress in the Gut of Weanling Piglets

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Abstract
Cereals and cereals-based products are frequently contaminated with ochratoxin A (OTA) and aristolochic acid (AA). The aim of this study was to compare the effect of OTA and AA on oxidative stress and inflammation in the swine intestine as major organ involved in the absorption of xenobiotics. Fifteen pigs were randomly distributed in 3 groups (control, OTA and AA) and were fed diets contaminated or not with 250 µg toxin/kg for 28 days. When compared with control group, consumption of a OTA and AA contaminated diet significantly increase the concentration of IL-1 beta and IL-8, as major cytokines involved in the inflammatory response. When compared with OTA intoxicated group, AA group has a higher concentration of IL-8 in the intestine. The exposure of piglets to AA significantly decrease the activity of enzymes involved in the oxidative stress response: CAT and GPx as well as the total antioxidant status. The consumption of the diets contaminated with OTA and AA increase in the lipid peroxidation, as showed by TBARS assay. In conclusion, our results have shown that at the intestine level, both OTA and AA induced inflammation and oxidative stress, AA having probably a more important inflammatory effect in the intestine.

Keywords: aristolochic acid, duodenum, ochratoxin A, pig

1. Introduction

Ochratoxin A (OTA) and aristolochic acid (AA) are toxins responsible for the Balkan endemic nephropathy (BEN), a tubulo-interstitial nephropathy described in Balkan Peninsula and in Romania [1].
Gut has an essential role in the digestion and absorption of ingested material in order to supply nutrients, vitamins, minerals, and electrolytes, essential for the good function of the body. The intestinal epithelium is the largest and most important barrier against the external environment and it acts as a selectively permeable barrier permitting the absorption of nutrients, electrolytes and water, while maintaining an effective defense against intraluminal toxins, antigens and enteric flora [2].
Oxidative stress has a critical role in the pathophysiology of many gastrointestinal diseases, and many complications of these diseases are mediated by oxidative stress, oxidative stress-related mediators, and inflammation [3, 4]. Recent studies have attributed an important role of oxidative stress and inflammation to OTA and AA mediated toxicity [5, 6].
The aim of this study was to compare the effect of OTA and AA on oxidative stress and inflammation in the swine intestine as major organ involved in the absorption of xenobiotics.

2. Materials and methods

Reagents. All chemicals, immunological reagents and media components were purchased from
Sigma (Sigma-Aldrich, Steinheim, Germany) unless otherwise stated.

**Animals and treatments.** For this study, a total number of fifteen cross-bred TOPIG hybrid [(Landrace×Large White)×(Duroc×Pietrain)] pigs with an average body weight of 10.9±0.77 kg were allocated to three experimental groups (5 pigs per group). The piglets were exposed to one of the three treatments: control group (C), ochratoxin group (OTA) and aristolochic acid (AA) for 28 days. The piglets were fed a maize-soybean-meal-based diet contaminated or not with 250 µg OTA and respectively AA/kg feed (Sigma). Pigs had free access to feed and water during the experimental period. At the end of the experiment (day 28), animals were slaughtered by exsanguination in an EU-licenced abattoir according with the EU Council directive 2010/63/CE. After slaughtering, samples of duodenum were taken on ice and stored at –80°C until analysed for cytokine concentration, activity of enzymes involved in oxidative stress, total antioxidant capacity and lipid peroxidation.

**Determination of total antioxidant status.** Total antioxidant capacity (TAC) assay was based on the absorption of ABTS+ cation [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] as already described [7] in samples of plasma, liver and kidney and inhibition percentages were converted into trolox equivalent antioxidant capacity (TEAC), expressed as µmol TEAC/g tissue.

**TBARS assessment.** For the determination of thiobarbituric acid reactive substances (TBARS), frozen liver and kidney samples were homogenized in Tris HCL buffer containing thiobarbituric acid, as already described [7]. The absorbance at 532 nm was measured using a photometer (Tecan Sunrise, Austria). TBARS are reported as nmol/mg protein.

**Cytokine measurement.** Samples of duodenum for each animal were homogenized in phosphate buffer containing 1% igepal, 0.5% sodium deoxycholate, 0.1% SDS and complete (EDTA-free) protease inhibitor cocktail tablets, as already described [7]. The homogenates were kept 30 min on ice, and then centrifuged at 10,000 g at 4°C for 10 min. The supernatants were frozen at -20°C, until analyzed for cytokine content by ELISA. Total protein content was measured using Bradford assay. Monoclonal anti-porcine antibody from: i) R&D Systems (Minneapolis, USA): IL-1β (MAB6811), IL-8 (MAB5351), TNF-α (MAB6902) ii) or Invitrogen (Camarillo, Canada): IFN gamma (ASC4934), were used as capture antibody in conjunction with anti-porcine cytokines-biotinylated antibodies: IL-1β (BAF681), IL-8(BAF535), FNγ (ASC4839), TNF-α (BAF690). Streptavidin-HRP (Invitrogen, Camarillo, USA) and TMB (tetramethylbenzidine) were used for detection. Absorbance was read at 450 nm using a microplate reader (SUNRISE TECAN, Austria). Recombinant swine IL-1β, IL-8, IFN-γ, TNF-α were used as standards and results were expressed as picograms of cytokine/mL, after normalization to the total protein content of the samples.

**Statistical analysis.** All the results are expressed as mean±standard error of the mean (SEM). ANOVA tests followed by a Fisher PSLD test were used to analyse the differences. (StatView software 6.0, SAS Institute, Cary, NC). Values of P<0.05 were considered significant.

### 3. Results and discussion

As compared with the control piglets, the exposure of piglets to OTA and AA significantly decrease (P<0.05) the activity of enzymes involved in the oxidative stress response when compared with control group: CAT (-31% for OTA and -35% for AA); GPx (-29% for OTA and -43% for AA) (Figure 1). Exposure to OTA and AA doesn’t significantly affect the SOD activity in the gut. Assessment of the total antioxidant capacity in the gut of the piglets showed that both OTA and AA induced a decrease of TAC by -21% (P<0.0001) and respectively by -16% (P<0.0001). Lipid peroxidation as resulted from the assessment of thiobarbituric acid reactive substances was increased by both OTA and AA by 33% respectively by 38% in the pig duodenum. The consumption of the diets contaminated with OTA and AA significantly increase the synthesis of IL-1 beta (3323 pg/mL in OTA group, respectively 4422 pg/mL in AA group vs 19 pg/mL in control group) and of IL-8 (3623 pg/mL in OTA group, respectively 4932 pg/mL in AA group vs 1997 pg/mL in control group) (Figure 2).
When compared with OTA intoxicated group, AA group has a higher concentration of IL-8 in the intestine (846±194 pg/mL vs 636±158 pg/mL). The synthesis of TNF alpha was not affected after the exposure to both toxins, while the synthesis of IFN gamma tended to decrease in pigs exposed either to OTA or AA (66±27 pg/mL, respectively 68±17 pg/mL vs 128±22 pg/mL in control group).

In the last years, there is an increase of the general awareness concerning the toxicity of different food and feed contaminants. AA and OTA are cereal contaminants with nephrotoxic effects, but other organs than kidneys, can be also affected by their toxicity. The intestinal epithelial cells are exposed to different xenobiotics and can be the first cells affected by the interaction with the toxins. Aristolochic acid is absorbed through the gastrointestinal tract into the blood stream. OTA is rapidly absorbed and remains long time in the body (72-120h in pigs) [8] due to renal tubular secretion and reabsorption of the toxin [9]. This mechanism plays an important role in toxin accumulation and development of nephrotoxicity [10]. However, many studies have shown that feed contaminants (eg. mycotoxins) are able to affect key functions of the gastrointestinal tract, including decreased surface area available for nutrient absorption, modulation of nutrient transporters, or loss of barrier function [11]. In addition, some mycotoxins facilitate persistence of intestinal pathogens and potentiate intestinal inflammation. Indeed, the intoxication of piglets with 250 ppm OTA and respectively AA significantly increase the synthesis of IL-1 beta and IL-8. IL-1 beta is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis [12]. IL-8 is a chemokine with important functions in immune surveillance, inflammation, and it is released from several cell types in response to an inflammatory stimulus [12].

**Figure 1.** Effect of OTA and AA on the oxidative stress in the pig gut
The study of the immune response and of the oxidative stress could provide important information concerning the first reaction of defense of the organism to the toxins; this information could be used for the risk assessment studies and the establishment of guidance levels for mycotoxins [13]. Our results indicated a decrease of the antioxidant system in pigs, resulted as a consequence of the oxidative stress induced by the toxins. Also, the toxins induced an increase of lipid peroxidation as shown in the figure 1. There is some evidence in support of oxidative stress and lipid peroxidation being factors responsible for the toxicity of feed contaminants at the intestine level [6], and our results show that OTA and AA induce oxidative stress, an increase of lipid peroxidation and inflammation, and with the exception of IL-8, similar effects were observed at the same concentration (250 ppb) for both toxins. More studies are necessary in order to fully understand the role of AA and OTA at the intestinal level.

4. Conclusions

Our results show that OTA and AA induce oxidative stress, an increase of lipid peroxidation and inflammation, and with the exception of IL-8, similar effects were observed at the same concentration (250 ppb) for both toxins. More studies are necessary in order to fully understand the role of AA and OTA at the intestinal level.

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