ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Substantiation of use of Photodynamic Therapy in Experimental Research in Vitro with Strains of Periodontopathogenic Bacteria and Fungi Candida

Vadim O. Samusenkov[1*], Viktor N. Tsarev[2], Evgenii V. Ippolitov[2], Mikhail S. Podporin[2], Alexei V. Yumashev[1], Natalia N. Vlasova[1], Vitaliy V. Borisov[1], Tatyana V. Tsareva[2]

1. *The State Education Institution of Higher Professional Training The First Sechenov Moscow State Medical University under Ministry of Health of the Russian Federation, Moscow, Russian Federation.*
2. *A.I. Yevdokimov Moscow State University of Medicine and Dentistry, Moscow, Russian Federation.*

***Corresponding Author: Vadim O. Samusenkov**

Abstract

Photodynamic therapy is used for the treatment of inflammatory periodontal diseases. This study was aimed at determining the effectiveness of the treatment of periodontitis and gingivitis with the use of a photosensitizer that interacts with the light flux, has the ability to adsorption on the surface of the bacterial cell. To determine the sensitivity of photodynamic effects, the following priority strains of microorganisms were selected: *Aggregatibacter actinomycetemcomitans, Streptococcus constellatus, Candida glabrata*. For the cultivation of microorganisms in the bioreactor, two types of tubes were used: 50 ml tubes with a membrane filter (TubeSpin) and 50 ml tubes of Falcon type with a lid without a membrane. 20 ml of nutrient medium was added to each tube. A. actinomycetemcomitans (37°C; RPM: 500 min–1; measurement freq.: 3 h-1; Rev. spin period: 1 sec.; volume: 15-16 ml; λ: 850 nm); S. constellatus (37°C; RPM: 1500 min–1; measurement freq.: 3 h-1; Rev. spin period: 2 sec.; volume: 15-16 ml; λ: 850 nm); C. albicans (37°C; RPM: 1300min–1; measurement freq.: 3 h-1; Rev. spin period: 3sec.; volume: 15-16 ml; λ: 850 nm). After bacterial suspension inoculations, the samples were irradiated with semiconductor laser devices with different wavelengths-630 nm and 662 nm, at a power of 100 j/cm2. The basis of experimental studies was automated cultivation of isolated strains of microorganisms with control of growth curves of microbial populations using different modes of exposure and photosensitizers.

Keywords: *Periodontopathogenic microorganisms, Photodynamic effects, Photosensitizers, Gingivitis, periodontitis.*

Introduction

Inflammatory diseases of periodontitis-gingivitis and periodontitis - are a serious medical and social problem; in recent years a progressive tendency to growth of disease incidence is found among younger persons [1]. Furthermore, just periodontological treatment becomes a foundation on which tactics of treatment of other dental diseases is quite often built up.

Nowadays the most confirmed and substantiated model of emergence of inflammatory diseases of periodontitis with considerably marked microbial and inflammatory-immune components is the multi-factorial model thereof [2, 3].

Emergence and development of various forms of periodontitis is caused by activity of a "periodontopathogenic community" as well as by a complex of other unfavorable factors what determines the multiple-factor character of this disease. One can with good reason believe that periodontitis must be considered as a complex disease which emerges under impact of a cumulative

influence of exogenous factors (microbial biofilm, anomalies of attachment of frenulums, defects of filling and prosthesis, anomalies of position of teeth, occlusion abnormalities) and endogenous ones (diseases of gastrointestinal tract, hormone

disruptions, blood diseases etc.), and under influence of general and local factors; this disease is infectionally induced by immune damage of periodontal complex with a high probability of genetic predisposition; i.e. it is a result of imbalance between factors of aggression (periodontopathogenes) and factors of protection of microorganism [2].

Experience, which has been gathered in recent years, show that the leading role in forming of an inflammatory process in oral cavity is played by resident obligate anaerobic and microaerophilic microflora. Many groups of researchers studied composition and properties of bacterial biofilm of oral cavity by means of microbiological and molecular research methods in order to estimate microbial risk factors of periodontium diseases. Modern technologies allow to extract genetic material of more than 700 species or phenotypes of microbes in oral cavity and a half of them cannot be cultivated [4, 5].

At the same time, role of an only small number of bacteria is nowadays proved as etiological factors of periodontium diseases. Among these bacteria are Porphyromonas gingivalis, Prevotella intermedia, Aggregatibacter actinomycetemcomitans (Actinobacillus actinomycetemcomitans), Tannerella forsythia (Bacteroides forsythus), Campylobacter rectus as well as Treponema denticola [5].

Most authors also believe that yeast like fungi of genus Candida spp. belong to the group of periodontopathogenic species of microorganisms too, which must not be present onto a healthy gingiva, except for 6-12% cases of a "healthy carriage" [6]. Exactly in fungi of genus Candida the mechanisms of aggression and protection (variability and considerable lability of morphological properties of the cell; adhesion receptors and lytic enzymes) were determined which directly and indirectly - through immune reactions - damage tissues of periodontium and cause negative changes in system of local immunity of oral cavity [7].

Nowadays carrying-out of the whole complex of therapeutic measures in case of inflammatory-destructive character of diseases of periodontium tissues does not secure a long remission effect of the pathologic process [8]. Growing resistance to antibacterial preparations and choice of treatment technique of this pathology will always be a topical problem in dental practice. Just wide and unjustified use of antimicrobial preparations increasingly frequently results in increase of number of resistant microorganisms, selection of antibiotic-resistant strains and, as a consequence, causes difficulties in choice of an appropriate antibiotic therapy.

Examples of various forms of periodontitis and microbiological examinations show that reinfection of periodontium tissues begins after 1.5-2 months following a course of conducted complex treatment, and reach of high values of concentration of pathogenic microflora is detected already by the end of the second month after carried-out therapeutical measures. This fact confirms a low clinical effectiveness of treatment and confirms recurrent marked chronic inflammatory process in periodontium tissues [9]. During treatment of patients with periodontium diseases the modern practice is based on use of a combined therapy.

Recommended complexes of therapeutical measures are mainly combinations of various components of pharmacotherapy which allow reaching only a short-term effect. At the same time, there is a risk of emergence of complications from pharmacotherapy in case of inflammatory diseases [10, 11].

Dental market is in constant search for alternative methods of treatment of this pathology and one of the most promising photochemotherapeutic methods, which are based on a complex use of light and chemical compounds of various purposes, is photodynamic therapy (FDT). Although at the beginning, FDT was used solely for treatment of patients with malignant neoplasms, today a growing number of reports are published about successful application of the method in other fields of medicine what considerably widens range of its use [12, 13]. The first component of this method is a photosensitizer. It is a substance which interacts with light flux and has ability of adsorption on the surface of a

bacterial cell. The second component of FDT is a low-energy light radiation whose wave length theoretically must conform to absorption peak of this photosensitizer, thereby enhancing effectiveness of this method [14].

Source of such radiation can be multipurpose or specialized lasers. Under influence of light radiation a photochemical reaction develops in organism tissues with release of singlet oxygen and active radicals which are cytotoxic agents for cells which quickly propagate [15, 16].

But outcomes of this therapy can be different and depend on several factors: on intensity of generation of active forms of oxygen due to influence of radiation with a certain wave length, on activity of anti-stress proteins and antioxidant bacteria and on correctly selected photosensitizer which must conform to the specific clinical presentation [17, 18]. Goal of the research is to evaluate effectiveness of use of photodynamic therapy in experiments in vitro in respect of microaerophilic group of causative agents of periodontitis and fungi Candida by means of light activation with different wave lengths.

Materials and Methods

To conduct the research we selected patients suffering from chronic generalized periodontitis of medium severity - 62 persons aged from 35 to 46 years who did not have a marked somatic pathology. Before treatment a complex clinical examination of all patients' state of periodontium tissues was conducted, it included an obligatory examination of microbial profile of periodontal pocket by means of method of PCR diagnostics (set of reagents Multi-dent-S of company Genlab, Russia). Obtained data allowed determining high-priority species of microorganisms for this nosology which were used in the further experiment.

To determine sensitivity of photodynamic influence the following high-priority microorganism strains were selected: *Aggregatibacter actinomycetemcomitans, Streptococcus constellatus, Candida glabrata.* We used technique which had been developed in Department of Microbiology, Virology and Immunology of A.I. Yevdokimov Moscow State University of Medicine and Dentistry of the Ministry of Health of the RF [19]. Initial inoculation for extraction of obligate and facultative anaerobic bacteria was performed on Columbia 5% Blood Hemin Agar with addition of defibrinated sheep blood and selective additives for extraction of gram-negative anaerobic bacteria and gram-positive microaerophilic streptococci. Fungi of genus Candida were cultivated on a chromogenic nutrient medium.

All inoculations were placed into a thermostat at temperature 37 °C for 48 hours (for anaerobic cultures - into an anaerobic jar for 14 days). Before conducting the experiment for a pure culture an enrichment medium for microorganisms was used to let the cultures grow for preparation of a bacterial suspension. Base for conducting the experiment was an automatic system of microorganisms' real-time cultivation Bioreactor Reverse-Spin® RTS-1 (BioSan, Latvia). Results were interpreted by change of turbidity of cellular suspension on the basis of evaluation of optical density (OD) at wave length 850 nm.

To cultivate microorganisms in the bioreactor we used two types of tubes: tubes 50 ml with a membrane filter (TubeSpin®) and tubes 50 ml of type *Falcon* with lid without a membrane. 20 ml nutrient medium, a beforehand prepared bacterial suspension and photosensitizers were added into each tube. After inoculation of the bacterial suspension the examination specimens were irradiated by semiconductor laser apparatus at different wave lengths - 630 and 662 nm and at power 100 J/cm2.

The tubes were placed into the bioreactor and necessary cultivation parameters were created by means of software for each species of microorganisms:

- ***A. actinomycetemcomitans*** (37°C; RPM: 500 min–1; measurement freq.: 3 h-1; rev. spin period: 1 sec.; volume: 15-16 ml; λ: 850 nm);
- ***S. constellatus*** (37°C; RPM: 1500 min-1; measurement freq.: 3 h-1; rev. spin period: 2 sec.; volume: 15-16 ml; λ: 850 nm);
- ***C. albicans*** (37°C; RPM: 1300min-1; measurement freq.: 3 h-1; rev. spin period: 3 sec.; volume: 15-16 ml; λ: 850 nm).

Table 1: Quantitative parameters of specimens

tube 1	tube 2	tube 3	tube 4	tube 5	tube 6
C-	C+	FDT_630nm	FS_630nm	FDT_662nm	FS_662nm
broth 15	broth 15	broth 15	broth 15	broth 15	broth 15
	1 ml suspension	0.5 ml suspension	0.5 ml suspension	0.5 ml suspension	0.5 ml suspension
		0.5 ml FS	0.5 ml FS	0.5 ml FS	0.5 ml FS

Results of the experiment were presented on a diagram of the dynamics of change of optical density during the whole period of cultivation. At the boundary of each phase of development of a microbial culture the quantitative inoculations were aseptically made onto dense nutrient media for subsequent calculation of microbial concentration in 1 ml nutrient broth. Key points of curves of growth of microbial populations were selected in order to make a statistical treatment of data in five iterations; the data were processed by the method of parametric statistics for a small sample according to Mann–Whitney ($p \leq 0.05$)

Results of Clinical and Laboratory Research

From the total number of examined persons a high frequency of occurrence of genetic markers (DNA) of *Aggregatibacter actinomycetemcomitans* was detected in the contents of periodontal pocket in 61.5%. This microorganism belongs to first-order periodontopathogenes and is detected in cases of disease forms with a marked destruction of periodontium tissues [20]. Species *Streptococcus constellatus* had a high frequency of detection too; its genetic markers were extracted in periodontal pocket in 53.8%. This microorganism belongs to group anginosus, and since it is a representative of normal flora of oral cavity, it is most often extracted in association with periodontopathogenic species of microorganisms and causes proinflammatory processes in oral cavity. Frequency of occurrence of fungi *Candida* in these patients was 26.9% what conforms to research data about increase of cases of Candida-associated periodontitis [21]. In connection therewith we conducted an experimental study for determination of effect of photodynamic therapy with substantiation of choice of an optimal mode of exposure and photosensitizer.

Results of Experimental Study

Basis of the experimental study was automated cultivation of extracted microorganisms' strains with monitoring of curves of growth of microbial populations in case of use of different modes of exposure and photosensitizers.

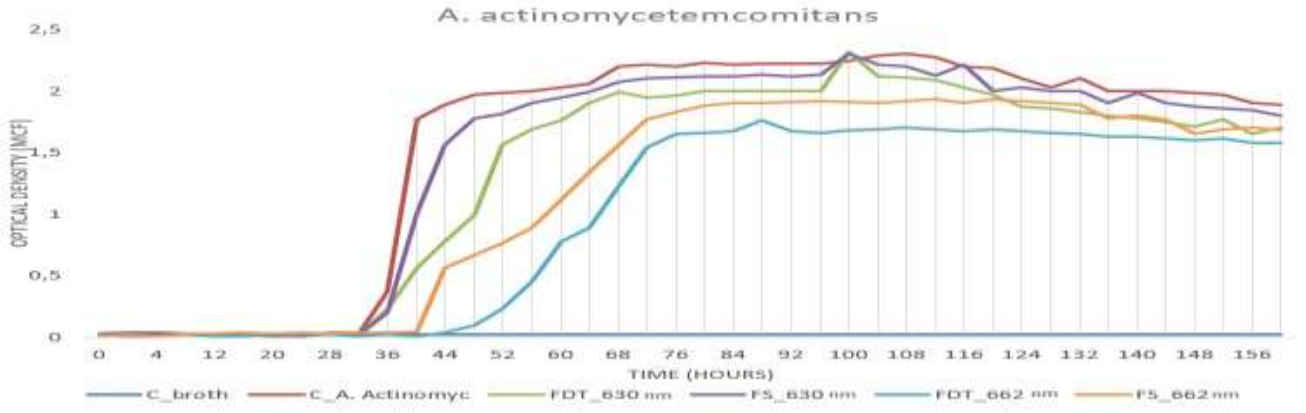

Figure 1: Result of cultivation of clinical isolate A. actinomycetemcomitans

After cultivation of clinical isolate of *A. actinomycetemcomitans* (Fig. 1) in a control tube the lag-phase lasted for more than 24 hours. Obviously marked acceleration of bacterial increase was absent therefore a picture of logarithmic jump was observed at once.Exponential growth, which was characterized by maximal rate of bacteria division in the control specimen, was observed between the 32nd hour (0.03±0.3 Mcf) and the 40th hour (1.7±0.3 Mcf - parameter α). In this phase the growth of cells takes place with a constant specific rate, i.e. in a time unit a unit of microbial biomass increases by the same value. However, in the first half of this phase the division of cells passes more quickly than their growth and the cells become smaller but in the second

half the rates of growth and division of cells get equal.

During the whole exponential phase the cells continue to keep a high physiological activity which is characteristic for young populations. Phase of inhibition or slowed increase, which is characterized by a steady rate of increase of biomass (number of cells) during the period of linear growth, was quite long - from the 40th to the 68th hour of cultivation, with transition to stationary phase of cultivation with value 2.2±0.3 Mcf (parameter ϐ). Proportion of dying-off cells, newly formed ones and resting ones becomes equal and gradually becomes stabilized. Mean value of the stationary phase was 2.23 ±0.3 Mcf.

Activation of Photosensitizers (FS) by Wave Length 630 nm

FS Fotosan. No prolongation of phase of adaptation is detected. Exponential phase had an intensive generation of bacterial populations, rate of this generation almost conformed to the control specimen, however end of this phase and hence the peak value were reached with a delay (8 hours later). Maximal total concentration of cells (M concentration) was 2.1±0.3 Mcf what was not statistically significant as compared with the control specimen. Phase of deceleration of bacterial increase and stationary phase in their tendency were identical with the control specimen. Mean value in the stationary phase was 2.15 ±0.3 Mcf.

FS Fotoditazin, Lag-phase was identical to the one of previous specimens. Tendency concerning character of logarithmic phase remained, but rate of bacterial increase was lower than in the control specimen; the mean change of optical density was 0.35±0.3 Mcf. End of phase of logarithmic growth was even more prolonged; the peak value was reached by the 52nd hour (1.56±0.3 Mcf). Phase of deceleration of bacterial increase was shorter as compared with preceding specimens; its mean value was 2.01±0.3 Mcf.

Activation of Photosensitizers (FS) by Wave Length 662 nm

FS Fotosan. A prolongation of lag-phase is detected in this specimen while culture develops to the 40th hour. Exponential increase can be conventionally divided into 2 stages having different rates of gain of biomass. While in the first 4 hours the rate of increase of bacterial populations was comparable with previous specimens, after the 45th hour of cultivation the rate began to decrease gradually.

Maximal peak value at the end of logarithmic growth was reached only by the 72nd hour and amounted to 1.77±0.3 Mcf. When M concentration was reached, no considerable increase of cells was detected too; already 8 hours later the culture entered into stationary balance. Mean value of optical density was 1.8±0.3 Mcf what was by 19% lower than in the control specimen.

FS Fotoditazin. Lag-phase had a considerable prolongation with obviously marked period of accelerated growth. Character of logarithmic phase was identical to the one of the preceding specimen, however the parameter α was slightly lower, its value was 1.54±0.3 Mcf. End of phase of logarithmic growth and entering into stationary balance took place on the interval between the 72nd and 76th hours. Mean value of optical density in stationary phase was 1.6±0.3 Mcf what was by 30 % lower than in the control specimen.

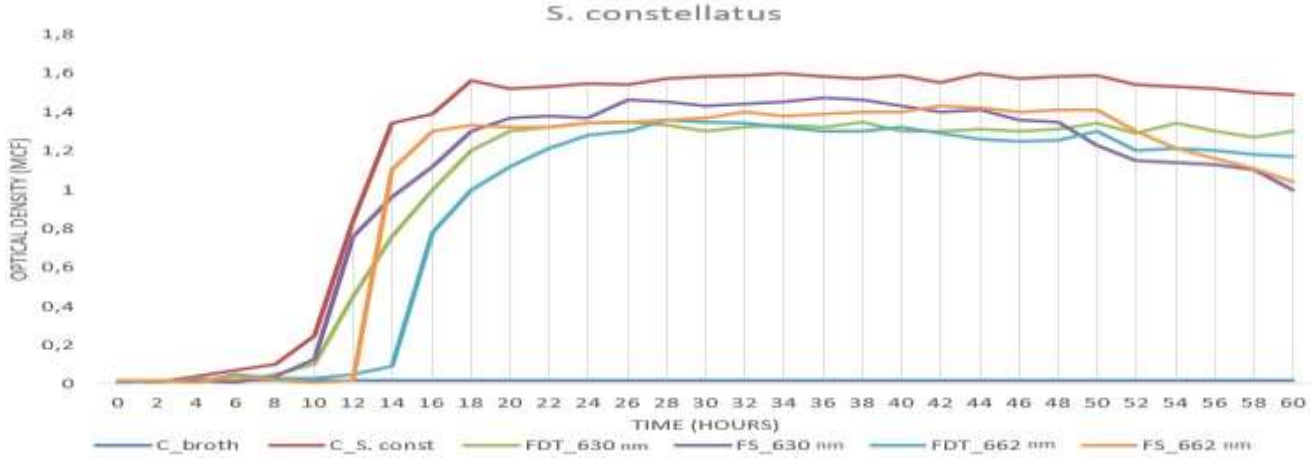

Figure 2: Result of cultivation of clinical isolate *S.* constellatuss

Lag-phase in the control specimen lasted up to the second hour of cultivation and from the third hour the bacterial culture entered into the phase of accelerated growth. This phase was long; during it a gradual growth of bacterial mass took place. Logarithmic phase had an explosion-like character of quick growth and by the 14th hour of cultivation the optical density reached its maximal value for this interval (parameter α), this value was 1.34 Mcf; maximal value of optical density throughout the whole period of culture growth (parameter ß) was 1.56±0.3 Mcf. Mean value of change of optical density in log phase was 0.41±0.3 Mcf. Stationary phase started at the 18th hour of cultivation and lasted to the 52nd hour. No bacterial increase was detected. Mean value of optical density in the stationary phase was 1.56 ±0.3 Mcf.

Activation of photosensitizers (FS) by wave length 630 nm

FS Fotosan. Adaptive phase and phase of accelerated growth statistically did not differ from the control specimen. Exponential growth up to the 12th hour of cultivation distinguished itself by high rate of generation of new cells; however rate of division began to decrease on the interval between the 12th and 18th hours. Maximal value of optical density in this phase was reached much later than in the control specimen (by the 18th hour), this value was 1.30±0.3 Mcf. Stationary phase was shorter (12 hours) than in the control specimen and after the end of this phase the bacterial cells entered into the phase of dying off. Mean value of optical density in the stationary phase was 1.45 ±0.3 Mcf.

FS Fotoditazin. Adaptive phase and phase of accelerated growth statistically did not differ from the control specimen. Exponential phase was characterized by a stable rate of generation of new cells; maximal value of optical density throughout the whole period of culture growth was 1.35±0.3 Mcf what is statistically insignificant as compared with the control specimen.

Activation of photosensitizers (FS) by wave length 662 nm

FS Fotosan. Adaptive phase lasted up to the 12th hour of cultivation and after that an abrupt transition into phase of logarithmic growth took place. Phase of accelerated growth was absent. Exponential growth was marked by an intensive increase of number of bacterial cells with the peak value of optical density 1.1±0.3 Mcf (parameter α). Maximal value of optical density (parameter ß) was lower than in the control specimen too; however end of log phase and subsequent entering of culture into stationary phase took place more quickly than in the preceding specimens. Stationary period lasted for 34 hours; thereafter the phase of death of cells began. Mean value of the stationary phase was 1.37 ±0.3 Mcf.

FS Fotoditazin. Prolongation of adaptive phase up to the 14th hour of cultivation was observed. Exponential phase was short and ended by the 20th hour of the experiment. Maximal value at the peak of this phase (parameter α) was 1.12 Mcf; maximal value of optical density throughout the whole period of culture growth (parameter ß) was 1.38±0.3 Mcf. Stationary phase started at the 26th hour of cultivation and lasted to the 50th hour. No bacterial increase was detected. Mean value of optical density in the stationary phase was 1.31 ±0.3 Mcf.

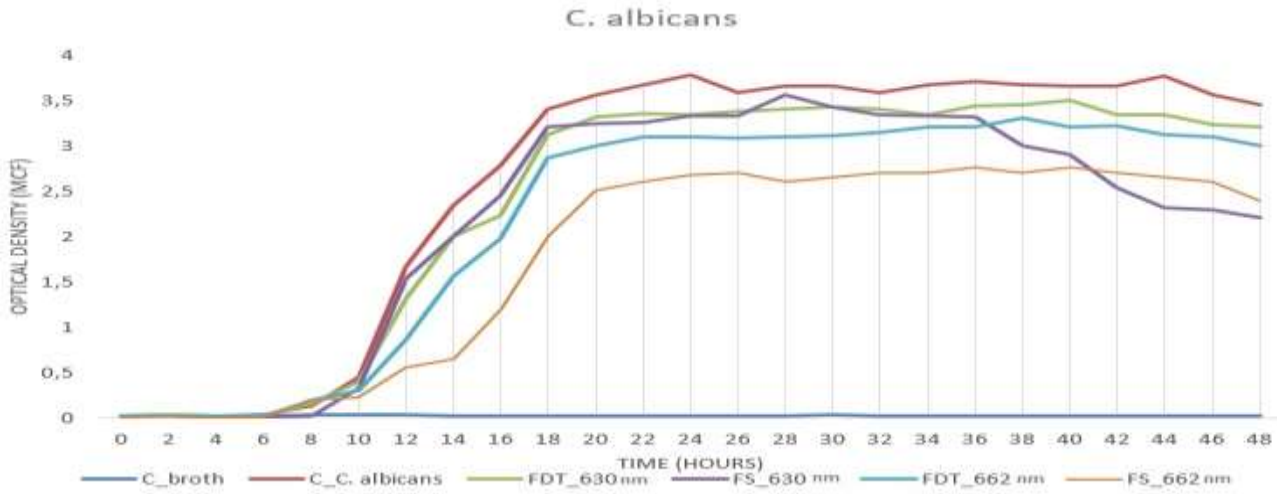

Figure 3: Result of cultivation of clinical isolate C. albicans

Adaptive phase in the control specimen lasted to the 6th hour of cultivation, after end of this phase a gradual growth of bacterial increase was detected. From the 6th 10th hour of cultivation the rate of division of cells has tendency which is approximate to logarithmical growth; however in this stage, to a greater extent, an increase of sizes of bacterial cells takes place without a significant growth of number of units in population. Exponential phase is detected on the interval between the 10th and the 18th hour; it is characterized by an abrupt increase of rate of culture reproduction. Time intervals between emergence of a preceding and next generation are constant.

Mean value of change of optical density in log phase was 0.75±0.3 Mcf. In the period from the 18th to the 24th hour of cultivation a phase of negative acceleration is observed, during it the rate of reproduction of bacteria is not maximal any more. Peak value of optical density of log phase (parameter α) is 3.4±0.3 Mcf; maximal value of optical density throughout the whole period of culture growth (parameter ß) is 3.78±0.3 Mcf. Stationary phase is long (20 hours), bacterial increase is absent. Mean value of optical density is 3.67 ±0.3 Mcf.

Activation of photosensitizers (FS) by wave length 630 nm

FS Fotosan. Adaptive phase and phase of accelerated growth statistically did not differ from the control specimen. Logarithmical growth distinguished itself by a slight lowering of rate of cells reproduction as compared with the preceding specimen and by presence of diauxy on the interval between the 12th and 18th hour of cultivation. Maximal value of optical density at the end of logarithmic phase is lower than in the control specimen, this value is 3.12±0.3 Mcf. Phase of deceleration of bacterial increase was almost absent and the cells passed over to the stationary phase immediately (by the 20th hour). Mean value of optical density in the stationary phase was 3.38 ±0.3 Mcf.

FS Fotoditazin. Lag-phase is prolonged by 2 hours and the start of phase of accelerated growth is detected at the 8th hour of the experiment. Exponential phase and stationary phase do not differ from the control specimen and FS Fotosan, however length of the stationary phase is shorter (it lasts from the 18th to the 36th hour), after that the picture of accelerated death of culture is observed.

Activation of photosensitizers (FS) by wave length 662 nm

FS Fotoditazin. Lag-phase and beginning of logarithmic phase did not have special differences from the control specimen. In exponential phase the rate of cells increase continued to decrease as compared with the preceding specimens and the duration remains the same. Peak value of optical density of logarithmic phase was relatively lower than in specimens with activation by wave length 630 nm and amounted to 2.86±0.3 Mcf (the 18th hour).

FS Fotosan. Adaptive phase lasted up to the 6th hour of cultivation and after that a transition to a prolonged phase of accelerated growth took place. This phase was observed up to the 14th hour of the experiment and lasted almost twice as long as in the preceding specimens. Exponential growth distinguished itself by a great increase of bacterial cells with peak value of optical density 2.5±0.3 Mcf (parameter α) that is by 26% less than in the control specimen. Maximal value of optical density (parameter ß) was lower than in the control specimen too, however end of log phase and subsequent entering of culture into stationary phase were longer by 2 hours. Stationary period lasted for 20 hours; thereafter the phase of death of cells began. Mean value of the stationary phase was 2.68 ±0.3 Mcf.

Conclusion

Results of the conducted experiment showed that in the stage of photoactivation of a photosensitizer the use of different wave lengths promotes presence of differential effectiveness with respect to bacterial populations.

Different combinations of photosensitizer and variants of its subsequent activation differently influence different groups of microorganisms by causing the changes in culture development of both bactericidal and bacteriostatic character. At the same time, we believe that it is very important that use of one single type of photosensitizer is not effective with respect to different microbial contaminations and no total stop of growth and death of all microorganisms take place in fact, how it was hypothetically supposed or

stated in articles of some authors who analyzed effectiveness of FDT. After analysis of growth curves the different intervals of development of culture are detected which allow evaluating effectiveness of use of photodynamic therapy and consequently allowing making necessary corrections during use of this technique in complex treatment of patients with periodontium diseases [22].

References

1. Kuriakina N V, Kutepova TF (2000) Diseases of periodontium. Moscow: Medical Book.
2. Tsepov LM, Mikheeva EA, Goleva NA, Nesterova MM (2010) Chronic generalized periodontitis: remarks to modern ideas. Periodontics, 1(15): 3-7.
3. Vorobyev VI, Vorobyev DV, Zakharkina NI, Polkovnichenko AP, Safonov VA (2019) Physiological status of'king'squab pigeon (Columba Livia gm. Cv.€'king') in biogeochemical conditions of low iodine, selenium and cobalt levels in the environment. Asia Life Sciences, 28(1): 99-110.
4. Aas JA, Paster B.J, Stokes LN, Olsen I, Dewhirst FE (2005) Defining the normal bacterial flora of the oral cavity. Journal of clinical microbiology, 43(11): 5721-5732.
5. Nikolaeva E N, Tsarev VN, Ippolitov EV (2011) Periodontopathogenic bacteria - indicators of risk of emergence and development of periodontitis (part I). Dentistry for all, 3: 4-9.
6. Ushakov RV, Tsarev VN (2018) Use of antibiotics in dentistry. Moscow: FSBEI SPE - RMACPE.
7. Lamont R J, Hajishengallis GN, Koo HM, Jenkinson HF (Eds.) (2014) Oral microbiology and immunology. John Wiley & Sons.
8. Orekhova LI (2004) Diseases of periodontium. Moscow: Poly Media Press.
9. Bulkina NV, Vediaeva AP (2012) Rapidly progressive periodontitis: new aspects of pathogenesis and complex therapy. Periodontics, 4(17): 13-18 (in Russian).
10. Shkarednaya OV, Goryacheva TP, Chunikhin AA, Bazikyan EA, Gazhva SI (2017) Optimizing the early diagnosis of oral mucosal pathologies. Sovremennye Tehnologii v Medicine, 9(3): 119-124.
11. Kalaeva E, Kalaev V, Efimova K, Chernitskiy A, Safonov V (2019) Protein metabolic changes and nucleolus organizer regions activity in the lymphocytes of neonatal calves during the development of respiratory diseases. Veterinary World, 12(10): 1657-1667.
12. Ulashchik VS (2013) Photodynamic therapy-technology of the 21st century. Physiotherapy, balneology and rehabilitation, 1: 36-43.
13. Kholikov K (2018) Enhanced Singlet Oxygen Generation and Antimicrobial Activity of Methylene Blue Coupled with Graphene Quantum Dots as an Effective Photodynamic Therapy Agent. Western Kentucky University.
14. Bazikyan EA, Syrnikova N V, Chunikhin AA, Zayratyants OV (2018) Morphological evaluation of singlet phototherapy in the treatment of periodontal diseases in an experimental study. Stomatologiia, 97(1): 22-26.
15. Aboshnaf F (2018) The synthesis and application of dendritic polymer for photodynamic therapy (Doctoral dissertation, University of Sheffield).
16. Singh K (2018) Small Molecule Probes for Photodynamic Therapy (PDT). Master, University of Toronto, Mississauga.
17. Sundara Raj B (2018) Pharmaceutical and Biological Evaluation of Nano Photosensitizers for Cancer Therapy (Doctoral dissertation, Curtin University).
18. Mousavi M (2019) Luminescence Spectroscopy for Biomedical Applications. Lund University.
19. Tsarev VN, Podporin MS, Ippolitov EV (2017) Use of scanning electron microscopy and a bioreactor for evaluation of eradication of microbial biofilm of root canals during endodontic treatment. Clinical Laboratory Diagnostics, 8(62): 506-512.
20. Timoshin AV, Sevbitov AV, Drobot GV, Yumashev AV, Timoshina MD (2018) Use of bioresorbable plates on the basis of collagen and digestase for treatment of diseases of oral mucosa (review of clinical cases). International Journal of Green Pharmacy, 12(1): 290-96.
21. Voloshina IM, Borisov VV, Sevbitov AV, Davidyants AA, Mironov SN, Kuznetsova M

Yu, Ergesheva EV (2018) Distinctive features of microcrystallization of mixed saliva in children with different levels of activity of carious process. Asian Journal of Pharmaceutics, 12(S3): 1017-1020.

22. Tsarev VN, Nikolaeva EN, Arutiunov DS, Fomicheva EM, Unanian AA, Arutiunov SD (2010) Experimental substantiation of use of biopolimer films which contain preparations having immunomodulatory and antibacterial action for treatment of periodontium diseases. Periodontics, 1(15): 57-60.

