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

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Conclusion

• No antibacterial effect was noticed with compound concentrations identical to the antihistosomal 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 antihistosomal.
• The potential of these materials on histomonosis was subsequently tested in vitro 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 needing 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 five 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 dependant inhibitory activity against all six mono-eukaryotic cultures (Figure 1).

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

Discovers

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

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 as extraction media.

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 (11). Preparation of inoculum followed the CLSI Direct Colony Suspension Method (2).
• A volume of 20 µl of each test solution per disc) which had no antibacterial effect on the bacterial strains isolated from the clonal histomonal cultures. This in agreement with a study no antibacterial effect of 100 µg/disc artemisinin was found on E. coli. Table 1. Bacteria isolated from the different clonal cultures of H. meleagridis.

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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<tbody>
<tr>
<td>Artemisia meleagridis/Mucoid E. coli</td>
<td>Proteus sp.</td>
</tr>
<tr>
<td>Artemisia meleagridis/Non-mucoid E. coli</td>
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</tbody>
</table>

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 shown 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. 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 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 Entrecococcus 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 antihistosomal.

Ext-DCM and artemisinin were found to have the strongest antihistosomal 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.