Study Regarding the Effect of Some Remineralizing Products Used in the Treatment of Erosion Induced by Sport Drinks

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The aims of the study were to evaluate and to compare the effects of some commercial sport drinks on enamel and dentine when using remineralizing products before the use of these beverages. Enamel and dentine fragments were immersed five times, 3 min each during 2 h, fourteen days, in three sport drinks: Gatorade beverage (PepsiCo), Isostar (Isostar) and Cyto Max (CytoSport). On other tooth fragments three remineralizing products were applied before the immersion in sport drinks: Colgate Total® toothpaste (Colgate Company), a fluoride gel (Densell Company), and MI Paste Plus product (GC Company). In all the samples the enamel and dentine hardness were determined using a digital device CV 400 DAT (Namicon). Gatorade beverage leaded to the highest demineralization of the enamel and dentine, followed by Isostar and CytoMax. All three remineralizing products used before the acid attack determined increased values of enamel and dentine hardness when compare to the values obtained when only the acidic beverages were applied. The highest enamel and dentine protection were recorded when MI Paste Plus product was applied, followed by fluoride gel and toothpaste products.

Keywords: remineralization, erosion, sport drinks, enamel, dentine

Sport drinks are considered to be water and mineral substitutes used during or after sport training. In low initial level of carbohydrates or hardly intense training that last more than an hour, these beverages are considered to be very efficient [1]. Their efficiency is doubtable especially in low or moderate effort. Liquid intake before and during the training will minimized the effects of dehydration on cardiovascular system, temperature regulation and physical performance. The question is if sport drinks consumption has any additional good effects when compare to water. It was stated that for many persons who perform physical activities sport drinks consumption do not offer additional benefits [2]. The major role of these beverages is related to the liquid intake in the condition of more than a half fluids lost during the sport training.

In 2014 more than 50 millions of dollars were spent on sport drinks and it was estimated that the sales will increase up to 30% until 2019, reaching the value of 64,1 millions of dollars [3]. In SUA over 1.5 billions of dollars are spent on sport drinks during one year. Despite the fact that these beverages were indicated to the persons who are making high physical effort during intense training, nowadays they are used by a higher number of the population. The consumption of sport beverages exceeded the consumption of water during training probably due to the good taste and smell of the products [2].

Sport drinks are acidic beverages having the pH lower than the critical pH of enamel [4]. Previous studies reported that risk for dental erosion onset was four times higher in persons that consume sport drinks weekly [5]. Their erosive potential is increased when the beverages are consumed during the periods of dehydration and when salivary flow rate is low as it occurs during sport training [6. 7]. Also, many studies showed a direct correlation between dental erosions and sport drinks consumption [8-10]. On a contrary, the results of other studies did not established a significantly correlation [11, 12]. Due to these conflicting results, more studies to evaluate and compare the effects of sport drinks on dental hard tissues are needed.

Attempts were made during the time to decrease the erosive potential of the beverages. Adding calcium or phosphate ions [13], increasing the beverage *p*H [10] or adding additional ingredients such as casein phosphopeptide-stabilized amorphous calcium phosphate (CPP-ACP) [14, 15] were reported to be effective in reducing the erosive aggression. In previous studies the protective effect of some fluoride products [16-18] or some food [19] applied before the acidic aggression was also demonstrated.

The aims of the study were to evaluate and to compare the effects of some commercial sport drinks on enamel and dentine when using remineralizing products before the use of these beverages.

Experimental part

Samples preparation

Thirty three healthy third molars, extracted for orthodontic reason were included in the study. Until the start of the study the teeth were kept in distilled water. Two horizontal sections of 2 mm each were obtain by cutting the teeth with diamond disks (Komet Dental, Brasseler GmbH&Co, Germania) at low speed and under water cooling. Both sections were cut bucco-lingualy in order to obtain four tooth fragments. The fragments were randomly split in thirteen groups. In group 1 (control) 10 samples were kept in artificial saliva. Thirty samples were exposed to sport drinks action (groups 2-4). Three sport products were chosen in this study: Gatorade beverage (PepsiCo), Isostar lemon tablets (Isostar) and Cyto Max orange powder (CytoSport). Isostar beverage was obtained by placing one tablet in 250 mL of mineral water (Borsec, Romaqua Group). Cuto Max beverage resulted by mixing one spoon of powder (25 mg) in 250 mL of mineral water (Borsec, Romaqua Group). The composition of the products included in the study is presented in table 1.

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Table 1
THE COMPOSITION OF THE SPORT DRINKS

Beverage	Composition	Producer
Gatorade	water, sucrose, dextrose, citric acid, salt, sodium citrate, monopotassium phosphate, natural flavor, modified food starch, calcium silicate, yellow 6	Pepsi Co.
Isostar	water, sucrose, glucose syrup dehydrated DE47, maltodextrin DE19, citric acid, sodium citrate, natural flavoring, calcium phosphate, magnesium carbonate, sodium chloride, potassium chloride, ascorbic acid, modified corn starch, coconut oil, vitamin B1.	Isostar
CytoMax	Maltodextrin, crystalline fructose, dextrose, alpha-L-polylactate, citric acid, natural and artificial flavor, malic acid, L-alanine, L-glutamine, guar gum, xanthan gum, stevia (rebiana), carmine.	CytoSport

In groups 2-4 the tooth samples were immersed in Gatorade beverage (group 2), in Isostar beverage (group 3), and in Cyto Max beverage (group 4) five times, 3 min each during 2 hours, fourteen days. The protocol was chosen in order to simulate the beverage consumption during sport training. Each tooth sample was immersed in 15 ml of fresh sport beverage. Between the beverages periods of action, the samples were immersed in artificial saliva (AFNOR, standard S90-701) that was renewed every day.

In groups 5-7 the tooth samples were immersed in Gatorade beverage according to the protocol in group 2, but before the first immersion *Colgate Total®* toothpaste (Colgate Company) was applied for three minutes and then washed with running water (group 5), a fluoride gel (Densell Company) was applied one minute (group 6) and MI Paste Plus product (GC Company) was applied for three minutes (group 7). In groups 8-10 the tooth samples were immersed in Isostar beverage according to the protocol in group 3, but before the first immersion *Colgate Total®* toothpaste (Colgate Company) was applied for three minutes and then washed with running water (group 8), a fluoride gel (Densell Company) was applied one minute (group 9) and MI Paste Plus product (GC Company) was applied for three minutes (group 10). In groups 11-13 the tooth samples were immersed in Cyto Max beverage according to the protocol in group 4, but before the first immersion *Colgate Total®* toothpaste (Colgate Company) was applied for three minutes and then washed with running water (group 11), a fluoride gel (Densell Company) was applied one minute (group 12) and MI Paste Plus product (GC Company) was applied for three minutes (group 13). The composition of the three products applied before the first contact with the sport drinks is presented in table 2.

Surface hardness determination

For all the samples the enamel and dentine hardness were determined using a digital device CV 400 DAT (Namicon). Indentations resulted at 50 g loading of the device. For each sample three indentations were obtained in enamel and dentine respectively. The distance between two indentations was 1mm. The mean surface hardness values (MPa) was recorded for enamel and dentine as a result of three determinations.

Results and discussions

The mean enamel and dentine surface hardness (MPa) and standard deviation in groups 1-13 are presented in table 3.

In groups 2, 3 and 4 the enamel hardness values were lower than group 1. The lowest hardness values were obtained in group 2, followed by group 4 and group 3. The same tendency of hardness value variation was recorded also in dentine. In all groups the dentine hardness values of were lower than that of enamel. Statistical tests Kruskal-Wallis and Mann-Whitney were used to compare the results between groups. Statistically significant results were obtained when compare the hardness values between groups, except the dentine values in groups 2 and 3 (table 4).

All three remineralizing products used before the acid attack determined increased values of enamel and dentine hardness when compare to the values obtained when only the acidic beverages were applied. MI Paste product and fluoride gel application before Isostar acid attack leaded

Table 2
THE COMPOSITION OF REMINERALIZING PRODUCTS

Product	Ingredients	Company
Colgate Total Advanced Deep Clean	sodium fluoride 0.24%, triclosan 0.30%, hydrated silica, water, glycerin, sorbitol, PVM/MA copolymer, sodium lauryl sulfate, cellulose gum, flavor, sodium hydroxide, carrageenan, propylene glycol, sodium saccharin, titanium dioxide	Colgate
Fluoride Gel	sodium fluoride 0,4%, ascorbic acid, carbomer, phosphoric acid, glycerin, colorant, flavouring	Densell
MI Pate Plus	sodium fluoride 0,2%, pure water, CPP-ACP (Casein phosphopeptide – Amorphous Calcium Phosphate), D-sorbitol, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, flavouring , zinc oxide, sodium saccharin, ethyl p-hydroxybenzoate, magnesium oxide, guar gum, propyl p-hydroxybenzoate, butyl p-hydrobenzoate	GC Company

	Enamel hardness(MPa)±SD	Dentine hardness(MPa)±SD
Group 1	203.05±0.87	57.23±0.87
Group 2	105.66±1.21	22.90±0.80
Group 3	113.80±0.50	36.56±0.37
Group 4	110.30±0.56	37.13±0.48
Group 5	130.76±0.77	37.70±0.68
Group 6	138.96±0.90	38.83±0.37
Group 7	146.26±0.41	39.53±0.20
Group 8	135.70±0.55	26.13±0.41
Group 9	158.30±0.22	45.00±0.57
Group 10	204.00±0.80	46.06±0.29
Group 11	186.96±0.33	41.26±0.91
Group 12	202.16±1.70	56.53±1.02
Group 13	234.66±3.6	56.83±0.27

Table 3
MEAN ENAMEL AND DENTINE HARDNESS VALUES IN GROUPS 1-13

Table 4
MANN-WHITNEY STATISTICAL TEST RESULTS

	Groups comparison					
	1-2	1-3	1-4	2-3	2-4	3-4
	Dentine					
P values	0.00	0.00	0.00	0.058	0.00	0.00
	Enamel					
P values	0.00	0.00	0.00	0.000	0.00	0.00

to higher surface hardness when compare to the toothpaste (table 3). The same tendency of hardness variation was also recorded when CytoMax and Gatorade products were evaluated. The highest surface hardness values in enamel and dentine were recorded when MI Paste Plus product was applied, followed by fluoride gel and toothpaste.

Statistical ANOVA and Bonferroni post hoc statistical tests were used to analyse the data. Statistically significant results were obtained when compared the hardness values between groups, except the values obtained in enamel when fluoride gel and MI Paste Plus product were applied before acid attack provided by Gatorade and Isostar beverages. Also, no statistically significant results were obtained when compare the dentine hardness values in case of fluoride gel and MI Paste Plus product were applied before Isostar beverage action (table 5).

Gatorade beverage leaded to the highest demineralization of the enamel and dentine, followed by Isostar and CytoMax. The composition is very important on determining the erosive potential of the beverages. Although many products are available on the market, little or no difference in their composition is present. The most

Table 5
STATISTICAL BONFERRONI TEST RESULTS

	Groups comparison								
	5-6	5-7	6-7	8-9	8-10	9-10	11-12	11-13	12-13
	Dentine								
P values	0.00	0.00	0.00	0.00	0.00	0.054	0.00	0.00	0.00
	Enamel								
P values	0.00	0.02	0.12	0.00	0.00	1.00	0.00	0.00	0.00
	Groups comparison								
	2-5	2-6	2-7	3-8	3-9	3-10	4-11	4-12	4-13
	Dentine								
P values	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Enamel								
P values	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

important carbohydrates are glucose, fructose, sucrose and maltodextrin. In order to keep the fluid-mineral balance and to improve the rate of absorption, small amount of sodium, potassium and chloride ions were added. Factors like acidic components and low concentrations of calcium and phosphate are known to cause dental erosion [20]. Despite that some sport products contain more than 5 mM inorganic phosphate (as Gatorade), they still have an erosive potential due to the fact that they remain undersaturated to enamel and dentine minerals [21]. Another component having a high erosive effect is the citric acid. In addition to erosive action gave by the protons release, citrate anion has the ability to chelate calcium ions [22]. The enamel erosive potential of Gatorade powder and liquid was also demonstrated in an in situ study [23]. The *p*H and titratable acidity can also determine the erosive potential of the beverages. In a previous study was reported that the *p*H of eight popular brands of sports drinks ranged 2.4 to 4.5 with a high variability in the titratable acidity [21].

It is known that due to the porous structure enamel allows ion changes with oral environment, favoring the regain of mineral components. If calcium and phosphate ions are present during acid attack, they have the ability to inhibit the demineralization and to provide the remineralization. Dentine is more prone to erosive dissolution when compared to enamel due to higher organic content, lower mineral content, and much smaller apatite crystals [24]. All the remineralizing products that were tested provided a protective effect of enamel and dentine against acidic challenge. Most of the products such as fluoride [25-30] aim to reduce the apatite dissolution rather than promote mineralization by apatite crystallization or replacement of the lost mineral. It was demonstrated that frequent application of a high concentration of topical fluoride may prevent further demineralization and increasing the toot resistance against erosions [31]. Enamel and dentine hydroxyapatite is responsible for the mechanical behavior of the dental tissues. If hydroxyapatite is dissolved by erosive challenge, enamel will not be able to be spontaneously remineralized. Previous studies showed that sport drinks induce severe dental erosion, and fluoride ions added to sport drinks decrease the evolution of erosive lesions and lead to partial occlusion of dentinal tubules [32]. Also, saturation of acidic beverages with calcium fluoride ions leaded to a decrease of erosion onset [33].

The presence of fluoride in the oral cavity during de- and remineralization cycles will lead to florapatite or fluorhydroxyapatite formation, which are less prone to dissolution. Many beverages, due to their *p*H and composition, are undersaturated to tooth minerals and even the superficial enamel layer containing fluor(hydroxy)apatite will be dissolved. Therefore, the protective effect of this layer is less important in preventing erosion lesion onset. Application of 2.26% fluoride varnishes for 24 h and 1.2% fluoride mouthrinsing solution before acid attack offered a good protection against erosive challenge [8]. It was supposed that this protective effect is gave by calcium fluoride-like particles. By their dissolution and ion release, the underlaying enamel will be protected [25]. All fluoride products tested in this study leaded to a good enamel and dentine protection against erosive challenge.

Phosphoprotein casein (CPP) and calcium phosphate (ACP) provided a good protection action against acid attack [34]. CPP have the ability to keep the calcium and phosphate in an amorphous, non-crystalline state. The cluster sequence -Ser(P)-Ser(P)-Ser(P)-Glu-Glu- from CPP stabilizes ACP in a stable solution. Also, CPP binds to form nanoclusters of ACP due to the multiple phosphoseryl residues, preventing their growth to the critical size required for nucleation and phase transformation [35]. In this way calcium and phosphate ions that remain soluble and biologically available can enter the tooth surface and enhance remineralization [36]. It was stated that the protective effect of CPP-ACP relies on keeping the calcium and phosphate available at supersaturated level and inhibiting this way the enamel demineralization [37]. The CPP-ACP paste application on the enamel surface determined the formation of a layer that covers partially the prisms and fills the area between the enamel prisms, thus preventing a future acid challenge. This action is evident in enamel, but it was observed also in dentine. MI Paste product demonstrated the ability to prevent the demineralization even in dentine [38]. One previous study demonstrated the formation of apatite precipitate on the surface of the dentin matrix as a result of remineralization process [24]. A complete remineralization is not possible and as a result of this process the dentine hardness did not reached the initial values in the present study. CPP - ACP application on dentine leads to a layer formation that fills the intratubular areas [39] and which might be a barrier against acid attacks. By using CPP - ACP paste, a composite material will result which is more or less selectively eroded by the $H+$ ions and which is in direct relation to the chemical properties of the solution [40]. CPP-ACP has been successfully incorporated into oral health products such as mouthrinses, sugar free chewing gums, and sport drinks to reduce enamel erosion [41].

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