Abstract

Background. Mixed saliva contains many antibacterial agents, i.e. peroxidase (SP) and myeloperoxidase (MP). The activity of SP and MP is inhibited by fluorine (F–).

Objectives. Evaluation whether fluorides obtained from fluoridated drinking water would influence the activity of salivary SP and MP.

Material and methods. 118 12–14-year-old subjects living from birth in areas with low (group I) and optimal (group II) fluoride content in drinking water were examined. In the supernantant of resting mixed saliva SP and MP activity, concentration of F–, total protein (P) and salivary flow rate (V) were estimated. Moreover, the influence of variable concentration of NaF (0.01–20 mmol/L F–) and pH (4.6–7.0) on salivary SP and MP activities were examined in vitro.

Results. The mean values in group I were as follows: V – 0.19 ml/ml, F– 4.01 µmol/l, P 1.41 mg/ml, SP 1.91 mIU/ml, MP – 0.72 mIU/ml, and in group II: 0.25 ml/ml, 8.84 µmol/l, 1.65 mg/ml, 1.78 mIU/ml, 0.73 mIU/ml, respectively. In vitro studies displayed an inhibition of SP and MP dependent on F– concentration and pH.

Conclusion. Higher salivary F– concentration in subjects from water fluoridated area in comparison to non-fluoridated one causes no significant reduction of SP and MP activities in saliva (Dent. Med. Probl. 2003, 40, 1, 63–68).

Key words: saliva, peroxidase, myeloperoxidase, fluoridated water.

Streszczenie

Wprowadzenie. Ślina mieszana zawiera wiele czynników antybakteryjnych, m.in. peroksydazę (SP) i mieloperoxdazę (MP). Aktywność SP i MP jest hamowana przez jony fluorowe (F–).

Cel pracy. Ocena, czy fluorki uzyskane z fluorkowanej wody pitnej mogą wpływać na aktywność ślinoj SP i MP.

Materiał i metody. Zbadano 118 dzieci 12–14-letnich mieszkających od urodzenia w rejonach z niską (grupa I) i optymalną (grupa II) zawartością fluoru w wodzie pitnej. W supernatancie spoczynkowej śliny mieszanej oszacowano aktywność SP i MP, stężenia F–, białka (P) i szybkość wydzielania śliny (V). Ponadto, in vitro zbadano wpływ zmiennego stężenia NaF (0,01–20 mmol/L F–) i pH (4,6–7,0) na aktywność ślinoj SP i MP.

 Wyniki. W grupie I uzyskano średnie wartości: V – 0,19 ml/ml, F– 4,01 µmol/l, P 1,41 mg/ml, SP 1,91 mIU/ml, MP – 0,72 mIU/ml, a w grupie II odpowiednio 0,25 ml/ml, 8,84 µmol/l, 1,65 mg/ml, 1,78 mIU/ml, 0,73 mIU/ml. W badaniu in vitro wykazano hamowanie SP i MP zależne od stężenia F– i pH.


Słowa kluczowe: ślina, peroksydaza, mieloperoksydaza, woda fluorokowana.
cells [1, 2]. Peroxidase activity in saliva is derived from salivary peroxidase (SP) and myeloperoxidase (MP). The family of described human peroxidases includes aside from the mentioned enzymes lactoperoxidase, eosinophilic peroxidase, uterine peroxidase, thyroid peroxidase and prostaglandin H1/2 synthases [3]. The salivary enzymes catalyse the oxidation of thiocyanate ions (SCN–) by hydrogen peroxide (H2O2), produced mainly by oral bacteria, to generate the oxidized forms of hypo-thiocyanous acid (Nbs) or hypothiocyanite anion (OSCN–). The reaction end-product – hypothiocyanite (OSCN–) is a strong oxidising agent. It plays an important role in the control of bacteria. The enzymes also prevent the oral accumulation of potential cytotoxic levels of the hydrogen peroxide (H2O2). Peroxidase is secreted by the acinar cells of salivary glands. Myeloperoxidase is produced by polymorphonuclear leukocytes (PMNs) and its concentration increases in the gingival fluid during inflammation and that increase may be reflected in whole saliva too. Peroxidase and myeloperoxidase differ in their substrate specificity so that MP can oxidize chloride whereas SP cannot [1, 2, 4]. Fluorides, commonly used for many years in caries prevention, are well-known inhibitors of many enzymes among which they influence the salivary peroxidase activity in concentration- and pH-dependent manner [5–9].

The aim of this study was to evaluate whether fluoride obtained from fluoridated drinking water could influence the activity levels of salivary peroxidase and myeloperoxidase.

Material and Methods

One hundred and eighteen 12–14-year-old schoolchildren lifetime residents in the areas with low (0.1–0.2 mg F/L – group I) and optimal (1.0 mg F/L – group II) content of fluoride in drinking water were examined. Group I consisted of 62 subjects, out of which 22 were caries-free (DMFS = 0) and 40 caries–susceptible (DMFTS ≥ 4) and group II of 26 and 30, respectively. The subjects had clinically healthy periodontal tissues and oral mucosa. Samples of resting mixed saliva were collected from the floor of the mouth by the aspiration technique using a soft plastic pipette. Before collection which took place between 9.00. and 10.00. a.m. the subjects neither ate nor drank anything for at least 1 hour.

Salivary flow rate was calculated (mL/min). Chemical assays were performed in the salivary supernatant obtained after centrifugation of saliva samples (18 000 g, 15 min). The fluoride concentration (µM) was estimated using ion selective electrode. The total protein content was measured in accordance with the Lowry et al. method [10]. Salivary peroxidase activity was assayed by measuring the rate of oxidation of 5-thio-2-nitrobenzoic acid (Nbs) to 5-dithiobis-2-nitrobenzoic acid (Nbs2) by OSCN– ions generated during the oxidation of SCN– by salivary peroxidase [11]. The same assay system, but with SCN– ions replaced by Cl–ions, was used for salivary myeloperoxidase measurements [11]. The levels of peroxidase and myeloperoxidase were expressed in concentative-volumetric units (mIU/ml) and specific activity (per 1 mg protein in saliva). The enzymes activities were calculated in relation to salivary flow rate or salivary flow rate and protein concentration as well (named as enzyme activity adjusted for flow rate or flow rate and protein content). The obtained data were analysed by ANOVA.

Moreover, in vitro experiments the effect of variable NaF concentration (0.01–20 mM F) at pH ranged from 4.6 to 7.0 on the activity level of salivary peroxidase and myeloperoxidase was examined. The values (mean of 5 measurements) were expressed as percentages of the enzymes activity without adding of fluoride – control.

Results and Discussion

Mean values of analysed salivary parameters are presented in the Table 1. Higher salivary flow rate (p < 0.05), fluoride concentration (p < 0.001) and total protein content (p < 0.05) in the saliva of subjects living in the fluoridated drinking water area (group II) in comparison to the non-fluoridated one (group I) were observed.

The level of peroxidase activity expressed in concentative-volumetric units (mIU/ml) was only slightly higher in non-fluoridated area (group I) than in fluoridated one (group II). However, the level of specific peroxidase activity turned out to be significantly lower in group II than in group I. Moreover, the enzyme activity adjusted for salivary flow rate and for both flow rate and protein content displayed significantly higher values in the saliva of subjects living in non-fluoridated area (group I) in comparison to water fluoridated one (group II).

The activity of myeloperoxidase expressed in concentative-volumetric units (mIU/ml) was on the same level in both groups. Contrary to SP, the level of specific myeloperoxidase activity was found to be significantly higher in group II than in group I as well as the enzyme activity adjusted for flow rate and both flow rate and protein content.

The analysis of correlation revealed only negative significant correlation between salivary flow rate and myeloperoxidase activity (mIU/ml)
in group I and positive between peroxidase and myeloperoxidase activities (mIU/ml) in group II.

In vitro experiments displayed the inhibition of SP and MP activity by fluorides in dose- and pH-dependent manner (Fig. 1 and 2). Reduction of peroxidase activity was higher than that of myeloperoxidase. For example, SP was inhibited with 0.5 mM F– (10 ppm) by 62.5% at pH 4.6, 61.5% at pH 5.0, 80.4% at pH 5.5, 79.3% at pH 5.6, 77.5% at pH 6.0, 82.3% at pH 6.5, 80.8% at pH 7.0 and MP by 72.8%, 70.95%, 86.7%, 87.3%, 94.3%, 100.0% and 105.0%, respectively.

The ranges of obtained mean values of fluorides are in accordance to previous data [12, 13] and point out the higher fluoride concentration in the saliva of subjects living in water-fluoridated area. It may be assumed that in our study fluorides derived from fluoridated water are the only additional source of salivary fluorides as the subjects from both groups used fluoridated toothpastes daily. The suggestion is supported by the results of early published data [14] in which salivary SP activity in 3–4-year-old children living in water fluoridated and non-fluoridated areas who very seldom used fluoridated toothpastes was compared. It was found significantly lower peroxidase activity in the subjects drinking fluoridated water probably caused by the increase of fluoride content in the saliva.

Mean level of peroxidase activity (mIU/ml) obtained in our study is slightly lower than the values for stimulated mixed saliva presented by Grahn et al. [15] – 1.00±0.31 and Kirstilla et al. [16] – 1.37±0.69 using the same assay method. Therefore, our findings can confirm the data obtained by Tenovuo at al. [17] and suggest that salivary peroxidase system is significantly lower in stimulated than in resting saliva.

The obtained in our study level of myeloperoxidase activity (mU/ml) is close to values presented by Grahn et al. [16] for stimulated mixed saliva (0.66±0.20 μM). However, Kirstilla et al. [16] received half lower, than in our data, level of MP in the supernatant of stimulated mixed saliva. Similarly to Grahn et al. [15] in the presented study observed no significant correlation between the level of enzyme activity and salivary flow rate. However, Smith and Yang [18] suggested some correlation of MP activity with flow rate as they found lower activity in mixed and parotid saliva at higher rate of stimulation. Moreover, at higher flow rate of both secretions they did not detect the enzyme activity at greater numbers of examined subjects. Schiott and Loe [19] observed that even in subjects with healthy periodontal tissue leucocytes are found in the saliva and become the source of enzyme. Their findings were confirmed in the presented study as in the resting saliva of all subjects with healthy periodontal tissue the MP activity was detected.

### Table 1. Means of salivary parameters

<table>
<thead>
<tr>
<th>Group (Grupa)</th>
<th>I</th>
<th></th>
<th>II</th>
<th></th>
<th>Significant difference (Istotność różnic)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SD</td>
<td>mean</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>Flow rate (Szybkość wydzielania) ml/min</td>
<td>0.19</td>
<td>0.10</td>
<td>0.25</td>
<td>0.15</td>
<td>0.05</td>
</tr>
<tr>
<td>Fluoride (Fluorki) µM</td>
<td>4.01</td>
<td>0.38</td>
<td>8.84</td>
<td>0.76</td>
<td>0.001</td>
</tr>
<tr>
<td>Protein (Białko) mg/ml</td>
<td>1.41</td>
<td>0.68</td>
<td>1.65</td>
<td>0.64</td>
<td>0.05</td>
</tr>
<tr>
<td>SP activity (Aktywność SP) mIU/ml</td>
<td>1.91</td>
<td>0.60</td>
<td>1.78</td>
<td>0.53</td>
<td>–</td>
</tr>
<tr>
<td>SP specific activity (Aktywność właściwa SP) mIU/mg P</td>
<td>1.62</td>
<td>0.79</td>
<td>1.18</td>
<td>0.48</td>
<td>0.001</td>
</tr>
<tr>
<td>SP activity adjusted for flow rate (Aktywność SP dostosowana do szybkości wydzielania) mIU/ml/min</td>
<td>13.03</td>
<td>10.61</td>
<td>9.26</td>
<td>6.61</td>
<td>–</td>
</tr>
<tr>
<td>SP activity adjusted for flow rate and protein content (Aktywność SP dostosowana do szybkości wydzielania i stężenia białka) (mIU/ml/min/mg/ml)</td>
<td>10.95</td>
<td>8.02</td>
<td>5.86</td>
<td>2.78</td>
<td>–</td>
</tr>
<tr>
<td>SP activity adjusted for flow rate and protein content (Aktywność właściwa SP dostosowana do szybkości wydzielania i stężenia białka) (mIU/ml/min/mg/ml)</td>
<td>0.72</td>
<td>0.18</td>
<td>0.73</td>
<td>0.24</td>
<td>–</td>
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<tr>
<td>SP activity adjusted for flow rate and protein content (Aktywność SP dostosowana do szybkości wydzielania i stężenia białka) (mIU/ml/min/mg/ml)</td>
<td>0.48</td>
<td>0.29</td>
<td>0.65</td>
<td>0.39</td>
<td>0.01</td>
</tr>
<tr>
<td>MP activity (Aktywność MP) mU/ml</td>
<td>3.73</td>
<td>1.58</td>
<td>9.26</td>
<td>6.61</td>
<td>0.05</td>
</tr>
<tr>
<td>Myeloperoxidase specific activity (Aktywność właściwa MP) mU/mg P</td>
<td>2.38</td>
<td>1.18</td>
<td>4.60</td>
<td>3.43</td>
<td>0.001</td>
</tr>
<tr>
<td>Myeloperoxidase activity adjusted for flow rate (Aktywność MP dostosowana do szybkości wydzielania) mIU/ml/min</td>
<td>3.73</td>
<td>1.58</td>
<td>9.26</td>
<td>6.61</td>
<td>0.05</td>
</tr>
<tr>
<td>Myeloperoxidase activity adjusted for flow rate and protein content (Aktywność MP dostosowana do szybkości wydzielania i stężenia białka) mIU/ml/min/mg/ml</td>
<td>2.38</td>
<td>1.18</td>
<td>4.60</td>
<td>3.43</td>
<td>0.001</td>
</tr>
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</table>
Biological importance of myeloperoxidase in the saliva is not fully elucidated. It can indirectly influence the OSCN– formation using hydrogen peroxide necessary for the oxidation of SCN– during formation of OCl– from chloride ions. However, this reaction has probably minor importance because of greater affinity of the enzyme to SCN– than to Cl– in salivary pH conditions. Therefore, the presence of myeloperoxidase in saliva has to be taken into consideration during the study of salivary peroxidase system.

The data obtained from in vitro study are in accordance with earlier reports that SP and MP inhibition depends on fluoride concentration and pH and that reduction of SP activity is higher than MP [6–8]. The rest activities of SP and MP are nearly the same as observed by Hannuksella et al. [8] and Abbeele Van den et al. [6]. Our results display 37.5% reduction of SP activity followed by adding 0.5 mmol/l F– at pH 4.6, and 19.2%. at pH 7.0. Instead, myeloperoxidase shows such percentage of inhibition at adding a 10-fold higher concentration of fluorides (37.3% at 5 mmol/l F– and pH 4.6, and 16.4% at 5 mmol/l F– and pH 7.0). Fluoride ions at 0.01 mmol/l concentration, i.e. close to the observed in the saliva of examined subjects do not reduce the enzymes activity. However, as fluorides are concentrated in the plaque fluid after intraoral exposure up to 0.05–10 mmol they may inhibit the activities of SP and MP in a pH and dose-dependent way in vivo conditions.

It could be suggested that the obtained data do not point out the importance of increase of fluorides in saliva as a results of drinking fluoridated water in decreasing of activity of peroxidase and myeloperoxidase. However, it is known, that fluorides can accumulate in the dental plaque up to levels many times exceeding their concentration in the saliva [13, 20], and after acidifying become ionised and are released [12]. Therefore, in caries prevention topical application of highly concentrated fluoride compounds (acidified especially) can result in transient high concentration of fluoride ions causing significant inhibition of peroxidases activity in the oral cavity. For example, after usage of conventional toothpastes containing 1000 ppm F (50 mmol/l), on assumption that toothpaste is diluted 1:3 by saliva fluorides will come into concentration ca. 250 ppm F [21]. Ekstrand and Vogel [20] described that rinsing of the oral cavity by 48 mmol/l NaF will cause the accumulation F– in dental plaque ranging from 0.9 to 3.0 mmol/l. Therefore, fluorides in spite of indisputable and fixed efficacy in caries prevention can reduce the activity of salivary peroxidases at acidic pH and diminish the enzymes role in the control of dental plaque bacteria.

Tenovuo et al. explained [22] the role of salivary peroxidase system in vivo conditions. They suggested that as a result of oxygenic metabolism of both extrinsic (delivered with diet) and intrinsic origin carbohydrates (glycoprotein, mono- and disaccharides present in the saliva) is generated hydrogen peroxide by some oral bacteria species. It is used up in the reaction catalysed by peroxidase, which causes the oxidation of thiocyanate (SCN–) present in the saliva to hypothiocyanite ions (OSCN–). The oxidation products inhibit bacterial metabolism by reduction of sugar transport (through cell membrane) and by inactivation of glycolytic enzymes, which diminishes the production of acids. Dropping of metabolism results in decreased formation of hydrogen peroxide and OSCN–. At lower OSCN– levels there may be spontaneous or thiol-induced recovery of inhibited microorganisms. Because peroxidase system does

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**Fig. 1.** Influence of NAF different concentrations (0.01–20 mmol/l F–) and pH (4.6–7.0) on peroxidase activity (% activity)

**Fig. 1.** Wpływ różnych stężeń NaF (0,01–20 mmol/l F–) i pH (4,6–7,0) na aktywność peroksydazy (% aktywności)

**Fig. 2.** Influence of NAF different concentrations (0.01–20 mmol/l F–) and pH (4.6–7.0) on myeloperoxidase activity (% activity)

**Fig. 2.** Wpływ różnych stężeń NaF (0,01–20 mmol/l F–) i pH (4,6–7,0) na aktywność peroksydazy (% aktywności)
not inhibit the growth of all oral bacteria, and besides *in vivo* conditions OSCN− concentrations are low, the rate of acid production depends mainly on individual bacterial flora and saliva composition. It could be supposed that levels of SP system constituents alone are not direct determinants of susceptibility or resistance to dental caries. Probably, the whole system acts more efficiently than each of its components. It should not skip its cooperation with other salivary defensive factors. It can be suggested that the results of studies on functional aspect of all antibacterial salivary factors evaluating their bactericidal potential, ability to agglutination and prevention of bacterial adherence to hydroxyapatite and capability to buffering would be the clue of defence mechanisms against caries forming factors. Therefore, basing on the own data and results of the research by authors mentioned above one can only suggest some complex influence of fluoride supplementation on salivary peroxidase activity.

As saliva is a complex system of quantitative and qualitative relationships of its constituents it seems useful to consider their variable influence on the level of the studied salivary components.

**References**


