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Thøfner, Ida; Hess, C; Liebhart, D; Hess, M; Schou, Torben Wilde; Ivarsen, Elise; Frete, Xavier; Christensen, Lars Porskjær; Grevsen, Kai; Engberg, Ricarda Greuel; Christensen, Jens Peter

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Effects of artemisinin and Artemisia annua extracts on xenic bacteria isolated from clonal cultures of Histomonas meleagridis.

Thøfner ICN1, Hess C2, Liebhart D2, Hess M2, Schou TW3, Ivarsen E4, Fretté XC4, Christensen LP4, Grevens K5, Engberg RM6 and Christensen JP1

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

Conclusion

• No antibacterial effect was noticed with compound concentrations identical to the antimhistomonal screening.
• Since no antibacterial effects were observed on the bacteria isolated from the xenic flora of six clonal H. meleagridis cultures the observed inhibition of histomonal multiplication is regarded as directly antimhistomonal.
• The potential of these materials on histomonosis was subsequently tested in vivo in chickens and in turkeys without success.

Background

Infection with the protozoa Histomonas meleagridis in poultry has re-emerged since the ban of effective drugs (7). Consequently efforts are set to find alternatives to chemotherapeutics to combat histomonosis. At present histomonads need accompanying bacteria when cultured in vitro, probably serving nutrient supply due to their appearance in parasitic food vacuoles. However, the relationship of the parasite and the bacteria is not fully clear. Six previously established clonal cultures of H. meleagridis (5) were used to evaluate the effect of the Artemisia annua derived materials (i.e. dry leaves, artemisinin, and hexane, dichloromethane or methanol extracts). Dry leaves, artemisinin, hexane and dichloromethane extract displayed significant dose dependent inhibitory activity against all six mono-eukaryotic cultures (Figure 1).

Aim

The aim was to assess whether the observed inhibitory effects on H. meleagridis multiplication could be accounted for as direct or indirect.

Methodology

Artemisia annua compounds.

• Dry leaves from Artemisia annua, artemisinin (purity ≥99%), crude essential oil fractions from A. annua leaves (Ext-oil-HEX; Ext-oil-DCM; and Ext-oil-MeOH), made using hexane, dichloromethane or methanol extraction methods.

Isolation and sensitivity testing of xenic bacteria.

• Bacteria present in the same mono-eukaryotic Histomonas cultures as in the antiprotozoal setting were isolated using selective media and biochemical characterisation methods.
• The antibacterial activity was assessed using the disc diffusion method (1,2).
• Preparation of inoculum followed the CLSI Direct Colony Suspension Method (2).
• A volume of 20 µl of the test solutions in concentrations identical to those in the antiprotozoal assay were loaded onto ampicil Sani-discs. Negative controls were loaded with 20 µl PBS and positive controls contained 10 µg.

Discussions

• In total 19 bacterial strains were isolated from the six mono-eukaryotic H. meleagridis cultures. E. coli (8/19) was isolated at least once from all six H. meleagridis cultures, including four APEC isolates (O1, O2, or O78). Streplococcus spp. (5/19) or Proteus spp. (5/19) were isolated from four protozoal cultures. Staphylococcus was isolated once.

Table 1. Bacteria isolated from the different clonal cultures of H. meleagridis.

<table>
<thead>
<tr>
<th>Clonal cultures</th>
<th>Bacterial isolates</th>
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<tr>
<td>Histomonas meleagridis - Bacterial isolates</td>
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</tr>
<tr>
<td>H. meleagridis - CARDB/91/HEX</td>
<td>E. coli, E. cloacae, E. coli-MeOH</td>
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<tr>
<td>H. meleagridis - CARDB/91/DCM</td>
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</tr>
</tbody>
</table>

• No inhibitory effect of any of the compounds (dry plant, artemisinin; Ext-oil-HEX; Ext-oil-DCM; and Ext-oil-MeOH) was observed in any of the 19 isolated bacterial strains from any of the six investigated histomonial clones (see example on Figure 3).

Methodology

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Figure 1: In vitro response of Histomonas meleagridis clone 6b to Artemisia annua dry leaves, artemisinin, and hexane, dichloromethane or methanol extract of Artemisia annua

Discussion

The present susceptibility testing at compound concentrations as used in the antihistomonal setup revealed no inhibitory effect on bacterial growth when treated with dried A. annua leaves, artemisinin or either of these extracts.

It is known that artemether, a derivative of artemisinin, has no antibacterial effect on human hospital strains of E. coli and S. aureus (4). Similar investigations found that artemisinin had no antibacterial effect on S. aureus (3,9). However, artemisinin showed antibacterial properties at 1 mg/ml against E. coli, E. coli NCTC 9002 and Proteus vulgaris (3). In our study, the amount of artemisinin loaded onto the discs ranged between 100-300 µg/disc (20 µl of each test solution per disc) which had no antibacterial effect on the bacterial strains isolated from the clonal histomonal cultures. This is in agreement with a study where no antibacterial effect of 100 µg/disc artemisinin was found on E. coli or S. aureus (8).

To the best of our knowledge, only a single study has addressed the antibacterial effect of essential oil components extracted from A. annua (6) in which no inhibitory effect on E. coli and S. aureus was shown, whereas complete inhibition was obtained for Enterococcus hirae at 0.1 mg/ml.

Combining the results of the antiprotozoal screening with the antibacterial tests, it is reasonable to assume that the observed inhibitory effect of dried A. annua leaves, artemisinin, Ext-HEx and Ext-DCM, is attributed to a direct effect on histomonads and could be regarded as antimhistomonal. Ext-DCM and artemisinin were found to have the strongest antimhistomonal effect in the in vitro studies and were therefore selected for further in vivo testing. Despite promising in vitro properties no effect on experimental H. meleagridis infection could be demonstrated.