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Investigation on Influence of Singlet Oxygen on Periodontal Microorganisms

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Microbiological experiments have not demonstrated any noticable direct antimicrobial activity of the both steam-water mixture and «activated water» generated by device «MIT-C» for singlet oxygen therapy against clinical strains of staphylococci, β-, α-haemolytic streptococci, *Candida* yeasts of periodontal origin. The possible ways to improve the clinical effectiveness of periodontal diseases singlet oxygen therapy by the local application of photosensitizers is discussed.

Keyword: Mood Singlet Oxygen Therapy, Antimicrobial Activity, Periodontal Microorganisms

1. Introduction

Singlet oxygen is a kind of molecular oxygen, the molecule of which being in an excited state, is characterized by excessive energy and has an overreacting property. It, along with other reactive oxygen species(hydrogen peroxide, superoxide anion, hydroxyl and peroxynitril radicals), plays a vital role in the development of many physiological and pathological processes [7]. Different biomolecules are oxidated with the participation of singlet oxygen(first of all nucleic acids, lipids, amino acid residues of methionine, histidine, tryptophan, which form functional centers of proteins) that underlies inactivation of microorganisms in the phagosome of phagocytes and free radical processes of the body tissue structures damage in inflammation. Singlet oxygen is produced by a number of body biological systems (by peroxidase catalysed oxidation of halogen ions in processes of lipoxygenase and cyclooxygenase oxidation of long-chained fatty acids, microsomal oxidation) [2, 7].

Determining the biological role of singlet oxygen resulted in the development of a new treatment approach of various diseases associated with an imbalance of free radical processes and antioxidant protection of the body tissues, called singlet-oxygen therapy (SOT). Both in our country and abroad there have been developed the devices which induce a conversion of atmospheric oxygen into the singlet one under the influence of hard ultraviolet. Oxygen molecules exist in a singlet state for the limited time and then recover to a normal state, accompanied by electromagnetic waves radiation of ultraviolet range. It is considered that a chain of biochemical and biophysical processes activates under the influence of the released energy aimed at resolution of metabolic and oxidative processes in the body tissues. With this purpose, SOT is used in various internal and metabolic diseases

treatment[5], as well as in dentistry, with periodontitis, peri-implantitis, endodontal diseases[5].

Taking into account that singlet oxygen is one of the compounds the production of which is induced by membrane NADPH • H (NOX) oxidase in contact of phagocytes with microorganisms (called "respiratory burst") and provides their killing in phagosome [4, 7, 10], its direct antimicrobial effect is supposed to be one of the possible explanations for the therapeutic effect of SOT. However, this hypothesis requires experimental verification.

2. Results of the research

The results indicate that exposure of microbial cultures to steam and water mixture released from the tip of the inhalation device for SOT «МИТ-С», carries out impact on the viability of staphylococci, streptococci and Candide of periodontal origin. While exposing to steam mixture took 3 minutes, the colonies number of all microbial cultures of periodontal origin (staphylococci, β-and α-hemolytic streptococci and Candida) did not change absolutely on the experimental cups in comparison to test pattern (Table 1).

Table 1: Changes of microorganism's colonies number on agar

Microbial Culture	The Test Pattern	Exposure Time	
		3 min	9 min
Staphylococci			
Staphylococcus aureus 209-P (ATCC 6538-P)	1524,0±20	1520,0±32	1416,0±15*
S. epidermidis	1657,0±20	1649,0±18	1559,0±12*
β-hemolytic Streptococci			
Streptococcus group G	1139,0±16	1138,0±22	1034,0±10*
Streptococcus constellatus	1176,0±7	1163,0±13	1045,0±16*
α-hemolytic Streptococci			
Streptococcus salivarius	983,0±11	971,0±13	922,0±11
Streptococcus mitis	866,0±6	859,0±9	804,0±14*
Yeast Fungi			
Candida albicans	1140,0±14	1135,0±5	1119,0±7

Note: $p < 0.05$ compared to test pattern.

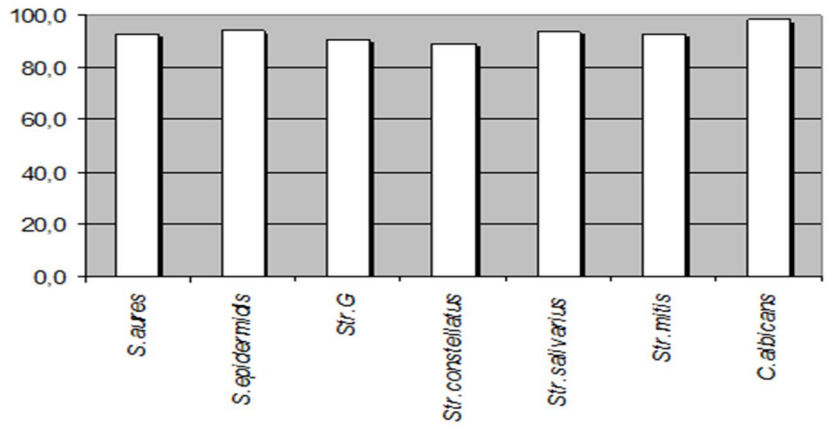

Fig. 1: Percentage of microbial cells survival after exposure to steam mixture generated by device for SOT «МИТ-С» during 9 min.

3. Materials and Methods of the Research

In this regard, the aim of this research work was to study the influence of steam and water mixture enriched with singlet oxygen generated by device for SOT «МИТ-С» (LLC " SRI Medinteh ", Kyiv, Ukraine), on the viability of the microflora types in the patients' periodontal pockets with generalized periodontitis.

We have used steam and water mixture and "activated water" enriched with singlet oxygen generated by device for SOT «МИТ-С» (LLC "SRI Medinteh", Kyiv, Ukraine).

A collection of Staphylococcus aureus strain 209-R (ATCC 6538-P), clinical strains of facultative anaerobic microorganisms isolated from periodontal pockets of patients with generalized periodontitis: S. epidermidis, β-hemolytic Streptococcus group G, β-hemolytic Streptococcus, Streptococcus constellatus, α-hemolytic Streptococci, Streptococcus salivarius and Streptococcus mitis, yeast-like fungus Candida albicans have been used as test strains. Clinical strains of microorganisms were identified on the basis of morphological, cultural properties using biochemical microtests sets "STAPHYtest 16", "STREPTOtest 16"

(Lachema, Czech Republic). Staphylococci cultivation has been carried out on an ordinary agar, Streptococci cultivation has been carried out on blood agar, fungi of the genus Candida cultivation has been carried out on Sabouraud's medium. Two series of experiments were performed which evaluated the impact on the viability of microbial cultures enriched with steam and water mixture and "activated water". In the first series of experiment, the effect of steam and water mixture generated by device for SOT «МИТ-С» on microorganisms was investigated. Standardized suspensions were prepared beforehand up to optical turbidity model (5×10^8 CFU/ml) of daily microbial test strains cultures. Working suspension of each culture with concentration of 5×10^4 CFU / ml was received by performing tenfold serial dilutions in sterile saline. 100ml of the working suspensions of every culture was plated in 3 cups of nutrient agar. Cultures on cups no. 2 and no. 3 were exposed to steam and water mixture released from the tip of the inhalation device for SOT during 3 and 9 min., respectively. Plated with culture cup no. 1 was the test pattern. After 24 hours of incubation in an incubator at 37°C, rate of culture growth in the test pattern was compared to the one in experimental cups. In the second series of experiments, the effect on the viability of microbial cultures during their exposure to the "activated water" enriched with singlet oxygen was studied. Working suspensions with concentration of 5×10^5 CFU/ml were used for research. 200 ml of standardized working suspensions of every culture was added into 4 sterile tubes. 1.8 ml of sterile saline was added into test tube no. 1 (test pattern), thoroughly mixed, and 100 ml of aliquot was selected for plating on the IPA. Applied sample was triturated carefully with a spatula along the surface of the agar. 1.8 ml of "activated water" immediately after the release from device for SOT «МИТ-С» was added into test tubes no. 2, no. 3 and no. 4. Exposure time of test cultures to "activated water" was 10, 30 min. and 1 hour respectively. Aliquots from each tube were plated similarly to IPA. The results of plating were analyzed after 24-hour incubation in an incubator at 37°C. To make experiments results more objective, digital images on cups cultures were taken, processing of which (counting colonies) was performed with the help of computer program TotalLab TL120 v 2008 (Nonlinear Dynamics Ltd.). Rate of culture growth in the test pattern was compared to the rate of growth of cultures in the experimental cups. The results were processed by methods of variation statistics.

A slight decrease in the microorganism's colonies number was observed on the cups after a 9-minute exposure to steam mixture (Fig. 1). Thus, the percentage of S. aureus cell survival in this experiment was 91.9%, of coagulase-negative S.

epidermidis was 94,1% (p <0,05). β-hemolytic Streptococci appeared to be slightly sensitive to steam mixture enriched with singlet oxygen. Percentage of S. constellatus cells survival was 88,9%, β-hemolytic Streptococcus of group G was 90,8% (p <0,05). However, α-hemolytic Streptococci S. salivarius and S. mitis, which represent the oral micro flora residents, maintained their viability at 93.8% and 92.9%, respectively. The number of yeast fungi of Candida genus has not practically changed after contact with steam mixture (survival rate was 98.1%). So generally bacteria of periodontal origin showed weak sensitivity to steam mixture which is released from the tip of the inhalation device for SOT «МИТ-С». We reported the death of only about 10% of β-cells hemolytic Streptococci and no more than 6-8% of the α-cells hemolytic Streptococci and Staphylococci, which is insignificant under high microbial load.

Analysis of plating showed that contact with "activated water" for 10 and 30 min. had no impact on the number of viable microorganisms cells at all (Table 2). Decrease of their number is observed only after 1-hour contact with the "activated water". The number of viable S. aureus cells decreased in suspension by approximately 30%, coagulase-negative S. epidermidis - by 40% (p <0,05). Culture of β-hemolytic Streptococcus group G appeared to be the most sensitive to the "activated water" among all the tested microorganisms, the number of viable cells of which decreased by 45,9% (p <0,05).

Table 2: Changes of microorganism's colonies number after contact with "activated water"

Microbial culture	The number of viable microbial cells lg CFU / ml			
	The test pattern	Exposure Time		
		10 min.	30 min.	1 h.
Staphylococci				
S. 209-P (ATCC 6538-P)	4,04±0,02	4,09±0,01	4,03±0,02	3,87+0,03*
S. epidermidis	4,10±0,01	4,09±0,01	4,09±0,01	3,85±0,03*
β-hemolytic Streptococci				
Streptococcus group G	4,06±0,01	4,06±0,01	4,06±0,01	3,79±0,03*
Streptococcus constellatus	4,07±0,01	4,06±0,01	4,07±0,01	3,87±0,05*
α-hemolytic Streptococci				
Streptococcus salivarius	4,02±0,01	4,02±0,01	4,01±0,01	3,89±0,02*
Streptococcus mitis	3,98±0,01	3,98±0,01	3,97±0,01	3,78±0,03*
Yeast Fungi				
Candida albicans	4,03±0,01	4,02±0,01	4,02±0,01	3,95±0,02

Note: p <0.05 compared to test pattern

The number of other species viable cells cultures of oral Streptococci decreased by approximately one third (p <0,05). Survival rates of Candida after 1-hour exposure to "activated water" was the highest among all test cultures. We reported the death of only 20.6% of fungi. We believe that the observed decrease in viability of all microbial cultures after their contact with the "activated water" for 1 hour in isn't associated with the presence of singlet oxygen in it. Singlet oxygen form is extremely unstable. Thus, it is proved that the lifetime of singlet oxygen generated by laser irradiation of porous silicon at room temperature in the gas phase is 15 ms and in biological systems is much shorter, approximately100 ns [5, 8]. Therefore, its bactericidal effect could have been expected only at the shortest time of microbial cells exposure to the "activated water". In biological systems there is observed a rapid effect of slaking singlet oxygen with antioxidant factors protection (carotenoids, tocopherols, glutathione), as well as its interactio`n with soluble biomolecules [1, 2, 3].

Therefore, it is obvious that the bactericidal effect of singlet oxygen in the body can be realized only in case of its prolonged generation in close proximity to the surface of microbial cells, i.e., during its absorption in the phagosome of phagocytes. In this situation prolonged production of singlet oxygen is provided by membrane NADPH • H oxidase, activation of which is consistent with the interaction of phagocytes receptors with immunoglobulins or complement components on the surface of bacterial cells opsonized by them (the so-called "zipper"mechanism). The decrease of viable cells number in microbial cultures which were in the "activated water" during 1 hour, can be explained by their lysis in hypotonic conditions. These considerations, together with the above mentioned experimental data indicate that the therapeutic effects of SOT are described in the clinical setting may not be related to direct antimicrobial effect of singlet oxygen.

4. Conclusion of Research

Steam and water mixture, and the "activated water" generated by the device for SOT «МИТ-С» did not show significant direct antimicrobial effect on clinical strains of Staphylococci, β-and α-hemolytic Streptococci and yeast fungi of Candida genus of periodontal origin.

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