Effect of supernatants from selected periopathogens cultures on ATP levels in human gingival fibroblasts

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Introduction: Periodontitis belongs to the most frequent chronic diseases in humans and leads to destruction of periodontal tissues. In etiopathogenesis of periodontitis a principal role play subgingival bacterial biofilm with specific species of pathogenic bacteria, termed periopathogens, among which a recognised clinical significance is manifested by *Porphyromonas gingivalis*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans*.

Aim: The study aimed at: 1) analysis of action manifested by supernatants obtained from cultures of *Porphyromonas gingivalis*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans* strains on ATP levels in human gingival fibroblasts (HGF-1), 2) evaluation of temperature effect on activity of studied supernatants.

Methods: In the presented experiments 21 bacterial strains were isolated from subgingival bacterial biofilm of the adult patients with periodontitis, including 11 strains of *Porphyromonas gingivalis* (Fig. 1), 6 strains of *Prevotella intermedia* (Fig. 2) and 4 strains of *Aggregatibacter actinomycetemcomitans* (Fig. 3). Changes in ATP level in HGF-1 gingival fibroblasts, taking place under effect of supernatants of every culture containing the isolated strains of bacteria were presented in means of the effects. Evaluation of ATP levels in cultures of gingival fibroblasts HGF-1 was performed using a luminescence test (CellTiter-Glo Luminescent Cell Viability Assay, Promega). In addition activity of the supernatants was analysed under effect of the temperatures of 56ºC for one hour and of 100ºC for 30 minutes.

Results: In control cultures mean levels of ATP in HGF-1 amounted to 4.84 ± 0.35 mln RLU. Heat-treated at 56ºC or untreated supernatants of *P. gingivalis* and *A. actinomycetemcomitans* cultures were found to significantly reduce production of ATP in HGF-1 (mean levels of ATP amounted to, respectively, 3.47 ± 0.43 and 3.52 ± 0.46 mln RLU). In turn, heat-treated at 56ºC or untreated supernatants of *P. intermedia* induced no significant alterations in ATP level in HGF-1. Supernatants of the bacterial cultures subjected to heat treatment at 100ºC induced no decrease in ATP levels in gingival fibroblasts. Results are summarized in Table 1.

Table 1. Mean levels of ATP (luminescence in millions of RLU) in HGF-1 gingival fibroblasts following 24 h incubation with supernatants of the examined periopathogens

Studied cells	Unheated supernatants	Supernatants exposed to the temperature of 56ºC for 30 minutes	Supernatants exposed to the temperature of 100ºC for 30 minutes
		Mean ± SD [range]	
Control (gingival fibroblasts HGF-1) with 10% PBS	4.84 ± 0.35 [4.37-5.45]	4.78 ± 0.51 [4.21-5.35]	4.81 ± 0.48 [4.26-5.29]
HGF-1 with 10% supernatant of *P. intermedia*	4.61 ± 0.57 [3.89-5.28]	4.72 ± 0.69 [4.12-5.07]	4.77 ± 0.56 [4.03-5.21]
HGF-1 with 10% supernatant of *A. actinomycetemcomitans*	3.52* ± 0.46 [2.83-4.28]	3.65* ± 0.45 [3.06-4.41]	4.68 ± 0.52 [3.64-5.06]
HGF-1 with 10% supernatant of *P. gingivalis*	3.47* ± 0.43 [2.57-4.15]	3.53* ± 0.36 [2.69-4.37]	4.61 ± 0.49 [3.48-5.15]

*Significant difference as compared to the control group

Fig. 1. *Porphyromonas gingivalis*

Fig. 2. *Prevotella intermedia*

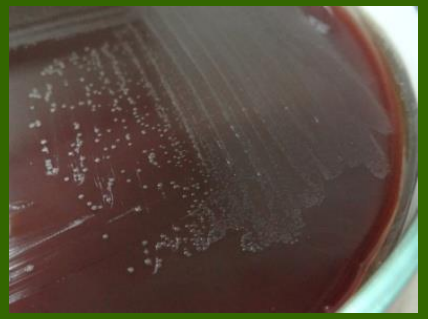

Fig. 3. *Aggregatibacter actinomycetemcomitans*

Conclusions: The results indicate that extracellular products of *P. gingivalis* and *A. actinomycetemcomitans,* representing most probably thermostable peptides, reduce synthesis of ATP in human gingival fibroblasts, which may inhibit their proliferation.

References:
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