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RESEARCH ARTICLE

Evaluation of the Potential of Commercial Vitamin Drinks to Induce Tooth Erosion

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Background: The market for vitamin drinks is expanding both in Korea and worldwide. However, it was difficult to find studies regarding the possibility of tooth erosion induction due to vitamin drinks. The purpose of the present in vitro study was to evaluate the effect of tooth erosion caused by a few commercial vitamin beverages on bovine teeth enamel in terms of erosion depth and fluorescence loss.

Methods: Three experimental groups (vitamin drinks), a positive control group (Coca-Cola), and a negative control group (mineral water) were established. Each group consisted of 5 specimens obtained from sound bovine teeth. The pH and titratable acidity of beverages were measured. Specimens were immersed in the beverages and artificial saliva for 6 and 18 hours, respectively. This cycle was repeated for 5 days. The depth of the tooth loss caused by tooth erosion (erosion depth) and maximum loss of fluorescence (Max ΔF) were measured using the microscope and quantified light-induced fluorescence-digital, respectively. For the statistical analysis, the Kruskal–Wallis test and ANOVA were used to compare the erosion depth and Max ΔF of the enamel surfaces. In addition, Spearman correlations were estimated.

Results: The pH of the three vitamin beverages ranged from 2.65 to 3.01, which is similar to that of the positive control group. All beverages, except mineral water, had sugar and acidic ingredients. Vitamin drinks and the positive control, Coca-Cola, caused tooth erosion lesions, and showed significant differences in erosion depth compared to mineral water ($p < 0.05$). The vitamin beverages with low pH were associated with high erosion depth and Max ΔF.

Conclusion: Vitamin drinks have the potential to cause tooth erosion.

Key Words: Fluorescence loss, Lesion depth, Tooth erosion, Vitamin drink

Introduction

As interest in health has increased recently, the focus has been shifting from developing thirst-quenching beverages to developing functional beverages that improve health and treat nutritional deficiencies[1,2]. Furthermore, consumers try to select drinks or food products with ingredients that are healthy. According to a previous study involving a cohort of Korean college students, male students consumed 4.6% of total calories, 22% of vitamin A, and 7.9% of vitamin B_2 through beverages, and female students consumed 4.8% of total calories, 6.1% of vitamin A, and 11.4% of vitamin B_2 through beverages[3]. This finding suggests that nutritional deficiency in meals can be supplemented with beverage intake.

In Korea, with the increase in health needs, the market for vitamin drinks is growing[2]. Furthermore, owing to the popularity of the Korean wave (Hallyu culture), the number of vitamin drinks produced in Korea is increasing, and the market for vitamin drinks is expanding both in Korea and worldwide[2].

Vitamin drinks contain water-soluble vitamins B and C, amino acid-based fatigue recovery substances, as well as other beverage components including sugars and acids, such as white sugar, liquid fructose, and citric acid. They also contain DL-carnitine hydrochloride, pyridoxine hyd-

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rochloride, sodium citrate, and carbon dioxide gas. Some of the ingredients of vitamin drinks may help to recover from fatigue and may have positive effects on the whole body's health, but the impact of vitamin drinks on oral health is not well known publicly. In particular, acid components contained in vitamin drinks may induce tooth erosion; therefore, studies are necessary to confirm this.

Tooth erosion refers to the loss of non-decayed tooth structures by chemical processes without the involvement of microorganisms[4]. Clinically, tooth erosion is caused by a defect in the enamel surface of the tooth, and is different from dental caries in which demineralization proceeds under the enamel surface[5]. There have been several studies on tooth erosion caused by beverages[6,7]. There has been a report on the possibility of tooth erosion caused by commercial beverages and the importance of the pH and titratable acidity of beverages[8]. A study that measured the degree of enamel erosion caused by energy drinks containing vitamins confirmed that low-pH beverages have the largest enamel erosion-inducing effect[9]. In addition, functional beverages used in previous studies of tooth erosion induction were sports beverages[10,11], energy drinks[9,12,13], and beverages containing lactic acid bacteria and calcium[14,15]. However, it was difficult to find studies regarding the possibility of tooth erosion induction due to vitamin drinks. Therefore, the present study aimed to identify the components of commercial vitamin drinks and to determine the possibility of tooth erosion induction due to commercial vitamin drinks.

Materials and Methods

1. Beverage selection

Among the vitamin-containing beverages marketed in Korea, three kinds of beverages, Bacchus-D (BD; Dong-A Pharm., Seoul, Korea), Vita 500 (V5; Kwangdong Pharmaceutical Co., Ltd., Seoul, Korea), and OronaminC (OC; Donga-Otsuka Co., Seoul, Korea), which have high sales and are easily available, were selected for the present study. Coca-Cola (CC; Coca-Cola Korea Co., Seoul, Korea) was used as a positive control, and mineral water (MW; Jeju Samdasoo; Jeju Province Development Co., Jeju, Korea) was used as a negative control (Table 1).

Table 1. The Major Ingredients of the Beverages Used in the Present Study, as Specified by Their Manufacturers

Group	Product name	Brand	Ingredients	
			Sugar and acid	Others
Experimental groups (vitamin drinks)	Bacchus-D	Dong-A Pharm., Seoul, Korea	Thiamine nitrate, DL-Carnitine hydrochloride	Taurine, Inositol, Nicotinic acid amide, Riboflavin phosphate sodium, Pyridoxine HCl (B_6) 5 mg, Caffeine anhydride
	OronaminC drink	Donga-Otsuka Co., Seoul, Korea	Sugar, Liquid fructose, Honey, Citric acid	Carbon dioxide gas, Vitamin B_2, Soluble vitamins P, Synthetic flavoring agent, Vitamin mix (Vitamin B_6 hydrochloride, Niacinamide, Phenylalanine, Threonine, Isoleucine, L-Sodium glutamate), Vitamin C, Caffeine
	Vita 500	Kwangdong Pharmaceutical Co., Ltd., Seoul, Korea	Liquid fructose, Concentrated apple juice, DL-Malic acid, Orange extract, Citric acid, Pectin	Taurine, Hyaluronic acid-KD, Vitamin B_2 1.2 mg, Synthetic flavoring agent, Sodium citrate, Vitamin C
Positive group (coke)	Coca-Cola	Coca-Cola Korea Co., Seoul, Korea	Liquid fructose, Sugar, Caramel color	Phosphoric acid, Natural flavoring agent, Caffeine, Flavor enhancer, Carbon dioxide gas
Negative group (mineral water)	Jeju Samdasoo	Jeju Province Development Co., Jeju, Korea	-	Calcium: 2.5～4.0 mg/L, Potassium: 1.5～3.4 mg/L, Sodium: 4.0～7.2 mg/L, Magnesium: 1.7～3.5 mg/L

HCl: hydrogen chloride, -: not available.

2. Chemical properties of the beverages

The ingredients of the beverages used in the present study are listed on the main ingredient list of the product. To measure the pH at the same temperature, the beverages were left at room temperature one day before measurement, and 50 ml was dispensed into a beaker. The pH was measured three times using a pH meter (Orion 3 star Benchtop pH meter; Thermo scientific, Beverly, MA, USA). Titratable acidity was determined by adding 40 μl of 1M NaOH (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) to 100 ml of each beverage that was stirred periodically until the pH of the solution was neutral. The measurements were repeated three times and the mean values were calculated.

3. Manufacture of tooth specimens

The permanent incisor of the cow with a healthy enamel surface was separated from the upper jaw and cut into 4 mm×4 mm sections. Subsequently, the surface of the bovine enamel was polished flat using a polishing machine (PB209 Minipol; R&B Inc., Daejeon, Korea) and a silicon carbide grinding paper gradually from 400 grit to 1,200 grit. The acid-resistant varnish (Nail top coat; Innisfree Corp., Seoul, Korea) was applied to the remainder of the specimen, leaving a 1 mm×2 mm window on the enamel surface (the portion to be exposed to the beverage). Five specimens were assigned to each group.

4. Immersion in the beverages

The beverage was opened immediately prior to immersion of the specimen and used for the experiment after confirming that the same pH was maintained. Five specimens per group were immersed in 100 ml of beverages of each group for 6 hours a day, and were immersed in 100 ml of artificial saliva for the remaining 18 hours. The artificial saliva consisted of gastric mucin (0.22%; Sigma-Aldrich, Saint Louis, MO, USA), KCl (14.93 mM), KH_2PO_4 (5.42 mM), NaCl (6.51 mM), and $CaCl_2 \cdot 2H_2O$ (1.45 mM). When the specimens were immersed in beverages and artificial saliva, they were stored in an incubator at 36.5°C. This process was repeated for a total of 5 days, and then, the specimens were washed with distilled water.

5. Measuring the degree of enamel erosion

1) Measuring the depth of enamel erosion

The acid-resistant varnish was removed to determine the degree of loss of enamel exposed to the beverage compared to the healthy enamel protected by the acid-resistant varnish. The window exposed to the beverage was cut vertically using a diamond disc (MultiCut; Edenta, Schaanwald, Liechtenstein). The depth of tooth loss caused by tooth erosion (erosion depth) of the window exposed to the beverage was compared with that of the healthy tooth using a microscope (CX21; Olympus Corp., Tokyo, Japan) and calibrated software (Excope X3; DIXI Science, Daejeon, Korea) on the side of the cut specimen. Three constant points were measured for each specimen.

2) Measuring the maximum ΔF value of the enamel surface

Quantified light-induced fluorescence-digital (QLF-D; Biluminator™; Inspektor Research Systems, Amsterdam, Netherlands) QLF was used to measure the maximum value of fluorescence disappearance (Max ΔF, %) of the tooth surface exposed to the beverage compared to the healthy tooth surface. The QLF-D imaging was performed with the surface of the specimen parallel to the floor and completely shielding the external light. The fluorescence images of each specimen were taken under blue light of the QLF-D camera with an International Organization for Standardization sensitivity of 1,600, a shutter speed of 1/45 s, and an aperture value of 3.2. Fluorescent images were analyzed using a dedicated software (QA2 version 1.15; Inspektor Research Systems) for Max ΔF values.

6. Statistical analysis

The homogeneity of variance was tested for erosion depth and Max ΔF of enamel surfaces. The Kruskal–Wallis test and ANOVA were used to compare the depth of enamel erosion and Max ΔF of enamel surfaces. The erosion depth and the Max ΔF value were compared between the beverage groups using the Bonferroni correction (α=0.005). Spearman correlations between the erosion depth and Max ΔF of the enamel surface were estimated for the beverages, except the negative control group

(α=0.05). All statistical analyses were performed using PASW Statistics ver. 21.0 (IBM Corp., Armonk, NY, USA).

Results

1. Chemical properties of the beverages

All drinks except MW had sugar and acidic ingredients (Table 1). The vitamin-containing beverages predominantly contained the two water soluble vitamins, i.e., the vitamin B group complex and vitamin C, as well as the amino acid taurine. Caffeine was present in all the beverages, except V5 and MW. The pH of each beverage was lowest in BD (2.65), followed by CC (2.76), OC (2.83), V5 (3.01), and MW (7.40). The titratable acidity was highest in V5 (15.60) and lowest in BD (6.80; Table 2)

2. Erosion depth caused by the beverages

The erosion depth in tooth specimens was found to be significantly different between the five beverage groups (p=0.001). Enamel disappearance was caused by all drinks, except MW. The specimens immersed in BD showed the greatest enamel loss; however, it was not significantly different from the enamel losses caused by other beverages, except MW (Table 3, Fig. 1).

3. Max ΔF of the enamel surface

The Max ΔF of the enamel surface was observed in all the beverage groups and there was a significant difference in the Max ΔF value among the beverage groups (p=0.003). CC (−0.42), V5 (−0.17), and BD (−0.17) showed higher Max ΔF than the other beverage groups ($p < 0.005$), and there was no significant difference in Max ΔF between OC and MW ($p > 0.005$, Table 3).

Table 2. The pH and Titratable Acidity of the Drinks Used in the Present Study

Group	pH	Titratable acidity[a]
Bacchus-D	2.65	6.80
OronaminC drink	2.83	11.06
Vita 500	3.01	15.60
Coca-Cola	2.76	10.02
Jeju Samdasoo	7.40	-

-: not available.
[a]The amount (ml) of 1M NaOH needed to make 100 ml of drinking water a neutral-pH solution.

Table 3. The Depths of Erosion Lesion and the Max ΔF Value after Immersing Specimens in the Beverages Used in the Present Study

Measure	Product name	n	Mean±standard deviation	p-value
Depths of erosion lesion (μm)	Bacchus-D	5	157.44[a]±18.29	0.001
	OronaminC drink	5	55.04[a]±17.26	
	Vita 500	5	67.28[a]±16.44	
	Coca-Cola	5	72.24[a]±11.46	
	Jeju Samdasoo	5	0.00[b]±0.00	
Max ΔF	Bacchus-D	5	−0.17[abc]±0.05	0.003
	OronaminC drink	5	−0.12[ac]±0.08	
	Vita 500	5	−0.17[abc]±0.09	
	Coca-Cola	5	−0.42[b]±0.09	
	Jeju Samdasoo	5	−0.04[c]±0.26	

Max ΔF (%) indicate the maximum value of fluorescence disappearance of the tooth surface exposed to the beverage comparing with the healthy tooth surface.
The differences in depth of erosion lesion and the Max ⊿F value were analyzed using ANOVA and the Kruskal-Wallis test, respectively.
[a~c]Values with the same letter superscripts are not significant in Bonferroni correction test at α=0.005.

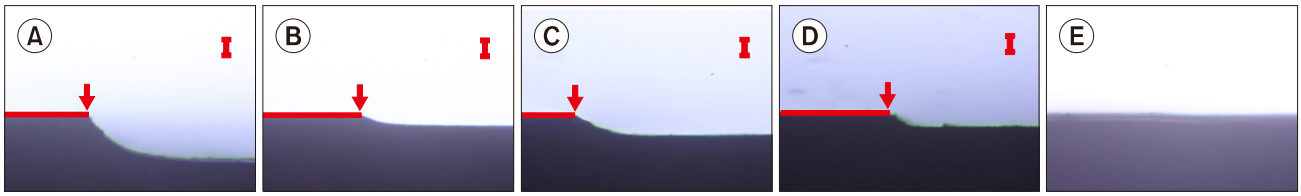

Fig. 1. Images of the sectioned enamel under a magnification of ×100 taken after 5 days of immersion in drinks. The left horizontal red lines indicate the baseline of sound enamel and the red arrows indicate the boundary between the sound enamel and treated side. Upper right vertical red bar represents 50 μm. (A) Bacchus-D, (B) OronaminC drink, (C) Vita 500, (D) Coca-Cola, (E) Jeju Samdasoo.

4. Correlation between erosion depth and Max ΔF of the enamel surface

The correlation coefficient between erosion depth and the Max ΔF value of the enamel surface was significant for all the beverages, except the negative control ($r=-0.612$, $p=0.004$).

Discussion

Studies on the prevalence of dental caries have primarily involved production workers who handle acidic substances[16-18]. In a previous study, while 37.7% of tooth erosion were occupation-related, 23.1% were diet-related; thus, dietary factors also account for a high percentage of tooth erosion[18]. Therefore, attention should be paid to ingestion of acidic drinks and food products that can cause tooth erosion. The purposes of the present study were to investigate the components of three commercially available vitamin beverages, which are sold domestically and exported abroad, and to investigate the possibility of tooth erosion induction due to these vitamin beverages.

The vitamin drinks used in the present study contained various vitamins and other substances that are not present in the beverages used as the negative and positive controls. Some of the vitamins present in the vitamin drinks used in the present study are as follows. BD, V5, and OC contain vitamin B_2 (riboflavin). Riboflavin is a water-soluble vitamin that plays an important role in enzymatic reactions, has antioxidant activity, has a neuroprotective effect, and is effective against cancer and cardiovascular diseases[19,20]. BD and OC contain vitamin B_6 (pyridoxine), and pyridoxine plays an important role in neural development and function[21]. V5 and OC contain vitamin C, and vitamin C lowers cholesterol, reduces cardiovascular mortality, and has a protective effect against lipid peroxidation[22].

Regarding ingredients other than vitamins, BD and V5 contained a common ingredient called taurine. Taurine is one of the most abundant free amino acids in mammalian tissue and is essential for the maintenance of the function of the cell membrane. Taurine is known to have the effects of preventing stroke and coronary heart disease, reducing cardiovascular risk, preventing obesity, preventing hypertension, and reducing cholesterol[23,24]. BD and OC, but not V5, contained caffeine. Adequate caffeine intake can increase the basal metabolic rate in the body, improve athletic performance, stimulate the central nervous system to reduce stress, promote gastric secretion, and promote digestion[25]. However, overdose can lead to health problems such as heartburn, excessive excretion of minerals in the body, promotion of gastric acid secretion, nausea, vomiting, hand tremor, and sleep disorder[25]. Moreover, all of the three vitamin drinks used in the present study contain significant levels of sugar, and the high calories in these vitamin drinks can increase the risk of obesity[26]. It is therefore necessary to limit its intake. As mentioned above, while vitamin drinks contain ingredients that are partly beneficial to health, they also contain ingredients that are not beneficial to your health; thus, it may be necessary to identify and consume the ingredients.

Ingredients of vitamin drinks contain acidic components such as cola, which is the positive control in the present study, and it is necessary to understand the effect of such components on the teeth. The threshold pH to determine the possibility of tooth erosion due to a beverage is based on the dissolution of the hydroxyapatite at pH 3.0; beverages having a pH value <3.0 have a high possibility of causing tooth erosion, and beverages having a pH of 4.0 or higher have less potential for causing tooth erosion[27]. The pH of all of the beverages used in the present study, barring MW, was found to be between 2.65 and 3.01, and thus, these beverages can cause tooth erosion. In the present study, when tooth specimens were immersed in beverages for a total of 30 hours (6 hours for 5 days) and immersed in artificial saliva for the rest of the time, enamel loss caused by erosion was observed in specimens immersed in all of the beverages, except MW. There was a significant difference in enamel loss between the negative control group and other groups. However, no significant difference was found in the erosion depth between the groups, except the negative control group ($p>0.005$). The average erosion depth was deepest at 157.44 ± 18.29 μm in BD, which had the lowest pH (pH 2.65). Enamel loss caused by CC, which had a slightly higher pH (pH 2.76) than BD, was found to be 72.24 ± 11.46 μm less than that caused by BD. Conversely, OC (pH 2.83), which had a

slightly lower pH than V5 (pH 3.01), caused slightly less enamel loss than V5. In a previous study, enamel loss of 78.81±7.95 μm was observed when the tooth specimens were immersed in a sports drink (Powerade®; Coca-Cola Korea Co.; pH 2.90), a kind of functional drink, for 7 hours in total for one week and immersed in saliva for the remaining time[11]. In another previous study, tooth specimens were immersed in a sports drink (Lemon-Lime Gatorade®), which has a pH of 2.84, and an energy drink (Red Bull®), which has a pH of 2.76, for 25 hours, resulting in enamel loss of 131±8 μm and 100±5 μm, respectively[28]. The results of the present study and those of previous studies indicate that the low pH of beverages may be one of the important factors affecting tooth erosion. The reason for the low pH of the vitamin drinks is that the added vitamin C is originally low in acidity, and acid is added to increase the taste and flavor, as well as to increase the shelf life. In the present study, citric acid, phosphoric acid, and malic acid were added to the beverages. Among the aforementioned acids, citric acid has been reported to have a greater effect on erosion of enamel and dentin than phosphoric acid in all pH ranges[29]. This is because the calcium chelation reaction of citric acid accelerates the erosion of the calcium and phosphorus crystals of the tooth. However, the manufacturers of the beverages used in the present study do not disclose the exact amount of acid added in these beverages; thus, it is difficult to determine the possibility of tooth erosion based on the type of acid used in the beverage.

The titratable acidity was considered to the crucial factors responsible for tooth erosion[30]. The titratable acidity refers to the value obtained by titrating the total acid amount in food with a standard alkali solution. In the present study, titratable acidity was determined by the amount of 1M NaOH required for 100 ml of a beverage to reach neutral pH. In terms of titratable acidity, V5 was highest at 15.60 ml and BD was lowest at 6.80 ml. A previous study has shown that a commercial orange juice with an initial pH of 3.96 reaches neutral pH after addition of 6.1 ml of 1M NaOH[31]. In another previous study, 13 commercially available sports drinks and 9 energy drinks showed a titratable acidity of 2.93～4.83 ml and 5.93～14.53 ml, respectively[32]. The titratable acidity of the vitamin drinks used in the present study was higher than that of orange juice and sports drinks, and was similar to that of energy drinks.

When the QLF system irradiates a blue visible light with a wavelength of around 405 nm on a tooth, the light is transmitted through the dentin-enamel junction and then reflected, resulting in natural fluorescence[33]. Tooth erosion is a lesion where teeth are shaved by acid. However, the content of calcium and phosphorus in the lower part of the erosion lesion is lower than that of the normal teeth, and the depth of this part is significantly correlated with the ΔF of QLF ($p < 0.01$)[34,35]. In addition, there was a significant correlation between erosion depth and the ΔF value ($p < 0.01$)[35]. Similar to these previous results, there was a significant correlation between erosion depth and the Max ΔF value in the present study ($r = -0.612$, $p = 0.004$). In the present study, the value of ΔF was very small; therefore, the Max ΔF value was used instead of ΔF. The CC group showed a higher Max ΔF value than the other groups. The reason was thought to be the coloring component, which blackens the color of the CC beverage, and in turn changes the ΔF value by discoloring the teeth. Some previous studies have supported this hypothesis, suggesting that beverages can cause coloration in the teeth[36,37], and that the ΔF value can vary significantly depending on the degree of tooth coloration[38,39].

The present study has limitations in that the number of specimens was insufficient to analyze the parametric statistics, and laboratory studies cannot accurately reflect the results of the research into actual oral conditions.

The vitamin drinks used in the present study had low pH and high titratable acidity. Vitamin drinks and the positive control, CC, caused tooth erosion lesions, showing significant differences from MW. There was no significant difference in Max ΔF between vitamin drinks and MW, but there was a difference in average values. In conclusion, the results of the present study indicate that vitamin drinks have a possibility to cause tooth erosion.

Notes

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Ethical approval

This study is an in vitro study and is not subject to IRB review.

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