Antioxidant activity of blood serum and saliva in patients with periodontal disease treated due to epilepsy

Sobaniec H[1], Sobaniec W[1], Sendrowski K[1], Sobaniec S[2], Pietruska M[2]*

[1] Department of Pediatric Neurology and Rehabilitation, Medical University of Białystok, Poland
[2] Department of Periodontal and Oral Mucosa Diseases, Medical University of Białystok, Poland

Abstract

Purpose: The aim of the study was to estimate the activity of chosen antioxidants in blood serum and saliva in patients with periodontal disease treated due to epilepsy.

Material and methods: Twenty-five epileptics and fifteen control persons were involved in the study. The activity of selected endogenous antioxidants were determined by spectrophotometric assay. Concentrations of vitamin A and vitamin E were measured using liquid chromatography.

Results: The analysis of the serum and saliva from patients with overgrown gingiva revealed: reduced activity of superoxide dismutase, glutathione peroxidase and glutathione reductase, elevated lipid peroxides, and decreased concentration of ascorbic acid and α-tocopherol. All values were statistically significant.

Conclusions: Our results indicate on the oxidant-antioxidant disturbances in epileptic patients, which can play an important role in the pathomechanism of periodontal disease in these persons. Further studies on the role of antioxidants in patients with epilepsy treated with antiepileptic drugs and afflicted with gingival hyperplasia will be continued.

Key words: epileptic patients, hyperplasia, antioxidants, antiepileptic drugs.

* CORRESPONDING AUTHOR:
Department of Pediatric Neurology and Rehabilitation,
Medical University of Białystok
15-274 Białystok, ul. Waszyngtona 17, Poland
Tel/fax: +48 85 7450812
e-mail: izapan@wp.pl (Wojciech Sobaniec)

Received 20.03.2007 Accepted 10.04.2007

Introduction

Among adverse effects of antiepileptic drugs (AEDs) used for the therapy of epilepsy gingival hyperplasia has been found as quite common complication. Since 1939 when Kimball as first [1] had described phenytoin (PHT)-induced hyperplasia of gums many theories trying to explain the gingival damage have been presented. Risk factors like stress, the effects of bio-antioxidants and prooxidants, dental plaque bacteria induced "oxygen shock" which can activate free radicals – these all reflect attempts at contemporary elucidation of the reasons of the gingival hyperplasia [2,3]. The action of free radicals brings about an increase in the protein degradation products, kinins, and activation of the arachidonic acid cascade (lipid peroxides, thromboxane, prostaglandins, leukotrienes) [4]. Free radicals atherogenic effects can induce disturbed microcirculatory hemostasis, and possibly through periodontal blood vessels, they can favour secondary gingival hyperplasia [5]. In an earlier publication we have described the disturbance of the oxidants-antioxidants balance in epileptic children [6]. Patients treated with antiepileptic drugs (AEDs) and suffering from gingival overgrowth can be an interesting model for studies of possible pathogenic mechanisms underlying the process of hyperplasia; the material for searches were their blood and saliva.

Material and methods

The present study included twenty-five patients of both gender aged 9 to 68 years (mean ±31.2). They were treated chronically due to epilepsy with PHT or with PHT combined with other AEDs. Average time of the therapy was 11.9 years. The study group consisted of 15 patients with gingival hyperplasia (GH) and 10 with gingivitis. Examination of oral health revealed 100% caries, softened, hyperplastic and bleeding gums, loosened teeth, lingual and buccal scars due to epileptic attacks. Blood samples and unstimulated saliva were taken at the same

***Table 1*. Endogenous antioxidants activity in blood sera and saliva of epileptic patients affected with periodontium disease**

	Superoxide dismutase (SOD) U/ml	Glutathione peroxidase (GSH-Px) IU		Glutathione reductase (GSH-R) IU	
	serum	serum	saliva	serum	saliva
Control (n=15)	4.00±0.45	261.76±44.18	195.45±53.39	37.00±7.86	9.72±2.90
Patients with periodontopathy (n=25)	2.78±0.85* * p<0.001	239.85±40.23* * p<0.02	167.33±34.24* * p<0.01	27.77±7.79* * p<0.001	5.82±2.17* * p<0.001

***Table 2*. Vitamin C and E levels in blood serum and saliva of epileptic patients affected with periodontium disease**

	Vitamin C mg/L		Vitamin E mg/L
	serum	saliva	serum
Control (n=15)	42.72±11.70	9.24±2.86	13.06±2.49
Patients with periodontopathy (n=25)	27.65±15.70* * p<0.01	6.94±3.15* * p<0.01	8.35±2.94* * p<0.001

***Table 3*. Blood serum and saliva concentrations of lipid peroxides in epileptic patients affected with periodontium diseases**

	Lipid peroxides (MDA) nmol/ml	
	serum	saliva
control (n=15)	1.03±0.13	0.97±0.16
patients with periodontopathy (n=25)	1.39±0.16* * p<0.01	1.17±0.24* * p<0.01

MDA – Malonyl dialdehyde

time in morning hours from fasting patients. Saliva samples collected under strict hygienic dietary regime conditions were frozen at -20°; prior to examination the samples were thawn and centrifuged. The sediment containing morphotic elements was discarded. Control group included 15 persons generally healthy, without periodontal disease. The following parameters were estimated in blood serum and saliva:

I. the activity of selected antioxidants (free radical scavengers): 1. endogenous enzyme systems (activities of superoxide dismutase (SOD), glutathione reductase (GSSG-R) and glutathione peroxidase). 2. low molecular mass endogenous substances: blood serum vitamin E (α-tocopherol) and vitamin C (ascorbic acid).

II. Malonyl dialdehyde (MDA) concentration in the blood serum and saliva.

Concentrations of both vitamins were measured using liquid chromatography [7]. Other parameters were determined by spectrophotometric assay according to the methods used in our earlier study [8]. The results have been elaborated statistically using the Student's t-test and compared with control values. A level of $p<0.05$ was considered statistically significant.

Results

Tab. 1 presents the activity of endogenous antioxidants in blood serum and in saliva. Significant decrease of SOD, GSSG-R and glutathione peroxidase is visible. *Tab. 2* illustrates the concentrations of ascorbic acid and α-tocopherol in the samples of blood serum and saliva. Comparing to control values the levels of these two free radical scavengers are reduced. Elevated levels of the blood serum and saliva lipid peroxides are seen in *Tab. 3*.

Discussion

The present findings support idea that oxydoreductive processes are involved in inducing gingival overgrowth in epileptic patients. Reduced activities of antioxidant enzymes like SOD, glutathione peroxidase and reductase, both in the blood and in saliva and decreased levels of C and E vitamins well correlated with an increase in the thiobarbituric acid reacting products (MDA) which are the products of enhanced peroxidation of arachidonic acid. It can be postulated that long-term PHT therapy inducing gingival hyperplasia is followed by disturbed redox system both in the blood and in saliva. Yu and Wells reported on catalytic effects of PHT upon cyclo- and lipoxygenase in their action on arachidonic acid [9]. According to these authors the resulting elevated hydroxyperoxides and prostaglandin H2 synthase should be responsible for teratogenic effects known as "fetal hydantoin syndrome". This syndrome includes myelocele, mental retardation, cleft lip and/or cleft palate, hypodactylia and heart defects. Pre-treatment with free radical scavengers (vita-

mins C and E and indomethacin) prevented these adverse effects [10]. Suppose, PHT acts directly as a modifier through the cytochrome P-450 system upon enhancement of lipid peroxidation processes in tissues, thus its effects upon gingiva through the saliva and crevicular fluid can be similar. According to earlier data [11,12] the PHT concentrations in saliva are high enough to estimate its levels during monitoring the therapy. Irrespective of direct effects of PHT on lipid peroxidation processes in the pathology of inflammatory and hyperplastic lesions of periodontium another pathomechanisms responsible for these changes can exist, too [13,14]. Local factors as bacterial plaque and calculus responsible for periodontal ill conditions which are followed by relative fluid stasis due to reduced blood flow, bleeding, hypoxia within the gingival sulcus and depressed redox potential can result in destruction of the periodontal tissues [13]. The pathogenic role of reactive oxygen species in the destruction of the periodontium during inflammatory periodontal diseases and the imbalance in oxidant/antioxidant activity in these processes have been described by many authors [15--17]. Under experimental conditions of acute oxygen shock vasodilatation and exudate from the periodontal blood vessels followed by increased gingival pocket fluid flow were observed [18]. This fluid showed antioxidant effects similar to the blood serum. Similar to our results are the findings which show diminished levels of blood serum ascorbic acid in most of patients with periodontitis and that this reduction progresses along with advancement of the disease. Also, clinical studies on early gingivitis confirmed the relation between decreased ascorbic acid concentration and the disease process [19]. In chronically PHT treated epileptic patients reduced blood serum calcium levels were found. This event results from prevalent calcium excretion over its enteral absorption that can influence calcium depletion in alveolar bone processes, in particular in the mandible distal parts. As a consequence, besides the alveolar recession pathologic pocketing and increased resorption of interdental septa of molar teeth have been observed [16]. Another important role of free radical scavengers includes conditions when at reduced levels they facilitate formation of atherosclerotic lesions and disturbed collagen synthesis [13,19].

Conclusions

The concept on the role of free radicals in the genesis of periodontopathies points to the purposefulness of including bioxidants and other bioregulatory substances in the pharmacological prevention of these diseases. Under present conditions these substances should find a proper place both in individual and professional oral hygiene. The importance of antioxidants in the process of periodontal diseases is the subject of further studies.

References

1. Kimball OP. The treatment of epilepsy with sodium diphenylhydantoinate. J Am Med Assoc, 1939; 112: 1244-5.

2. Dongari M, Mc Donnell HT, Langlais RP. Drug-induced gingival hyperplasia. Oral Surg Oral Med Oral Pathol, 1993; 76: 543-8.

3. Sobaniec H. Mechanism of phenytoin-induced gingival overgrowth – literature review. Magazyn Stomatol (in polish), 1995; 8: 36-8.

4. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or conseqence? Lancet, 1994; 344: 721-4.

5. Voskresenskii ON, Tkachenko EK. The role of lipid peroxidation in the patogenesis of periodontitis. Stomatologia (Mosk.), 1991; 4: 5-10.

6. Artemowicz B, Sołowiej E, Sobaniec W. The disturbance of the oxidants-antioxidants balance in epileptic children. IJNN, 2004; 1: 27-31.

7. Nierenberg DW, Lester DC. Determination of vitamins A and E in serum and plasma using a simplified plasma clarification method and high-performance liquid chromatography. J Chromatogr, 1985; 345: 275-84.

8. Sobaniec W, Sołowiej E, Kulak W, Boćkowski L, Śmigielska-Kuzia J, Artemowicz B. Evaluation of the influence of antiepileptic therapy on antioxidant enzyme activity and lipid peroxidation in erytrocytes of children with epilepsy. J Child Neurol, 2006; 21:1-5.

9. Yu WK, Wells PG. Evidence for lipoxygenase-catalysed bioactivation of phenytoin to a teratogenic reactive intermediate: in vitro studies using linoleic acid-dependent soybean lipoxygenase, and in vivo studies using pregnant CD-l mice. Toxicol Appl Pharmacol, 1995; 131: 1-12.

10. Sanyal S, Wells PG. Reduction in phenytoin teratogenicity by pretreament with the antioxidant d-α-tocoferol acetate (vitamin E) in CD-I-mice. Toxicologist, 1993; 13: 252-5.

11. Sobaniec W. A correlation between the levels of phenobarbital, phenytoin and valproic acid in the blood serum and in saliva from children treated due to epilepsy. Materia Medica Polona, 1989; 4: 323-6.

12. Sobaniec W. Lipid peroxidation in experimental and clinical epilepsy and the effect of sodium valproate and vitamin E on these processes. Neurosci Jap, 1992; 18: 123-6.

13. Kataoka M, Kido J, Shinohara Y, Nagata T. Drug-induced gingival overgrowth-a review. Biol Pharm Bull, 2005; 28: 1817-21.

14. Brunet L, Miranda J, Roset P, Berini L, Farre M, Mendieta C. Prevalence and risk of gingival enlargement in patients treated with anticonvulsant drugs. Eur J Clin Invest, 2001; 31: 781-8.

15. Tsai CC, Chen HS, Chen SL, Ho YP, Ho KY, Wu YM, Hung CC. Lipid peroxidation: a possible role in the induction and progression of chronic periodontitis. J Periodontal Res, 2005; 40: 378-84.

16. Voskresenskii ON,Tkachenko EK. The role of lipid peroxidation in the patogenesis of periodontitis. Stomatologiia (Mosk.), 1991; 4: 5-10.

17. Akalin FA, Toklu E, Renda N. Analysis of superoxide dismutase activity levels in gingiva and gingival crevicular fluid in patients with chronic periodontitis and periodontally healthy controls. J Clin Periodontol, 2005; 32: 238-43.

18. Smalley JW. Pathogenic mechanism in periodontal disease. Adv Dent Res, 1994; 8: 320-8.

19. Bobyrev VN, Rozkolupa NV, Skripnikova TP. Experimental and clinical bases for the use of antioxidants as agents for treatment and preventing periodontitis. Stomatologiia (Mosk.), 1994; 73: 11-8.