Quantitative assessment of bile ducts in turkeys treated with artemisinin: A model for liver toxicity?

Thøfner, Ida; Larsen, Jytte Overgaard; Nielsen, Ole Lerberg; Liebhart, Dieter; Hess, Michael; Schou, Torben Wilde; Hess, Claudia; Ivarsen, Elise; Fretté, Xavier; Christensen, Lars Porskjær; Grevsen, Kai; Engberg, Ricarda M.; Christensen, Jens Peter

Publication date:
2013

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Thøfner, I., Larsen, J. O., Nielsen, O. L., Liebhart, D., Hess, M., Schou, T. W., ... Christensen, J. P. (2013). Quantitative assessment of bile ducts in turkeys treated with artemisinin: A model for liver toxicity?. Poster session presented at Annual PhD day at the Faculty of Health and Medical Sciences, Copenhagen University, København, Denmark.

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Download date: 31. May, 2019
Quantitative assessment of bile ducts in turkeys treated with artemisinin: A model for liver toxicity?

Theofner IG1, JO Larsen2, OL Nielsen1, D Liebhart3, M Hess3, TW Schou4, C Hess4, E Iversen5, XC Fretté6, LP Christensen5, K Grevesen5, RM Engberg7 and JP Christensen1

1 Department of Veterinary Disease Biology, Faculty of Health and Medical Science, University of Copenhagen 2 Department of Neuroscience and Pharmacology, Faculty of Health and Medical Science, University of Copenhagen 3 Clinic for Avian, Reptile and Fish Medicine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, 4 DHI Environment and Toxicology, DHI Group, 5 Institute of Chemical Engineering, Biotechnology and Environmental Technology, Faculty of Engineering, University of Southern Denmark, 6 Department of Food Science, Aarhus University, and 7 Department of Animal Science, Aarhus University

Background

Development of resistance to anticoccidial drugs, removal of licensed anthelmintical formulations and a shift towards organic production has put the focus on natural occurring compounds for controlling protozoa infections in poultry.

Artemisinin originates from Artemisia annua and is widely used as an efficient antimalarial in humans. Inhibitory activity against coccidia of chickens was reported previously; however, toxic side effects, i.e. degeneration of the brain, liver and kidney, also were induced.

In a previous study investigating the effect of artemisinin against histomonosis in turkeys and chickens, artemisinin was administered in the feed to day old turkeys (Theofner et al. 2012). As a result, sudden deaths occurred on day 5 and 6 in the group receiving 2600 mg artemisinin per kg feed, and the remaining birds in the group were then euthanized.

The histological examination of haematoxylin and eosin stained sections indicated bile duct proliferation of the treated turkeys.

In the present study we examined liver samples from the 15 dead/euthanized turkeys and 10 untreated and age matched birds.

Aim

The aim was to quantify the volume fraction of bile duct epithelium and the number of bile duct profiles/area in turkeys suspected of artemisinin intoxication by unbiased stereology.

Methodology

Histological preparation

- Paraffin embedded formalin fixed liver samples from Artemisinin treated turkey chickens (5-7 days old, n=15) and untreated and age matched birds (n=10) were cut into 4 μm thick sections.
- Immunohistochemical detection of cytokeratin (Cytokeratin clone AE1/AE3, DAKO) was performed to distinguish the bile duct epithelium from liver parenchyma, connective tissue and blood vessels. Slides were counterstained with Mayer’s haematoxylin.

Stereology

- All fields of vision in all 25 sections were randomly selected within the delineated area of interest in steps of 400 μm (dx,dy).
- Determine the volume fraction of bile duct epithelium relative (Figure 1, red circles) to liver parenchyma using point grid counting (16 points per field of vision).
- Presence of lumen was noted for the transected bile duct profiles in each counting frame (7709.6 μm²).
- The relative number of lumen-less bile duct profiles per total number of bile duct transects was estimated as a function of the total observed area.

Results

The present findings suggest that artemisinin treatment changes the differentiation of the biliary duct system.

- The ratio of bile duct profiles with no lumen of the artemisinin treated birds is significantly higher than the same ratio in the control birds.
- No differences in the volume fraction of bile duct epithelium in relation to the volume of liver parenchyma between treatments.
- This is the first study that uses stereological microscopy for quantification of bile duct epithelium in turkey livers.

Discussion

The present findings suggest that metabolism or excretion of artemisinin in birds may take place in the liver. However, no analysis on bile or faecal matter was performed.

The differences in the relative ratio of bile duct profiles without lumen may be explained by a significant difference in the absolute number of bile duct profiles with lumen between treatments. Combined with the observed pleomorphic appearance of bile duct epithelium in artemisinin treated birds the present observation suggests that bile differentiation is abnormal as a response to toxic injury caused by artemisinin.

Turkey fetuses has been evaluated as suitable for assessing in vivo carcinogenicity showing hepatic changes similar to adult rodents (Williams, et al., 2011), however the histological evaluation is purely descriptive. This is the first study that uses stereological microscopy for quantification of bile duct epithelium in turkey livers.

Key words

Toxicty, artemisinin, turkeys

References
