Effect of photodynamic therapy on glutathione S-transferase activity in oral liquid of children with high risk of caries

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Abstract

Background: Pediatric dentistry is looking for new methods to influence the cariogenic microorganisms of dental biofilm without the use of antiseptics and antibiotics, which often have negative side effects. Photodynamic therapy (PDT) is a promising and effective method to influence on the cariogenic microorganisms without using antiseptics and antibiotics.
Material and methods: Forty five children aged 7 to 12 years with high risk of dental caries and pathology of central nervous system were observed during three years of the complex preventive measures, including multivitamins, minerals, antioxidants and PDT of dental biofilm. The activity of glutathione S-transferase (GST), content of reduced glutathione (GSH), thiocyanate (SCN) and total protein in the oral liquid (OL) were determined by spectrophotometry (Diasys Diagnostics, DE). The results were statistically processed using the program Excel Microsoft: Microstat 2007.
Results: In three years the protein content in OL of the children was below the initial content in all groups that may be the confirmation of PDT bacteriostatic effect. In all periods of the study significant changes in the content of GSH, thiocyanate and activity of GST in OL in the children were not observed.
Conclusions: Our results are indicating that complex preventive measures including the non-invasive method of PDT were effective, without any negative side effects and had bacteriostatic action. These complex preventive measures may be recommended for children with high risk of caries and pathology of CNS.

Key words: caries, glutathione, glutathione S-transferase, photodynamic therapy.

Introduction

Currently, pediatric dentistry is looking for new methods to influence the cariogenic microorganisms of dental biofilm to achieve sterility of cavity, root canal, the successful treatment of periodontal disease and oral mucosa without the use of antiseptics and antibiotics, which often have negative side effects. In dental practice photodynamic therapy (PDT) is non-invasive, effective, perspective, and safe method, with a bacteriostatic effect on pathogenic microflora of the mouth [1]. The basis of PDT is a chemical reaction which develops in biological tissues/cells after exogenous application of photosensitizer defined under the action of light energy dose.

The photosensitizer is applied to biological tissue or injected into the tissue where it selectively accumulates in the cells of the pathogen. Then this site is irradiated with a laser using a certain wavelength. As a result, the photosensitizer's photochemical reaction releases oxygen, which acts on the pathologically altered cells or pathogens, destroying them [2]. As a source of laser radiation for PDT the low-powerful semiconductor lasers are used.

In 1990, M. Wilson and G. Pearson demonstrated that PDT can kill the bacteria found in the infected dentin, root canals, and periodontal tissues. Since 1992, the PDT method has been used in clinical medicine, and since 2002 it is a standard of treatment in oncology [1, 5]. Lima J.P. et al. (2009) demonstrated the efficacy of PDT for killing pathogens in the treatment of dentine caries [3].

In the literature, there are a few publications about the usage of PDT in the treatment of dental diseases in children [4, 5, 6, 7], and therefore, further studies on the effect of PDT in children are very important. PDT destroys pathogens, removes the pigment, which are waste products of the microbiota. However, in the process of photochemical reaction the active singlet oxygen releases which is the initiator of radical chain reactions, the products of which are toxic substances, named Reactive Oxygen Species (ROS).

Reduced glutathione (GSH) itself directly and a coenzyme of glutathione transferase (GST), which use it in the conjugation reactions of toxins, are involved in the antioxidant protection of cells against the aggressive oxygen radicals and products of the radical chain reaction. In the literature there is no information on the influence of PDT on the activity of GST in the OL of children with severe disorders of the central nervous system (CNS) and high risk of dental caries.

Objective. Investigation of the PDT influence on the activity of glutathione transferase in the OL of children with severe pathology of CNS and high risk of dental caries.

Material and methods

The study involved 45 children aged 7 to 12 years with severe pathology CNS and high risk of dental caries. All clinical and biochemical studies have been conducted in accordance with ethical and legal standards. As the prevention of dental caries children underwent the complex preventive measures, including the appointment of multivitamins, minerals and antioxidants, as well as conducting PDT on dental biofilm. Children were observed during three years and were divided into four groups depending on the photosensitizer for application: 1st group – 2% methylene blue; 2nd group – 2% anthocyanin; 3d group – 2% toluidine blue, followed by laser irradiation LED 625-635 nm, and the 4th group served as a control group. OL of children were taken after PDT exposition in an hour,

week, month and 3 years. OL was centrifuged at 600 r for 10 minutes and after that the activity of GST [8] content of GSH [9], thiocyanate (SCN) [10] and the total protein (Lowry H., 1951) were determined spectrophotometrically (Diasys Diagnostics, DE). The results are statistically processed using the program Excel Microsoft: Microstat 2007.

Results and discussion

The protein content in OL of patients with caries correlated with the degree and amount of pathogenic microorganisms inhabiting in the oral cavity. One of the tasks was using PDT to destroy cariogenic microflora of dental biofilm in children with high risk of dental caries. Table 1 shows the results of the dynamics of the protein content in OL of children with CNS pathology and high risk of dental caries after PDT influence.

As the table shows, one hour after PDT content of the protein in OL of children slightly increased in the 1st, 3-d and 4th groups, that probably was a result of receipt of proteins and peptides carrying a protective function, in response to the use of PDT. However, during the follow-up after 3 years protein content in OL of children was below the initial content in all groups, that may be the confirmation of PDT bacteriostatic effect.

Active oxygen and its generated radicals due to a photochemical reaction are involved in the oxidative radical chain reactions which result in the formation of toxic products. This leads to the induction of protective antioxidant and antitoxic systems, such as superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase and others. GSH has the multifaceted role as an antioxidant. It participates directly in the neutralization reactions of oxygen radicals and their products Reactive Oxygen Species (ROS). Also it is a coenzyme of many glutathione-depending enzymes, including GST.

Figure 1 shows the dynamics of the content of GSH in the OL of children during three years of observation after PDT.

In all periods of the study significant changes in the content of GSH in OL in the children were not observed both in the content per litre of OL (mcmol/l), and per gram of protein (mcmol/g). Results of research of the GST activity of the OL in children varied during the course of PDT (fig. 2).

In an hour after PDT all groups showed a decrease of GST activity both per litre of OL, and per gram of protein. In a week of observation GST activity was also reduced as compared to the baseline in the OL of children before PDT. Apparently, the decrease of GST activity in OL may indicate a partial inactivation of the enzyme by methylene blue [11, 12], anthocyanin [13] and PDT. However, a month later GST activity increased in OL of children, correlating with an increase in the total protein content (tab. 1). After 3 years of observation activity of GST in the first three groups of children was compared with the initial level, which was at the first visit of children, before the influence of PDT. The exception was the 4th group (control) of children who had a low enzyme activity in the OL, but this is not statistically significant ($p_t > 0.05$).

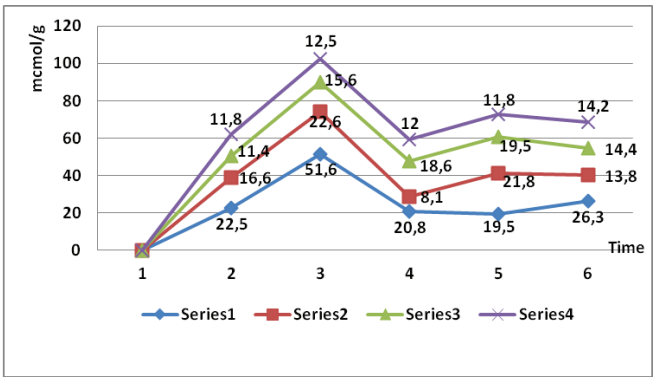

Fig. 1. Dynamics of GSH content in the saliva of children (mcmol/g protein) after PDT. The series correspond to number of groups.

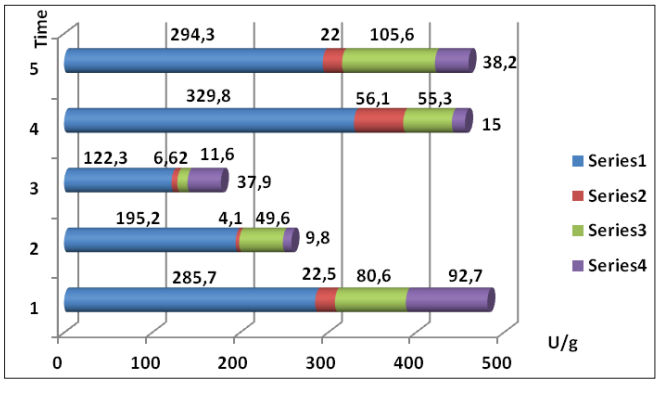

Fig. 2. Dynamics of GST activity in the saliva of children after PDT (U/g protein).

Time: 1- start; 2- in an hour; 3 – in a week; 4 – in a month; 5 – in 3 years. The series correspond to numbers of groups.

One of the defense systems of OL is lactoperoxidase system comprising lactoperoxidase, hydrogen peroxide (H_2O_2) and thiocyanate ions (SCN-), which inhibit cariogenic microflora

Table 1

PDT influence on the content of total protein in the oral fluid of children with pathology of central nervous system and high caries risk

Protein (g/L) inthesaliva	Time of determination/observation				
	1st day	1 hour	1 week	1 month	3 years
1st group	2.65 ± 0.31	2.93 ± 0.14	2.83 ± 0.16	2.82± 0.28	2.36 ± 0.37
2nd group	2.57 ± 0.17	2.39 ± 0.12	2.39 ± 0.46	2.88 ± 0.87	2.57 ± 0.17
3-d group	2.92 ± 0.32	3.27 ± 0.57	2.44 ± 0.25	2.28 ± 0.14	2.52 ± 0.45
4th control group	2.66 ± 0.16	2.93 ± 0.26	2.71 ± 0.19	3.05 ± 0.42	2.27 ± 0.08

Table 2

PDT influence on the content of SCN in the oral liquid of the children with pathology of central nervous system and high caries risk

SCN, mcmol Groups	Time of determination/observation				
	1st day	1 hour	1 week	1 month	3 years
1: mcmol/L	0.280 ± 0.032	0.110 ± 0.015	0.150 ± 0.020	0.185 ± 0.016	0.090 ± 0.028*
mcmol/g	0.102 ± 0.013	0.037 ± 0.005*	0.054 ± 0.010*	0.067 ± 0.009	0.040 ± 0.014*
2: mcmol/L	0.330 ± 0.050	0.219 ± 0.020	0.410 ± 0.056	0.308 ± 0.041	0.264 ± 0.035
mcmol/g	0.128 ± 0.017	0.092 ± 0.012	0.200 ± 0.064	0.124 ± 0.047	0.103 ± 0.012
3: mcmol/L	0.118 ± 0.098	0.217 ± 0.100	0.161 ± 0.047	0.227 ± 0.082	0.145 ± 0.045
mcmol/g	0.053 ± 0.041	0.071 ± 0.039	0.069 ± 0.025	0.102 ± 0.037	0.063 ± 0.024
4: mcmol/L	0.186 ± 0.094	0.106 ± 0.033	0.114 ± 0.063	0.114 ± 0.027	0.140 ± 0.035
mcmol/g	0.068 ± 0.032	0.040 ± 0.008	0.043 ± 0.026	0.039 ± 0.010	0.062 ± 0.015

Symbol * – $P_t < 0.05$

of the oral cavity [14]. Lactoperoxidase, using hydrogen peroxide as oxidant and thiocyanate ions (SCN-), catalyzes the reaction of formation of antimicrobial products, more actively than hydrogen peroxide, such as hypothiocyanate (OSCN-) [15]. The results of determination of the thiocyanate in OL of children after PDT are presented in table 2. In an hour after PDT thiocyanate content slightly decreased, which may indicate a partial inactivation of the lactoperoxidase system. However, subsequently the content of thiocyanate was increased in both methods of calculation and reached levels in the OL of children at their first visit before the PDT (except the first group).

Conclusion

Thus, on the basis of the obtained results, we can conclude that PDT did not have negative effects on the antioxidant systems of glutathione-glutathione S-transferase and antitoxic lactoperoxidase system, the state of which reflects the content of thiocyanate. Bacteriostatic effect of PDT is an established fact that also was confirmed by our results of reducing the protein content in the OL of children. This non-invasive and effective method is particularly important for using in children with disorders of the central nervous system, because it is safe and does not cause negative side effects that can occur with the usage of antibiotics and antiseptics.

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