Comparative analysis of the effect of preparations used in professional fluoride prophylaxis on the chosen parameters of human saliva

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Abstract

Regular supply of fluoride ions to the oral environment is one of the prophylactic actions against dental caries. Fluorides, whose exogenous action combines with saliva properties, condition the anticariogenic effect. Fluoride ions exhibit high chemical activity, can alter the oral environment parameters and inhibit the activity of enzymes.

Purpose: In the current study, the effect of fluoride preparations used in professional caries prophylaxis on chosen saliva parameters was studied. The levels of pH and fluoride ions, and the activity of cathepsin D in human saliva were determined.

Material and methods: Material for analysis contained resting mixed saliva collected before and 1, 4 and 24 hours after the application of Duraphat, Elmex Gel, Fluor Protector, Fluormex Gel and Fluoro-Gel.

Results: The fluoride-containing preparations inhibited the activity of cathepsin D in the way depending on the time that had passed since the application and altered the pH level of human saliva.

Key words: fluorides, saliva, cathepsin D.

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Introduction

Due to a number of functions, saliva plays a significant role both in physiology and pathology of the oral cavity. While the properties of saliva affect the course of various processes in the mouth, many factors can influence and alter the saliva parameters.

Caries prevention involves the arrest of etiological agents of dental caries and through application of fluoride compounds resistance of dental hard tissues is increased. The inorganic and organic fluoride compounds are found in preparations used both in everyday oral hygiene procedures and in professional fluoridation.

The major cariostatic mechanism of fluoride compounds is based on local action. They inhibit enamel demineralization, improve remineralization and decrease the activity of dental plaque bacteria [1]. Enamel stability in the oral environment depends on the pH of saliva and dental plaque, and is related to the concentrations of calcium, phosphate and fluoride ions. Depending on the concentration of a topically applied fluoride compound, the reaction of fluoride ions with enamel can yield fluorohydroxyapatite (after low-dose fluoride preparation) or calcium fluoride (after professional fluoridation) [2]. Fluoride ions contained in these compounds show high biochemical activity and can thus alter the oral environment parameters. Cathepsin D (EC 3.4.23.5) is a lysosomal endopeptidase that splits peptide bonds formed by carboxy groups of hydrophobic amino acids. It is isolated from tissues and organs, its trace amounts being found in blood plasma and body fluids including saliva. Cathepsin D takes part in pathological processes associated with myocardial ischaemia, liver disorders, muscular dystrophy, inflammatory states of joints and gums. It is involved in degradation of lipoproteins, parathyroid hormone, thyreoglobulin and glucagon [2,3].

In the study, the effects of the preparations commonly used for professional exogenous fluoride prophylaxis on the chosen parameters of saliva were compared. Levels of saliva pH and F^-, and activity of cathepsin D were determined.

Table 1. **Mean values of the saliva parameters studied**

		Duraphat				Elmex Gel				Fluor Protector			
		Mean	N	SD	Mediana	Mean	N	SD	Mediana	Mean	N	SD	Mediana
pH	0 h	7.06	6	0.33	7.00	7.46	6	0.43	7.47	7.27	6	0.15	7.29
pH	1 h	7.41	6	0.19	7.41	7.65	6	0.35	7.65	7.49	6	0.18	7.45
pH	4 h	7.42	5	0.15	7.48	7.53	6	0.26	7.43	7.39	6	0.16	7.34
pH	24 h	7.09	6	0.29	7.05	7.39	6	0.29	7.31	7.45	6	0.21	7.49
F^-[mg/dm^3]	0 h	18.96	6	12.74	16.46	2.11	6	1.33	2.09	0.40	6	0.13	0.39
F^-[mg/dm^3]	1 h	50.63	6	27.23	50.45	3.91	6	2.57	4.11	0.48	6	0.14	0.42
F^-[mg/dm^3]	4 h	51.89	5	21.80	56.23	3.73	6	1.85	3.51	0.46	6	0.12	0.46
F^-[mg/dm^3]	24 h	22.99	6	10.13	21.17	2.41	6	0.90	2.02	0.53	6	0.13	0.55
KAT D [nmol/ml]	0 h	136.67	6	29.90	137.00	158.00	6	54.10	129.50	97.67	6	38.40	83.00
KAT D [nmol/ml]	1 h	71.83	6	51.50	69.50	129.83	6	62.40	116.50	79.17	6	43.88	74.00
KAT D [nmol/ml]	4 h	80.00	5	16.25	76.00	125.40	5	55.94	105.00	147.83	6	59.38	132.00
KAT D [nmol/ml]	24 h	102.83	6	41.14	91.00	156.17	6	72.83	150.00	146.67	6	59.70	138.50

		Fluormex Gel				Fluoro-Gel				Razem			
		Mean	N	SD	Mediana	Mean	N	SD	Mediana	Mean	N	SD	Mediana
pH	0 h	7.12	6	0.26	7.09	7.03	6	0.12	7.08	7.19	30	0.31	7.13
pH	1 h	7.52	6	0.33	7.56	7.13	6	0.12	7.19	7.44	30	0.29	7.39
pH	4 h	7.41	6	0.23	7.38	7.18	6	0.13	7.24	7.38	29	0.22	7.30
pH	24 h	7.29	5	0.30	7.26	7.17	6	0.14	7.16	7.28	29	0.27	7.23
F^-[mg/dm^3]	0 h	8.86	6	5.66	6.84	16.14	6	7.44	15.55	9.29	30	9.97	6.65
F^-[mg/dm^3]	1 h	15.22	6	8.11	15.01	32.16	6	22.84	29.71	20.48	30	24.33	10.49
F^-[mg/dm^3]	4 h	12.96	6	3.59	13.78	26.00	6	14.43	23.75	17.87	29	21.02	9.12
F^-[mg/dm^3]	24 h	10.99	5	4.23	9.66	17.55	6	7.39	17.78	10.89	29	10.49	9.12
KAT D [nmol/ml]	0 h	164.17	6	29.46	166.50	93.67	6	53.56	71.50	130.03	30	49.59	127.00
KAT D [nmol/ml]	1 h	133.00	6	23.55	128.00	70.67	6	44.01	51.00	96.90	30	52.17	95.50
KAT D [nmol/ml]	4 h	133.17	6	61.24	108.50	75.67	6	48.33	56.50	113.11	28	56.42	104.50
KAT D [nmol/ml]	24 h	158.80	5	27.94	158.00	106.33	6	91.55	73.50	133.31	29	64.15	134.00

Material and methods

Material for analysis contained mixed saliva collected directly to test-tubes (ZPS, Medlab), two hours after a meal, from 30 dentistry students aged 19-24 years (21 women and 9 men). Samples were obtained before (baseline measurement) and directly after a single application of fluoride preparation, as well as after 4 and 24 hours. The tubes containing saliva were immediately frozen at -18 to -24°C. After defrosting, saliva pH was measured by ionoselective fluoride electrode. The activity of cathepsin D was determined by adding 0.1 ml of 6% haemoglobin to 0.4 ml of saliva. The samples were incubated for four hours at 37°C. The reagents used had a pH 3.5. The reaction was discontinued by adding 0.5 ml of 10% TCA and then the samples were centrifuged 2 700 x g, for 30 min, at 4°C. Samples that precipitated at baseline measurement were referred to as control. Acid-soluble tyrosine was determined in the supernatant using the method of Folin and Ciocalteau in copper modification. The amount of released tyrosine was read from the calibration curve designed according to the model solutions of this amino acid [4].

The following preparations were used for contact fluoridation procedures: Fluor Protector, Elmex Gel, Fluormex Gel, Duraphat, Fluoro-Gel. Results were statistically analysed using the two-factor analysis of variance for repeatable measurements with Fisher NIR post hoc tests, using Statistica 6.0 programme, Statsoft. The accepted level of significance was 0.005.

Results

Tab. 1 presents mean values of the saliva parameters determined in the current study, taking into account type of fluoride preparation used and time that passed since fluoridation. Groups were characterized by calculating the mean, median and standard deviation.

Saliva pH values after contact fluoridation increased as compared to those before application. Type of preparation and time that passed since fluoridation had a statistically significant effect on pH. *Fig. 1* shows saliva pH values after application of the preparations used, being the highest for Elmex Gel. Statistically significant differences in the increase in saliva pH between the preparations were found between Elmex Gel and Fluoro-Gel, Elmex Gel and Duraphat, and Fluor Protector and Fluoro-Gel.

The pH value was found to depend on the time of measurement after fluoridation. *Fig. 2* presents pH values averaged for all the preparations according to the time point. The highest increase in pH was observed directly and 4 hours after fluoridation procedure as compared to the baseline one. After 24 hours, the pH dropped. The differences were statistically significant in comparison to those before fluoridation. No statistically significant differences were noted between pH value directly and 4 hours after fluoridation

A comparison of the pH values for the respective prepara-

Figure 1. **Mean pH values for the parameters studied, 95% confidence**

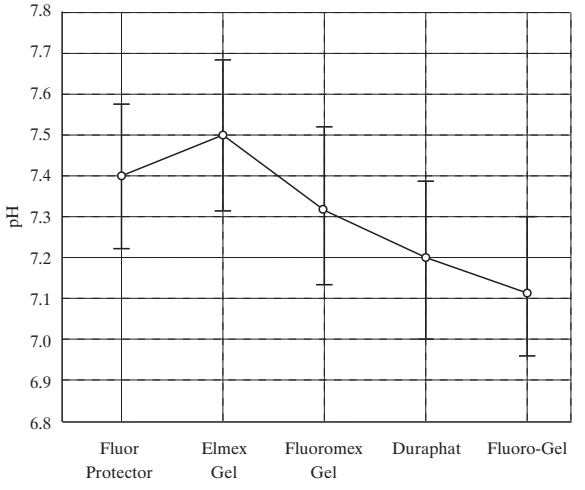


Figure 2. **Mean pH value for all preparations depending on time of measurement (95% confidence)**

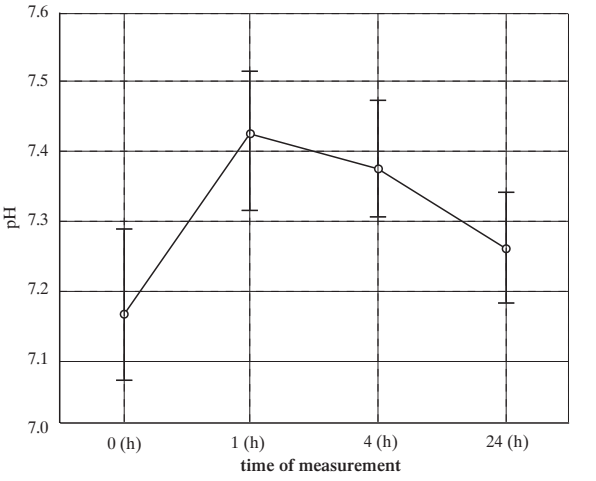


Figure 3. **Mean pH value for the respective preparation at a particular time point**

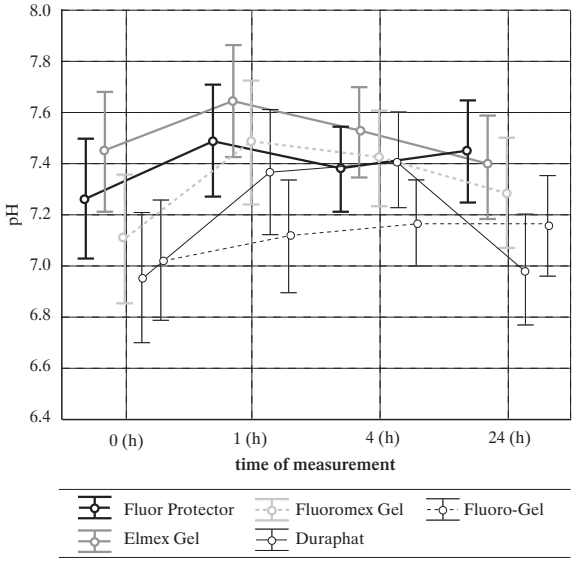
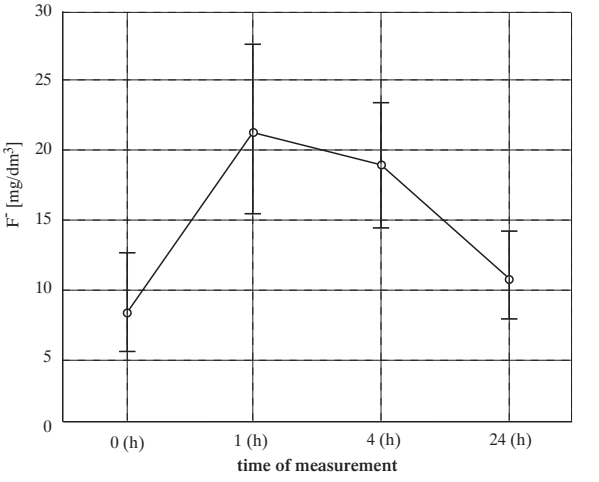


Figure 4. **Mean F^- values for the parameters studied, 95% confidence**



tion between various time points revealed a statistically significant increase for Fluor Protector at the time point directly after application as compared to baseline and between baseline and the level after 24 hours. The same tendency was noted when Elmex Gel, Fluormex Gel and Duraphat were applied. No statistically significant increase in saliva pH at all the time points was observed for Fluoro-Gel.

Fig. 3 demonstrates the mean pH value for the respective preparation at a particular point of measurement. A comparison between various preparations showed statistically significant differences only for Fluoro-Gel as compared to Elmex Gel directly after fluoridation. The level of fluoride ions measured before the application of fluoride preparations was found to undergo a marked increase directly after fluoridation, a slight decrease after 4 hours and a further drop after 24 hours, finally reaching a slightly higher level than before fluoridation (*Fig. 4*). However, the differences between the level of F^- before and 24 hours after application were not statistically significant. Analysis of variance indicates a statistically significant correlation of the level of F^- with type of fluoride preparation and time of measurement.

No statistically significant increase in saliva F^- was noted at any time points after application of Fluor Protector, Elmex Gel or Fluormex Gel. Duraphat caused the highest statistically significant rise in saliva F^- directly and 4 hours after fluoridation (*Fig. 5*). 24 hours after fluoridation, the level of F^- remained slightly but not statistically significantly elevated, as compared to that before fluoridation. However, drop in the level of F^- after 24 hours in comparison to examination directly and 4 hours after application was statistically significant. A similar distribution of F^- in saliva was observed after application of Fluoro-Gel (*Fig. 6*). No statistically significant differences were found between Elmex Gel and Fluormex Gel with regard to their effect on saliva fluoride ions. The increase in fluoride ions after application of Duraphat and Fluoro-Gel in comparison to

***Figure 5*. Mean F^- value for all preparations depending on time of measurement (95% confidence)**

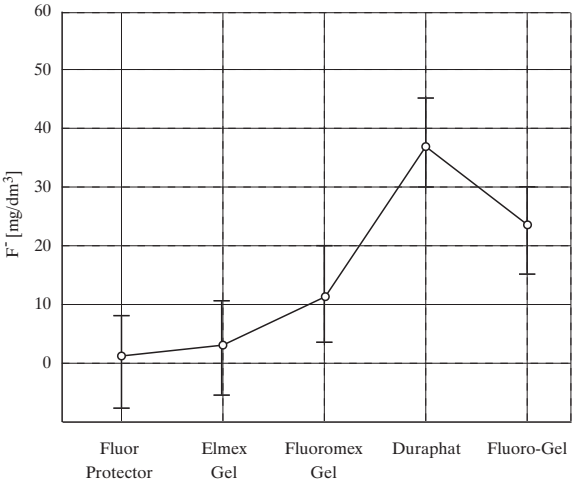


***Figure 6*. Mean F^- value for the respective preparation at a particular time of measurement**

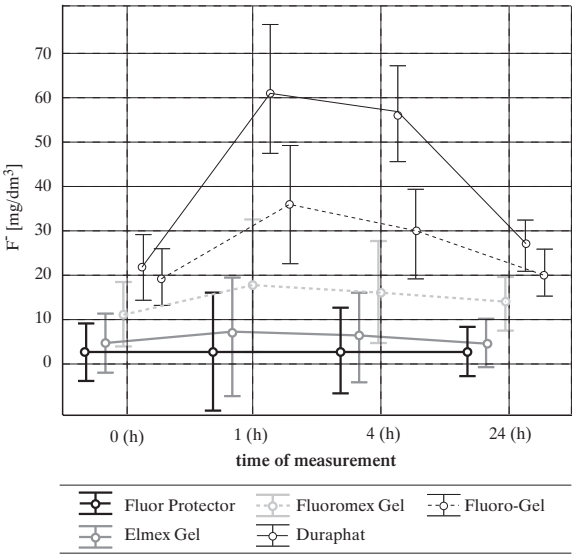


***Figure 7*. Mean cathepsin D level for all the preparations depending on time of measurement (95% confidence)**

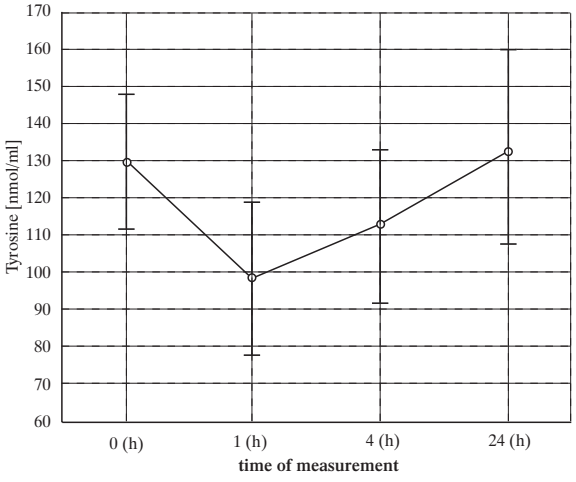


***Figure 8*. Mean cathepsin D level for the preparations studied, 95% confidence**

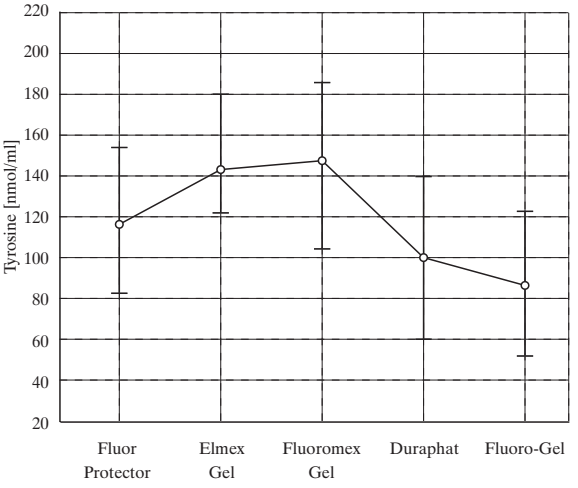
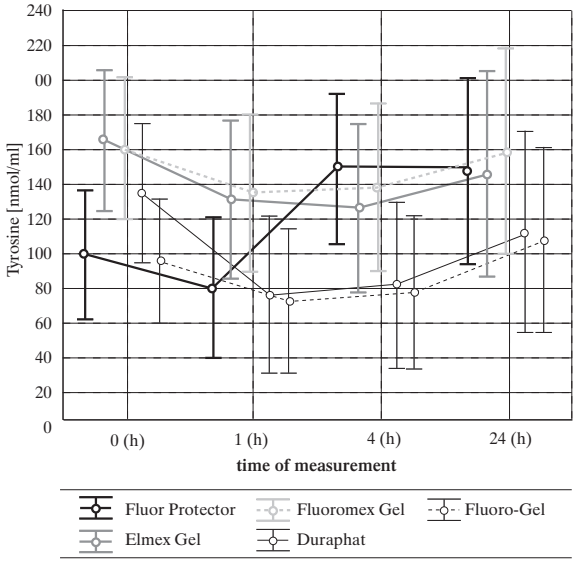


***Figure 9*. Mean level of cathepsin D for the respective preparation at a particular time point**



other preparations was statistically significant. A comparison of F^- level between various preparations at the respective time point revealed lack of statistically significant differences between Duraphat and Fluoro-Gel. The F^- values observed after application of the remaining preparations were lower as compared to Duraphat and Fluoro-Gel, the differences being statistically significant.

Measurements of saliva cathepsin D revealed its reduction directly after application of all the preparations studied (*Fig. 7, 9*). Differences in the effects between the respective preparations on cathepsin D in saliva were basically not statistically significant, and were only observed between Fluoro-Gel and Elmex Gel and Fluormex Gel (*Fig. 8*). The time point affected the level of cathepsin D and was a statistically significant factor. Statistically significant differences in the level of cathepsin D were found before and directly after fluoridation, when its level decreased, and between the examination directly

after and 24 hours after fluoridation, when its level increased (*Fig. 7*). The effects of various preparations on the level of cathepsin D at a particular time point showed no statistically significant differences.

Discussion

Fluoride compounds used for therapeutic reasons, thanks to their high chemical activity, can affect various biological systems. Affinity of fluoride ions for calcium and magnesium results in the effect on enzymatic activity. The compounds inhibit the action of oxidoreductases, transferases, hydrolases and the Krebs cycle enzymes [5-8]. The effect of fluorides on extracellular enzymes is also known. They inhibit, *in vitro* and *in vivo*, depending on their concentration and environmental pH, the activity of peroxidase and myeloperoxidase, the salivary antibacterial, non-immunological defence factors [9-11]. Endogenous and exogenous supplementation with fluoride compounds in caries prophylaxis is to ensure regular provision of fluoride ions to the oral cavity. The salivary level of fluoride ions increases on endogenous delivery or after topical application of fluoride preparations, but fluoride retention is unstable. In the current study, Duraphat and Fluoro-gel caused the most pronounced and statistically significant increase in the salivary F^- level immediately and 4 hours after application. This may be related to high fluoride content in these preparations and perhaps too low F^- availability to form calcium fluoride. The remaining preparations caused an increase in fluoride ions, which was, however, statistically insignificant. This would require further study.

In the current analysis, the pH value was elevated after contact fluoridation, being the highest immediately following the procedure and after 4 hours. The increase was statistically significant for all the preparations except for Fluoro-gel. Our results are consistent with those reported by other authors [12]. Dąbrowska et al. observed a slight increase in salivary pH after a single application of each of the preparations [13]. During saliva collection for analysis, the saliva secretion rate was found to increase directly after the use of prophylactic preparations (data not included in the report). Analysing the causes of the saliva pH increase even in the case of a slightly acidic pH (Elmex Gel), which promotes formation of calcium fluorides, a potential relationship between this increase and saliva secretion rate should be considered. Fluoridation can stimulate saliva secretion, resulting in elevated pH level. Engel-Brill et al. found significantly higher saliva secretion after brushing the teeth with an aminofluoride preparation in comparison to a monophosphate-containing preparation. The authors suggest increased saliva flow with an increase in the level of fluoride ions in aminofluorides [14].

Studies on the mechanism underlying the development of periodontal diseases have made an attempt to determine the role of proteolytic enzymes in the oral cavity. High activities of cathepsin B and L were noted in gingival tissue homogenates in periodontal patients [15-17]. Based on gingival biopsy, Jotterand and Cimasoni determined a statistically significant correlation between cathepsin D activity and gingivitis grade [18].

The study on the level of cathepsin D in the saliva showed its reduction directly after fluoridation, which was statistically significant. The inhibitory effect of oral hygiene preparations on proteolytic enzymes of the saliva was observed in vitro. A decrease was found in the activity of cathepsin D in human saliva in the presence of Blend-a-med toothpaste [19]. Dąbrowska et al. revealed a substantial reduction in cathepsin C activity in saliva following application of preparations containing amino fluorides [13].

Conclusion

In conclusion, it was found that professional and individual local fluoridation procedures caused a reduction in the activity of cathepsin D in human saliva and a transitory change in salivary pH.

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