Characterization of in vitro intestinal absorption of veterinary drugs and coccidiostats in the presence of mycotoxic detoxifiers using a porcine intestinal epithelial cell line: outline of the study

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Introduction and Aims
Adding mycotoxin binders and modifiers to feed is a widespread strategy to reduce the effects of mycotoxins on animals. These products are often registered as technological additives and are considered safe for the animal. However, only few studies are available that investigated the interaction of these additives with other substances added to the feed such as coccidiostats and veterinary drugs (Goossens et al., 2012; Ossefaere et al., 2012). A large scale screening method is also not yet available.

This study aims to develop and apply an in vitro model to characterize the absorptive behavior of xenobiotics in the presence of mycotoxin detoxifying agents and of mycotoxins. Therefore, an intestinal porcine intestinal epithelial cell line (IPEC-J2) will first be cultured on a Transwell® insert system (Devreese et al. 2013). Next, a mixture of veterinary active substances, together with a mycotoxin detoxifier and a mycotoxin will be applied on the Transwell®. The influence of twelve different detoxifiers on the cellular passage of twelve orally applied veterinary drugs in poultry and pigs, such as beta-lactams, aminoglycosides, tetracyclines, macrolides, lincosamides, pleuromutilins, sulfonamides, trimethoprim, fluoroquinolones and sodium salicylate will be studied. Furthermore, nine coccidiostats including ionophoric coccidiostats, nisarbazin, halofuginone, robenidine and diclazuril, also frequently used in poultry and pigs, will be assessed. The mycotoxin which will be included in this study is deoxynivalenol (DON). Control groups without mycotoxin and/or detoxifiers will also be included. The passage of the drugs and coccidiostats will be monitored using in-house developed LC-MS² methods and the permeability coefficients will be compared with the control groups. To exclude drug-drug interactions, the passage characteristics of the mixture of drugs and coccidiostats will be compared with the absorptive behavior of the single drug or coccidiostat.

Design of the first experiment:
The cells are cultured in a 96-well plate for 21 days. Next, they are exposed to various mixtures of the selected veterinary drugs. Toxicity will be assessed by a neutral red assay.

Design of the third experiment:
Xenobiotics, detoxifying agents and/or toxins are mixed and shaken with feed and liquid phase at pH 3. Gradually, the mixture is basified, simulating the pH variation in the GI-tract of the pig. At each stage a sample is analyzed to assess the direct binding at each pH. At pH 7.8, and after an exposure time of 4 hours, an aliquot is transferred to the basolateral side of a Transwell® insert, samples are collected at regular intervals from the basolateral and apical side. After the experiment (4 hours exposure), the membrane itself is also analyzed.

References

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