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HAEMOLYTIC ACTIVITY OF *ACTINOBACILLUS ACTINOMYCETEMCOMITANS* STRAINS ON DIFFERENT BLOOD TYPES

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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 haemolysin 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 [6].

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 [5, 7, 13]. 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 [11].

Papers on haemolytic activity of *A. actinomycetemcomitans* are few. SLOTS [9] and FARIAS et al. [3] showed no evidence of this activity in human strains. On the other hand, AVILA-CAMPOS et al. [2] showed haemolysis production (alpha-haemolysin) on human blood by nine strains of *A. actinomycetemcomitans* isolated from patients with and without periodontal disease and from spittoons.

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

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sites (radiographic evidence of alveolar bone loss and periodontal pockets deeper than 5mm). 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 coccobacilli, 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%). Defibrinated 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 10^6 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).

Haemolytic activity of the tested strains are shown in Table 1.

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 haemolysin 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
Haemolytic activity of 69 *A. actinomycetemcomitans* strains in different blood types.

Activity	Blood types									
	Animal		Human (Rh)							
	Rabbit	Sheep	A+	A-	B+	B-	O+	O-	AB+	AB-
Positive (%)	55 (79.7)	52 (75.4)	66 (95.7)	65 (94.2)	63 (91.3)	63 (91.3)	63 (91.3)	60 (86.9)	67 (97.1)	57 (82.6)
Negative (%)	14 (20.3)	17 (24.6)	3 (4.3)	4 (5.8)	6 (8.7)	6 (8.7)	6 (8.7)	9 (13.1)	2 (2.9)	12 (17.4)

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 [10].

However, TSAI et al. [10] have not observed haemolytic activity in *A. actinomycetemcomitans* FDC Y4, characterized as producing leukotoxin. It is not known yet if haemolysin 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, genetical and molecular characterization of haemolysin in *A. actinomycetemcomitans*, for an application in pathogenic and systematic terms.

RESUMO

Atividade hemolítica de cepas de *Actinobacillus actinomycetemcomitans* em diferentes tipos sanguíneos.

A atividade hemolítica de 69 cepas de *Actinobacillus actinomycetemcomitans* foi determinada em diferentes tipos de sangue animal e humano, usando como meio base ágar de soja tripticaseina, suplementado com extrato de levedura (0,5%). Foram utilizados sangue de coelho, carneiro e humano (grupos A, Rh+; A, Rh-; B, Rh+; B, Rh-; O, Rh+; O, Rh-; AB, Rh+ e AB, Rh-). As placas foram inoculadas e, incubadas em condições de microaerofilia, a 37°C, por 48 h. A atividade hemolítica das cepas testadas foi caracterizada como alfa-hemólise. Somente dois (2,9%) isolados não hemolisaram todos os tipos sanguíneos, duas cepas hemolisaram somente sangue humano (uma o grupo AB, Rh+ e outra os grupos A, Rh+ e AB, Rh+). Não foi observada alguma especificidade entre as hemolisinas produzidas e os tipos de sangue utilizados.

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REFERENCES

1. ALEXANDER, A. D.- *Actinobacillus actinomycetemcomitans*. **In**: BLAIR, J.E; LENNETTE, E.H. & TRUANT, J.P., ed. **Manual of Clinical Microbiology**. Bethesda, American Society for Microbiology, 1972. p. 220-222..
2. AVILA-CAMPOS, M.J; FARIAS, L. M.; CARVALHO, M. A. R. et al.- Atividade hemolítica de *Actinobacillus actinomycetemcomitans*. **Rev. Microbiol., 19**: 262-265, 1988.
3. FARIAS, L. M; CARVALHO, M. A. R.; DAMASCENO, C. A. V. et al.- Caracterização morfológica, bioquímica e fisiológica de *Actinobacillus actinomycetemcomitans* isoladas de lesões periodontais humanas. **Rev. Microbiol., 17**: 296-306, 1986.
4. HILMAN, J. D.; MAIDEN, M. F. J.; PFALLER, S. P. et al.- Characterization of hemolytic bacteria in subgingival plaque. **J. Periodont. Res., 28**: 173-179, 1993.
5. REIDER, J. & WHEAT, J.- Endocarditis caused by *Actinobacillus actinomycetemcomitans*. **Sth. med. J.** (Bgham, Ala.), **72**: 1219-1224, 1979.
6. ROGOLSKY, M.- Nonteric toxin of *Staphylococcus aureus*. **Microbiol. Rev., 43**: 320-360, 1979.
7. SLOTS, J.; REYNOLDS, H. S. & GENCO, R. J.- *Actinobacillus actinomycetemcomitans* in human periodontal disease: a cross-sectional microbiological investigation. **Infect. Immun., 29**: 1013-1020, 1980.
8. SLOTS, J.- Selective medium for *Actinobacillus actinomycetemcomitans*. **J. clin. Microbiol., 15**: 606-609, 1982.
9. SLOTS, J.- Salient biochemical characters of *Actinobacillus actinomycetemcomitans*. **Arch. Microbiol., 131**: 60-67, 1982.
10. TSAI, C.C.; Mc ARTHUR, W.; BAEHNI, P. et al.- Extraction and partial characterization of a leukotoxin from a plaque derived Gram-negative microorganisms. **Infect. Immun., 25**: 427-439, 1979.
11. TSAI, C.C; SHENKER, B. J.; DI RIENZO, J. M. et al.- Extraction and isolation of a leukotoxin from *Actinobacillus actinomycetemcomitans* with polymyxin B. **Infect. Immun., 43**: 700-705, 1984.
12. WELCH, R. A. & FALKOW, S.- Characterization of *Escherichia coli* hemolysin conferring quantitative virulence. **Infect. Immun., 43**: 156-160, 1984.
13. ZAMBON, J. J.; CHRISTERSSON, L. A. & SLOTS, J.- *Actinobacillus actinomycetemcomitans* in human periodontal disease. **J. Periodont., 54**: 707-711, 1983.

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