PL 1 - The Susceptibility of Anaerobic Bacteria to Chitosan and Metronidazole. In Vitro Study

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A total 32 strains anaerobic bacteria isolated from the infections in oral cavity, as well as 5 standard strains susceptibility to metronidazole and low-molecular-weight chitosan were determined. In the study were used: metronidazole (Pharma-Zentrale, GmbH) and low-molecular-weight chitosan (lmw, viscosity: ~ 100 mPas, Fluka Chemie AG, deacetylation grade: 75-85 %). Metronidazole and chitosan were dissolved in sterile distilled water immediately before the experiment to obtain the following concentrations: 100, 50, 25, 12.5, 6.2, 3.1 μg/ml (metronidazole) or 2000, 1000, 500, 250, 125, 62.5 μg/ml (chitosan) and were added into agar. The following anaerobes were investigated: *Bacteroides forsythus* (3 strains), *Prevotella intermedia* (6), *Prevotella loescheii* (1), *Prevotella oralis* (2), *Porphyromonas gingivalis* (2), *Porphyromonas asaccharolytica* (1), *Fusobacterium nucleatum* (4), *Peptostreptococcus anaerobius* (2), *Micromonas micros* (2), *Finegoldia magna* (2), *Actinomyces naeslundii* (1), *Propionibacterium acnes* (6) and standard strains: *Bacteroides fragilis* ATCC 25285, *Fusobacterium nucleatum* ATCC 25585, *Peptostreptococcus anaerobius* ATCC 27337, *Peptostreptococcus magnus* ATCC 29328, *Propionibacterium acnes* ATCC 11827. The susceptibility (MIC) of bacteria was determined by means of plate dilution technique in agar. Metronidazole and chitosan solutions were added to Brucella agar supplemented with 5% defibrinated sheep's blood, menadione and hemin. The plates were inoculated using a Steers multipoint inoculator. Inoculum contained 105 CFU/spot. In each experimental series, the growth of strains on the culture medium without the compounds investigated was checked. Incubation was performed for 48 h at 37°C (310 K) in anaerobic jars containing a mixture of 10% CO_2, 10% H_2 and 80% N_2, in the presence of palladium catalyst and indicator of anaerobiosis. The concentration at which no macroscopic growth of the microbes was observed on the medium was regarded as the lowest concentration inhibiting the growth of microbes (MIC).

In low concentrations (≤ 3.1 – 12.5 μg/ml) metronidazole inhibited growth of 25 (78 %) strains of investigated anaerobes. Metronidazole in range of examined concentrations did not show any antibacterial activity towards 7 strains of Gram-positive bacilli (MIC > 100 μg/ml). From amongst 32 examined strains of anaerobes the chitosan inhibited growth of 20 (62.5 %) strains in concentrations 62.5 – 2000 μg/ml. In low concentrations (MIC ≤ 62.5 – 125 μg/ml) chitosan inhibited growth of 8 (25 %) investigated strains of anaerobes.