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

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Quantitative assessment of bile ducts in turkeys treated with artemisinin

A model for liver toxicity?

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Background

Development of resistance to anticoiclidal drugs, removal of licensed antihistomonal 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. Its unique mode of action makes it attractive for use in veterinary medicine. A potential toxic effect of artemisinin is liver toxicity, which has been reported in adult rodents and is widely used as an efficient antimalarial in humans. 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 cytokinergan (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 Mayers haematoxylin.

Stereology

- All fields of vision in all 25 sections were randomly selected within the delineated area of interest in steps of 400 μm (dcdps).
- 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.

Figure 1: 16-point grid (black arrows) and counting frame (blue arrows) in a field of vision, p: portal vessel, S: sinusoid. Slide from Artemisinin treated bird (40x objective, oil immersion. Total magnification: 256X).

Discussion

The present findings suggest that metabolism or excretion of artemisinin in birds may take place in the liver. The relative number of bile duct profiles with no lumen of the artemisinin treated birds is significantly higher than the same ratio in the control birds.

Table 1. Quantitation of bile duct proliferation in Artemisinin treated turkey chickens and untreated control birds. Mean ± SEM values and unpaired t-test, p<0.05 (significance level).

<table>
<thead>
<tr>
<th></th>
<th>Control (n=15)</th>
<th>Artemisinin (n=15)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume fraction</td>
<td>0.0115 ± 0.0000369</td>
<td>0.0109 ± 0.0000354</td>
<td>0.4945</td>
</tr>
<tr>
<td>Examinated area (mm²)</td>
<td>2.605 ± 0.2239</td>
<td>2.203 ± 0.0145</td>
<td>0.0476</td>
</tr>
<tr>
<td>Lumen (%)</td>
<td>0.7056 ± 0.02225</td>
<td>0.7869 ± 0.02271</td>
<td>0.1917</td>
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<tr>
<td>No. of bile duct profiles with lumen/mm²</td>
<td>44.83 ± 3.114</td>
<td>39.59 ± 3.560</td>
<td>0.2809</td>
</tr>
<tr>
<td>No. of bile duct profiles with lumen/mm²</td>
<td>31.93 ± 2.744</td>
<td>31.97 ± 3.060</td>
<td>0.9986</td>
</tr>
</tbody>
</table>

Figure 3 Bile duct profiles without lumen (white arrows). A) Control bird. B) Artemisinin treated bird. Note the pleomorphic appearance (anisocytosis and anisokaryosis; intracellular vacuoles) and irregular organization of the bile epithelium in arterisinin treated birds. (B) compared to untreated birds. (A) (1:16, 45X objective, oil immersion. Total magnification: 380X).

Figure 2) A) Ratio of bile duct profiles with no lumen/total number of bile duct profiles. B) Total number of bile duct profiles. C) Number of bile duct profiles without lumen. D) Bile duct profiles with lumen. *, significant difference between control group and artemisinin treated group.

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Key words

Toxocca, artemisinin, turkeys

References

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