HAEMOLYTIC ACTIVITY OF ACTINOBACILLUS ACTINOMYCETEMCOMITANS STRAINS ON DIFFERENT BLOOD TYPES

Mario Julio AVILA-CAMPOS

SUMMARY

Haemolytic activity of sixty nine Actinobacillus actinomycetemcomitans strains on different animal and human blood types was examined by using a trypticase soy agar supplemented with yeast extract (0.5%). Blood types used were: rabbit, sheep and human (A, Rh+; A, Rh-; B, Rh+; B, Rh-; O, Rh+; O, Rh-; AB, Rh+; AB, Rh- groups). Plates were inoculated and, incubated in microaerophilic conditions, at 37°C, for 48 h. The haemolytic activity of the tested strains was characterized as alpha-haemolysis. Only two isolates were not haemolytic on all blood types (2.9%), two strains were haemolytic only on human blood (one strain on AB, Rh+ group and another one on A, Rh+ and AB, Rh+ groups). No specificity between haemolysis produced by the tested strains and blood type was observed.

KEYWORDS: Actinobacillus actinomycetemcomitans; Haemolysis; Pathogenicity.

INTRODUCTION

Haemolysins are extracellular proteins elaborated by bacteria that destroy membranes of human erythrocytes and other eukaryotic cells and are considered important pathogenicity factors for various microorganisms.

Actinobacillus actinomycetemcomitans, a capnophilic rod-shaped Gram-negative coccobacillus, is a pathogen isolated from monomicrobial and mixed infections associated with actinomycosis, endocarditis, brain and lung abscess and periodontal disease. This microorganism has the ability to produce a potent leukotoxin capable of destroying human polymorphonuclears. The participation of this factor in the pathogenesis of the localized juvenile periodontitis has been suggested.

Papers on haemolytic activity of A. actinomycetemcomitans are few. SLOTS and FARIAS et al. showed no evidence of this activity in human strains. On the other hand, AVILA-CAMPOS et al. showed haemolysis production (alpha-haemolysin) on human blood by nine strains of A. actinomycetemcomitans isolated from patients with and without periodontal disease and from spitoons.

The aim of this study was to examine the haemolytic activity of A. actinomycetemcomitans strains recovered from periodontally disease individuals, on different animal and human blood types.

MATERIALS AND METHODS

Microorganisms

A total of 69 Actinobacillus actinomycetemcomitans strains isolated from individuals with periodontal disease from Periodontic Clinic at the College of Dentistry, University of São Paulo, SP, Brazil. The specimens were collected from diseased
sites (radiographic evidence of alveolar bone loss and periodontal pockets deeper than 5 mm). The microorganisms were recovered in TSBV selective medium \(^8\) and identified as \(A.\) actinomycetemcomitans if they produced translucent colonies with a starlike inner structure, 0.5 mm to 1.0 mm in diameter, Gram-negative cocccobacilli, catalase-positive and if they did not ferment lactose, starch, sucrose and trehalose \(^9\). Reference strains \(A.\) actinomycetemcomitans ATCC 29522, ATCC 29523 and FDC Y4 were included in all tests. The strains were stored in freezer at -70°C.

**Haemolytic activity**

Haemolysin production was performed on trypticase soy agar (Difco Laboratories, Detroit, MI) supplemented with yeast extract (0.5%) and enriched with blood (5%). Dehydrated sheep blood, citrated rabbit and human blood (A, Rh+; A, Rh-; B, Rh+; B, Rh-; O, Rh+; O, Rh-; AB, Rh+; AB, Rh- groups) were used. The strains were grown in brain heart infusion medium (Difco Laboratories, Detroit, MI) supplemented with yeast extract (0.5%) and incubated in microaerophilic conditions (candle method), at 37°C, for 48 h. Aliquots of 20 µl of each culture (approximately 10^4 cells/ml) were then deposited on the agar media enriched with the different blood types. The inoculum size was verified by colony count. Plates inoculated in duplicate were incubated in the conditions described above. A positive haemolytic activity was indicated as a clear zone around the growth.

**RESULTS**

The haemolytic activity of the tested strains was characterized as an alpha-haemolysis. Of the 69 tested strains only two isolates were not haemolytic on all blood types (2.9%). On the other hand, two strains were haemolytic only to human blood, one strain only to AB, Rh+ group, and another one to A, Rh+ and AB, Rh+ groups. 52 strains were positive on both rabbit and sheep cells. No reference strains were haemolytic either on rabbit and sheep blood or human blood (O, Rh- and AB, Rh- groups).

**DISCUSSION**

There are very few reports in the literature about haemolytic activity of \(A.\) actinomycetemcomitans and in particular concerning its mechanism of action \(^4\). ALEXANDER \(^1\), testing strains isolated from swine, showed variable haemolytic activity against sheep, horse and cattle blood.

On the other hand, AVILA-CAMPOS et al. \(^2\) showed that of 41 tested strains of \(A.\) actinomycetemcomitans, eight were haemolytic on sheep and human blood, three of which were isolated from individuals without periodontal disease and five from localized juvenile periodontitis patients.

No specificity between haemolysis produced by the tested strains and any blood type was observed. \(A.\) actinomycetemcomitans when subcultured successively or treated with ethidium bromide in different concentrations did not lose haemolytic activity on various blood types. It suggests that the production of this haemolytic enzyme could be a stable factor (unpublished observations). Various aspects are not clear, such as the product interactions of Hly gene, transport and action of haemolysin, and molecular and genetic association the Hly gene with other virulence genes \(^12\).

**TABLE 1**

<table>
<thead>
<tr>
<th>Activity</th>
<th>Animal</th>
<th>Human (Rh)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rabbit</td>
<td>Sheep</td>
</tr>
<tr>
<td>Positive</td>
<td>55 (%)</td>
<td>(79.7)</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>(20.3)</td>
</tr>
</tbody>
</table>
On the other hand, loss of extracellular enzyme and toxin production is common with repeated laboratory passage of bacteria. It could well explain the difference between the reference strains and the fresh isolates, here tested. The pathogenic character of *A. actinomycetemcomitans* is poorly understood and an abundance of extracellular products are suspected of contributing to its virulence, e.g., leukotoxin.

However, TSAI et al. have not observed haemolytic activity in *A. actinomycetemcomitans* FDC-Y4, characterized as producing leukotoxin. It is not known yet if haemolysis contributes to the pathogenicity or virulence in some *A. actinomycetemcomitans* strains. Although the majority of *A. actinomycetemcomitans* described here express similar phenotypes for haemolysis, it has not been examined yet if they are all active by the same haemolytic mechanism. Certainly, further studies are needed for a functional, biochemical, genetic and molecular characterization of haemolysis in *A. actinomycetemcomitans*, for an application in pathogenic and systematic terms.

**REFERENCES**


This work was supported by grants from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Proc. 93/0056-0.

**ACKNOWLEDGEMENTS**

I wish to express my sincere gratitude to Dra. Maria Auxiliadora Roque de Carvalho and Dr. Flávio Zelante for their critical reading, and Andemir da Silva and João Paulo Ribeiro for their technical help.

Received for publication in 12/07/1994. Accepted for publication in 29/03/1995.