ADHERENCE AND EXPERIMENTAL INFECTION OF BACTERIA ASSOCIATED WITH PERIODONTAL INFECTIONS OF YOUNG CATTLE IN BRAZIL (“CARA INCHADA”)  

Barbara Grassmann, Jürgen Döbereiner, Iveraldo S. Dutra, Peter A. Kopp and Hans Blobel


In vitro- and in vivo-assays were conducted, to study the possible role of streptomycinn- and actinomycin-producing soil actinomycetes for the pathogenesis of “Cara inchada” in cattle (CI). Adherence of Bacteroides spp. to epithelial cells of the bovine gingiva, known to be associated with the progressive lesions of CI, was significantly increased by the addition of streptomycin, actinomycin or antibiotic culture supernatants of the soil actinomycetes. Applications of these mixtures together with Actinomyces pyogenes to the marginal gingiva of the upper premolar teeth of about 1 month old Holstein Friesian calves did not lead to progressive lesions of CI. Only one calf exhibited a slight diarrhea and a temporary retraction of the gingiva at the site of application.

INDEX TERMS: “Cara inchada”, cattle, periodontal infection, Bacteroides spp., streptomycin, actinomycin, adherence.

INTRODUCTION

Bacteroides spp. and Actinomyces (A.) pyogenes play a decisive role in the pathogenesis of “Cara inchada” in cattle (CI) (Blobel et al. 1984a,b, Blobel et al. 1987). It is of further interest that CI occurred in former forest areas and in the savanna (“cerrado”) recently cleared for pasture and other agricultural use after “liming” that led to a modification of the soil microbiota with an increase of streptomycin-producing actinomycetes (Baldani et al. 1982, Döbereiner 1990, Dutra et al. 1993). Streptomycin was shown to increase the adherence of...
Bacteroides spp. to the epithelial cells of the bovine gingiva significantly in vitro (Kopp et al. 1996).

In this study we investigated the influence of actinomycin and culture supernatants of soil actinomycetes with antibiotic activities on the adherence of Bacteroides spp. to the epithelial cells in vitro. Then these studies were extended to determine if streptomycin and actinomycin could also promote infection under in vivo-conditions.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Bacteroides spp., originally isolated from the gingiva of CI-lesions in young cattle (Blobel et al. 1984a,b, 1987), were cultured on CDC-blood agar plates (Dowell et al. 1977) under anaerobic conditions for 5 days at 37°C. For fluid cultivation a modified brain heart infusion (BHI) (Eley et al. 1985) was used. After adjustment to 10% transmission at 620 nm (i.e. 10⁸ bacteria/ml) Bacteroides suspensions were kept at -20°C until use.

A. pyogenes was cultivated on blood agar plates under microaerobic conditions for 48 h at 37°C (Blobel et al. 1984a,b, 1987). Subsequently, one colony of these cultures was suspended in 1 ml phosphate buffered sodium (0.15 mol/l NaCl, 0.05 mol/l disodiumhydrogenphosphate-dihydrate; pH 7.5).

Actinomycetes, isolated from soil samples of farms with a high prevalence of CI, were kindly provided by Carlos Gava (Embrapa-CNPAB, Seropédica, Rio de Janeiro, Brazil). For antibiotic production these actinomycetes were cultivated in fluid APM-medium (Berwick 1988) under aerobic conditions for 7 days at 30°C. Biochemical characterization of these strains was conducted according to the methods of Pridham & Gottlieb (1948), Perlman & O’Brien (1956), Berd (1973), Pridham & Tresner (1974), Gordon (1974) and Land (1992). Culture supernatants could be obtained by centrifugation for 10 min at 10.000x g with subsequent sterile filtration (0.45 µm, Millipore, Bedford, Massachusetts, USA).

Adherence assays (in vitro)

Adherence assays with Bacteroides spp. and bovine epithelial cells were conducted as previously described by Kopp et al. (1996). Actinomycin was added in increasing concentrations from 5 to 25 µg/ml. Culture supernatants of the actinomycetes contained 0.1 mg/ml protein according to the method of Lowry et al. (1951). All adherence assays were repeated 3 times.

Exposure of calves to the bacteria in the presence or absence of the antibiotics/culture supernatants (in vivo)

For the experimental infections 5 Holstein Friesian calves, 18 to 35 days of age, were used. Mixtures of the bacteria (Bacteroides spp. and A. pyogenes) with streptomycin and actinomycin as well as the antibiotic supernatants from actinomycetes in dosages indicated in Table 1 and 2 were applied to the marginal gingiva of the upper premolar teeth at the site of the interdentary papilla 3 times daily. Calves were examined daily for periodontal lesions and diarrhea indicating CI.

RESULTS

In vitro-adherence assays

Addition of actinomycin in concentrations of 5 to 25 µg/ml increased the adherence of Bacteroides spp. to the oral epithelial cells of calves for up to 335% (Fig. 1).

Exposure of calves

After experimental infections of the 5 calves no progressive periodontal lesions developed. Only 1 (no. 4688) of the 5 calves showed periodontal lesions and diarrhea indicating CI.

Table 1. Application-modus with Streptomycin

<table>
<thead>
<tr>
<th>Calf number</th>
<th>Bacteroides spp. 10⁹/ml</th>
<th>A. pyogenes 10⁹/ml</th>
<th>Streptomycin µg/ml</th>
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<tbody>
<tr>
<td>4688</td>
<td>400 µl</td>
<td>400 µl</td>
<td>20 µg/ml</td>
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<tr>
<td>4689</td>
<td>400 µl</td>
<td>400 µl</td>
<td>200 µg/ml</td>
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<td>4690</td>
<td>400 µl</td>
<td>400 µl</td>
<td>2 mg/ml</td>
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<td>4691</td>
<td>400 µl</td>
<td>400 µl</td>
<td>20 mg/ml</td>
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<tr>
<td>4692</td>
<td>400 µl</td>
<td>400 µl</td>
<td>200 mg/ml</td>
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</tbody>
</table>

Table 2. Application-modus with streptomycin, actinomycin and culture supernatants of soil actinomycetes with antibiotic activities

<table>
<thead>
<tr>
<th>Calf number</th>
<th>Bacteroides spp. 10⁹/ml</th>
<th>A. pyogenes 10⁹/ml</th>
<th>Supernatants of actinomycetes</th>
<th>Combinations Streptomycin/Actinomycin µg/ml</th>
<th>Streptomycin 200µg/ml</th>
<th>Actinomycin 200µg/ml</th>
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<tr>
<td>4688</td>
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<tr>
<td>4692</td>
<td>400 µl</td>
<td>400 µl</td>
<td>/</td>
<td>100 µg/ml each</td>
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</tr>
</tbody>
</table>
calves exhibited a slight retraction of the gingiva at the site of application and a diarrhea that persisted for a period of 6 days. Bacteriological examination of the feces of this calf revealed black pigmented colonies (Bacteroides spp.) on CDC-blood agar plates after cultivation under anaerobic conditions.

**DISCUSSION**

In the *in vitro*-assays actinomycin significantly increased the adherence of *Bacteroides* spp. to the oral epithelial cells of the calves as reported previously for streptomycin (Kopp et al. 1993). Both antibiotics (streptomycin and actinomycin) are produced by soil actinomycetes, isolated in increased numbers in soils of regions with a high CI-prevalence (Baldani et al. 1982, Döbereiner 1990, Dutra et al. 1993). Thus, there appears to be a correlation between the antibiotic activities in the soil and the development of CI-lesions in calves. Most of the actinomycetes isolated from soil of CI-farms were biochemically identified as streptomycetes. The antibiotics produced by these actinomycetes increased *in vitro* the adherence of *Bacteroides* spp. to the oral epithelial cells of the calves significantly. On the other hand, *in vivo*-exposure of calves to streptomycin, actinomycin or the antibiotic supernatants together with *Bacteroides* spp. and *A. pyogenes* within an observation period of 20 days did not lead to any progressive lesions in the periodontum. This could be explained by the relatively short time of exposure and low frequency of applications as well as possibly insuitable antibiotic concentrations in comparison to natural conditions. Nevertheless, it was confirmed *in vitro* that antibiotics produced by soil actinomycetes enhanced the adherence of CI-associated bacteria to the host epithelial cells and probably play a role as determinant factor in the pathogenesis of CI.

**REFERENCES**


