Oral Biology

Impact of modified acidic soft drinks on enamel erosion

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OBJECTIVE: To evaluate the enamel erosive potential of modified acidic soft drinks under controlled conditions in an artificial mouth.
MATERIALS AND METHODS: From each of 144 bovine incisors one enamel sample was prepared. Labial surfaces of the samples were ground flat, polished and covered with adhesive tape, leaving an exposed area. The samples were distributed among four (A–D) groups for treatment with A: Coca-Cola, B: Sprite; C: Sprite light, D: orange juice. Either 1.0 mmol l^{-1} calcium (Ca) or a combination (comb.) of 0.5 mmol l^{-1} calcium plus 0.5 mmol l^{-1} phosphate plus 0.031 mmol l^{-1} fluoride was added to the beverages. Samples of each group were subdivided into three subgroups (-original; -Ca and -comb.) for treatment with original and modified drinks. De- and remineralization cycles were based on a standard protocol described earlier. Surface loss of the specimens was determined using profilometry after test procedure.
RESULTS: In all subgroups, loss of enamel was observed. The enamel loss recorded for the samples rinsed with original Sprite and original orange juice was significantly higher compared with all other solutions ($P = 0.001$). Lowest enamel loss was recorded for the original Coca-Cola group ($P = 0.001$). With the exception of Coca-Cola, demineralization with the modified beverages led to significantly lower losses compared with the respective original solutions.
CONCLUSION: Modification of the test soft drinks with low concentrations of calcium or a combination of calcium, phosphate and fluoride may exert a significant protective potential with respect to dental erosion.
Oral Diseases (2005) 11, 7–12

Keywords: soft drinks; tooth; enamel; erosion; fluoride; dental erosion; acidic soft drinks; demineralization; remineralization

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Introduction

Frequent contact of the dentition with exogenous acids, originating from acidic food or beverages might result in dental erosion (Zero, 1996). Evidence shows that erosion is strongly correlated with the frequency and amount of soft drink intake (Lussi and Schaffner, 2000; Moazzez *et al*, 2000; O'Sullivan and Curzon, 2000; Al Majed *et al*, 2002; Johansson *et al*, 2002; Lussi *et al*, 2002). The consumption of soft drinks shows a continuing upward trend with a distinct increase in several countries (Attin *et al*, 2003). The increasing consumption of acidic soft drinks appears to be an increasingly important factor implicated in the etiology of dental erosion (Järvinen *et al*, 1991; Lussi *et al*, 1993, 2002; Millward *et al*, 1994; Johansson *et al*, 1996). Erosion may be prevented by reducing the intake of acidic foods and drinks and by modifying drinking habits (Grenby, 1996; Moazzez *et al*, 2000). Some modifications to acidic beverages have been suggested to reduce the potential of such drinks to demineralize and dissolve the mineral compounds of teeth (Sorvari *et al*, 1988; Sorvari, 1989; Rugg-Gunn *et al*, 1998; Hughes *et al*, 1999, 2002; Larsen and Nyvad, 1999; West *et al*, 1999, 2003; Bartlett *et al*, 2003). Additions of hydrocolloids, magnesium, calcium-citrate-malate, fluoride and calcium/phosphate to soft drinks have been tested. Studies have shown that high amounts of calcium, phosphate or fluoride were able to reduce the formation of erosive lesions in enamel distinctly (Beiraghi *et al*, 1989; Sorvari, 1989; Larsen and Nyvad, 1999). However, a comparison between different beverages demonstrated that even low differences in phosphate, fluoride or calcium content of beverages are responsible for distinct differences in erosive potentials of the beverages (Lussi *et al*, 2004). In a recent study, a 1% citric acid solution was supplemented with different concentrations of calcium, phosphate and/or fluoride to reduce erosive potential of the solution (Attin *et al*, 2003). These solutions were applied onto enamel utilizing an artificial mouth which allows for performing frequently alternating episodes of de- and remineralization. In this study, most effective reduction of enamel dissolution was achieved by adding either 1.0 mmol l^{-1} calcium or a combination of

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0.5 mmol l^{-1} calcium plus 0.5 mmol l^{-1} phosphate plus 0.031 mmol l^{-1} fluoride to the citric acid. However, with the added concentrations enamel dissolution could not be completely prevented. Beside the concentration of calcium, phosphate and fluoride, the erosive potential of soft drinks depends on various other factors, such as acid type, pH, amount of titratable acid or buffer capacity (Lussi *et al*, 1993, 2004; Larsen and Nyvad, 1999). While modifications of acidic solutions might lead to reduction of the erosive potential capacity of the solutions, it is not known, whether these modifications will also reduce erosive potential of commercial beverages with more complex compositions. Therefore, optimal adjustment of an acidic beverage with respect to reducing its potential for dental erosion should be individually checked for the respective beverage.

Consequently, the aim of the present study was to evaluate *in vitro* dental erosion following additions of low levels of calcium, phosphate and fluoride to different beverages under controlled conditions in an artificial mouth.

Materials and methods

Preparation of specimens
A total of 144 freshly extracted bovine incisors were stored in 0.5% thymol solution until required. A single cylinder (5 mm in diameter) was prepared from the central labial aspect of each tooth by means of a trephine mill (Komet, Lemgo, Germany). The specimens were embedded in acrylic resin (Palavit G, Kulzer, Wehrheim, Germany) using a steel mould of 25 mm diameter. The enamel surfaces of the specimens were ground flat and polished with water-cooled carborundum discs (800, 1000 and 1200, 2400 and 4000 grit; Water Proof Silicon Carbide Paper, Struers, Erkrath, Germany). Grinding and polishing was performed with a digitally controlled automatic grinding device ensuring complete flatness of the prepared surfaces (Exact Mikroschleifsystem 'Mikro 40', Exakt Apparatebau, Norderstedt, Germany). The preparation steps resulted in removal of around 100 μm of the outermost enamel layer. Finally, acrylic resin was applied onto the bottom of the embedded specimens in order to align the polished surface parallel to the bottom. Before inclusion in the experiment, the polished surfaces were checked for exact flatness and deficiencies with a profilometer (Perthometer Concept, Mahr, Göttingen, Germany).

The centre of the polished surface was marked with a water-resistant felt pen. On one-half of the surface, microhardness determination was intended to be performed later, and the other half of the surface was for profilometric analysis. Then a part of the enamel surface was covered with tape (Tesa®, Beiersdorf, Hamburg, Germany) exposing an area of 1.4 mm × 10.0 mm in the centre of the enamel specimens to stand as reference surfaces which were later not subjected to the de- and remineralizing solutions and allows for measuring the substance loss (Hunter *et al*, 2000a,b).

The specimens were evenly distributed among four experimental groups (A–D) with three subgroups each, according to their baseline microhardness values. Microhardness of the samples was determined using a Knoop diamond (Wild Leitz, Wetzlar, Germany) at a load of 1.961 N, applied for 30 s. Five indentations were performed on each specimen and averaged. Stratified random sampling was applied, so that the average microhardness in the four groups and the 12 subgroups was nearly equal.

De- and remineralization procedure
All samples were submitted to three alternating episodes of de- and remineralization in a so-called artificial mouth which was described in detail previously (Attin *et al*, 2003). Briefly, the artificial mouth allowed for alternating rinsing of tooth specimens with different solutions. The specimens of the four groups (A–D, $n = 36$) were demineralized with different beverages (Table 1): A: Coca-Cola®, B: Sprite®, C: Sprite® light, D: orange juice. Either 1.0 mmol l^{-1} calcium (Ca) or a combination (comb.) of 0.5 mmol l^{-1} calcium plus 0.5 mmol l^{-1} phosphate plus 0.031 mmol l^{-1} fluoride (0.6 ppm F) was added to the beverages. Calcium was added as CaCl$_2$-dihydrate, phosphate as KH$_2$PO$_4$ and fluoride as NaF. Samples of each group were subdivided into three subgroups (-original solution, -Ca, and -comb.) of twelve specimens each.

Remineralization was accomplished by rinsing with artificial saliva with a composition described in detail previously (Attin *et al*, 2000). For demineralization the respective solution was run for 1 min through the chamber, immediately followed by a remineralization period (1 min). The specimens were cycled through this alternating procedure five times within 10 min with a flow rate of the solutions at 3.25 ml min^{-1}. After cycling through this de- and remineralization procedure, the specimens were rinsed for 8 h with artificial saliva at a flow rate of 1.1 ml min^{-1}. The 8-h rest period was chosen in order to simulate remineralization overnight. Altogether, the de- and remineralization cycle (10 min) was repeated three times for each specimen interrupted by 8 h-remineralization periods.

Table 1 Compositions of the beverages according to the manufacturers

Coca-Cola (Coca-Cola Co., Essen, Germany)	Water, sugar, carbonic acid, colour E150d, phosphoric acid, flavour, coffein
Sprite® (Coca-Cola Co.)	Water, sugar, carbonic acid, citric acid, sodium citrate
Sprite® light (Coca-Cola Co.)	Water, carbonic acid, citric acid, sweeteners (Na-cyclamat, aspartam, Na-saccharin), flavour, sodium citrate
Orange juice (Summerhill, Penny-Markt, Cologne, Germany)	100% pure orange juice

Analysis of enamel loss

After removal of the tape, surface enamel loss was quantitatively determined at the end of the experiment with a stylus profilometer (Perthometer Concept). For profilometric measurements the stylus moved across the enamel surfaces, which had been protected by the tape during the experiment and the central part of the enamel, which had been exposed to the de- and remineralization regime. The assessment parameters of the profilometer were as follows: 0.5 mm s^{-1} speed, 0.23 μm vertical sensitivity and 0.69 μm distance between recorded points. A special jig allowed for repositioning of the specimens in the profilometer according to the tongue and groove principle. Five line scans were performed in the centre of each specimen at intervals of about 100 μm. The area under curve in the eroded area was recorded with the non-eroded parts serving as reference. Then, the average depth of the eroded part of the specimen was calculated with a specially designed software. The results of five scans were averaged for each sample.

Characterization of the beverages

In the original solution and the modifications, the concentrations of ionized calcium, phosphate and fluoride were determined. pH-value of the solutions was evaluated with a pH-meter at room temperature (pH/ion meter pmX 3000 WTW, Weilheim, Germany). Neutralizable acidity was determined by assessing the amount of 1 N NaOH (mval/l) needed to raise the pH of 10 ml of the solutions to 7.0 (Table 2).

For determination of the phosphate concentration the beverages were prepared according to the procedure developed by Fathi et al (2002). Phosphate concentrations were then colorimetrically assessed in a microplate reader at 650 nm (Molecular Devices, Ismaning/Munich, Germany). Calcium concentration was measured with a flame photometer (Eppendorf FCM 6341, Eppendorf, Hamburg, Germany). The concentration of fluoride was evaluated with a fluoride sensitive electrode (Merck Eurolab, Hanover, Germany) in 2 ml of the solutions after adjusting the pH to 5.4 by buffering with 1 N NaOH and addition of 1 ml TISAB III (Merck Eurolab) according to previous descriptions (Hellwig et al, 1985; Attin et al, 1995).

Additional experiment

Twenty-one volunteers (11 female, 10 male) aged between 25 and 45 years, who were all non-smokers, were asked for estimation of the taste of the four beverages and their modifications in a single-blinded test. The three blinded solutions (-original, -Ca, - comb.) of each of the four beverage (group) were offered to the subjects together with the original, non-blinded drink. The subjects were asked to check whether the solutions tasted differently as compared with the respective original drink. This procedure was performed on four different days. Per day, only one of the beverages and their respective modifications were tested at about 10 a.m. The volunteers were instructed to refrain from eating or drinking for 1 h prior to the test.

Statistical analysis

The software package STATISTICA 6.0 (Statsoft, Tulsa, OK, USA) was used for analysis. The data of the enamel loss were statistically analysed with the U-Test according to Mann and Whitney, since the data were not normally distributed. The data of the taste evaluation were analysed using chi-square tests. Significance in all tests was set at $P < 0.05$.

Results

The mean enamel loss of the specimens, as assessed using the profilometric analysis, is presented in Table 3. The enamel loss recorded for the samples rinsed with original Sprite and original orange juice was significantly higher as compared with all other solutions ($P = 0.001$). Lowest loss, as compared with the other original solutions, was recorded for the original Coca-Cola group ($P = 0.001$). In the Coca-Cola group, rinsing with the two modifications did not result in significantly less loss as compared with the original beverage. In the remaining groups supplementation of the drinks with calcium and with the combination of calcium, phosphate and fluoride led to significantly less enamel loss as compared with the respective original solution ($P = 0.001$). For orange juice, this effect was significantly more pronounced for the combination group than for the calcium group ($P = 0.014$). This was not true for Sprite light and Sprite, where enamel loss was not significantly different between the calcium and the combination group ($P = 0.467$ and $P = 0.266$, respectively).

In Table 4 the percentage distribution of the volunteers is given who perceived a difference in taste between the blinded solutions (-original, -Ca, -comb.) and the respective non-blinded original drink. The statistical analyses showed that the percentage of volunteers already tasting differences between the non-blinded

Table 2 pH, content of calcium, phosphate and fluoride, and amount of 1 N NaOH needed to raise the pH to 7.0 in the different original beverages

	Calcium (mmol/l)	Phosphate (mmol/l)	Fluoride (mmol/l)	pH	NaOH (mval/l)
Coca-Cola®	0.94	5.24	0.011	2.53	48
Sprite®	1.25	0.00	0.013	2.69	70
Sprite® light	0.89	0.00	0.027	2.81	100
Orange juice	2.67	2.85	0.023	3.70	100

Table 3 Mean enamel loss (μm) and standard error of mean ($\pm$s.e.m.) in the specimens rinsed with different original drinks or drinks supplemented with calcium (-Ca) or a combination of calcium, phosphate and fluoride (-comb.)

	Original	Ca	Comb.
Coca-Cola®	0.19 ± 0.03	0.31 ± 0.04	0.27 ± 0.03
Sprite®	1.59 ± 0.23	0.94 ± 0.16	0.80 ± 0.08
Sprite® light	0.72 ± 0.18	0.31 ± 0.06	0.26 ± 0.04
Orange juice	1.60 ± 0.22	0.99 ± 0.14	0.77 ± 0.09

Table 4 Percentage of volunteers ($n = 21$) perceiving a difference in taste between the non-blinded beverage and the respective blinded original and modified (-Ca, -comb.) solution

	Original	-Ca	-Comb.
Coca-Cola[®]	71	80	80
Sprite[®]	66	90	76
Sprite[®] light	71	95	95
Orange juice	52	61	76

original drinks and the respective blinded original solutions were lower, but not significantly different from the percentages of volunteers tasting a difference between the modified solutions and the original ($P > 0.0601$). Also, no significant difference in taste was reported for the drinks modified with calcium or the combination ($P > 0.2142$). This means that the volunteers were not able to differentiate clearly between the original drinks and the respective blinded original and modified solutions.

Discussion

In the present study the effect of commonly available beverages with additions of either calcium or calcium, phosphate and fluoride on enamel surfaces was tested. The beverages tested contained a basic concentration of these minerals and any additional amounts of calcium, phosphate and fluoride were in a range reflecting the average basic concentration of these minerals in drinks and foodstuffs (Lussi et al, 1993).

The beverages tested were different with respect to composition, such as type of acid, pH and amount of titratable acid. This may explain the different erosive potentials of the solutions. The least erosive loss of enamel was observed for Coca-Cola, which was the only beverage containing phosphoric acid, while the other beverages contained citric acid. In former studies, it was already shown that citric acid is an ingredient of acidic beverages, which demonstrate severe demineralizing potential (Lussi et al, 1995; Attin et al, 1997). The erosive potential of citric acid is pronounced because of the fact that citric acid acts as a chelator able to bind minerals of the apatite, such as calcium. However, as the pK_a-values of citric acid are 3.1, 4.74 and 6.42 chelating is not relevant at low pH, especially not at pH of 2.5–3.7, which were the pH range of the drinks tested in the present study.

In the present study, a cycling model simulating physiological conditions during sipping of a beverage by subjecting enamel samples to alternating de- and remineralization periods was utilized. This model and also the use of bovine samples and artificial saliva have been discussed in detail previously (Attin et al, 2003). However, it is noteworthy to mention that the chosen in-vitro-model did not completely reflect the intra-oral situation, where other factors have an impact on the initiation and progression of dental erosive lesion. For example, presence of the acquired salivary pellicle on tooth surfaces attenuates the erosive destruction induced by acidic solutions (Hannig and Balz, 2001; Hannig et al, 2003). On the other hand, abrasive influence exerted on erosively altered dental hard tissue, such as toothbrushing or tongue movement may add to the loss of the hard tissue (Attin et al, 2001, 2004). It should therefore by noted that the findings observed in the present study may not reflect the situation in vivo.

The profilometric analysis for determining erosively induced dental hard tissue loss is a well-established and suitable method for evaluation of the erosive potential of various acidic substances (Bartlett et al, 1997; Ganss et al, 2000; Hughes et al, 2002). The enamel loss in the different groups showed some variations as expressed by the high values of the standard error of means in Table 3. These variations among the specimens of the respective groups might have occurred because of the reason that susceptibility of enamel specimens to an erosive challenge is different. In previous studies determining enamel loss or hardness changes of erosively altered enamel, high variations among the samples of the same treatment group were also recorded (Ganss et al, 2000; Lussi et al, 2000).

The additions to the beverages had proved to be effective in reducing the erosive potential of 1% citric acid in a recent study (Attin et al, 2003). The results of this study revealed that addition of calcium or calcium/phosphate/fluoride was effective in reducing the erosive potential of the different tested beverages with the exception of Coca-Cola which, however, revealed the lowest erosive potential in its original version compared with the other soft drinks. For the other beverages, it was shown that the modified beverages decreased the loss of enamel. In order to prevent dental hard tissue damage because of acidic drinks completely it would be optimal to supplement acidic beverages with high amounts of calcium and phosphate so that the drink is saturated with respect to apatite (Larsen and Nyvad, 1999). It was shown that orange juice supplemented with calcium (42.9 mmol l^{-1}) and phosphate (31.2 mmol l^{-1}) did not erode enamel after immersion for 7 days (Larsen and Nyvad, 1999). The present results show that even lower concentrations of calcium, phosphate together with fluoride were able to reduce enamel dissolution and demineralization. Lussi et al (1995, 2004) had also proved that minimal differences in mineral contents (calcium, phosphate, fluoride) of beverages might have an impact on the erosive potential of soft drinks. With respect to the amount of fluoride added to the drinks it has to be noted that fluoride is able to reduce enamel dissolution when added to a demineralizing solution of pH 4.3 (Margolis et al, 1986). However, the enamel erosions were noted with beverages with lower pH-values. In demineralizing solutions with very low pH the addition of fluoride, such as administered in the present study, does not seem to be able to completely prevent enamel demineralization (Ten Cate and Duijsters, 1983; Larsen, 2001).

Moreover, it may be speculated that by rinsing enamel with fluoridated acidic solutions, a CaF$_2$-like layer is formed on the enamel surface, which to exposure to artificial saliva may lead to further remineralization

and protection against further erosion (Rølla and Saxegaard, 1990).

Beside the concentration of calcium, phosphate and fluoride, the erosive potential of soft drinks depend on various other factors, such as acid type, pH, amount of titratable acid and buffering capacity (Lussi *et al*, 1993; Larsen and Nyvad, 1999). Therefore, erosive potential solutions must be individually checked for the respective beverage.

In the taste determination of the beverages tested in the study, up to 71% of the volunteers already perceived differences in taste between the blinded original solution and the non-blinded original drink. Although a slightly higher percentage reported differences in taste of the modified solutions (especially for Sprite light), no statistically significant difference was seen for the modified drinks as compared with the original solutions. Since the ability of the volunteers to identify the blinded original solution was not different from the ability to taste differences between the modified drinks and the original, it is concluded that the modifications only exert minimal effect on the taste of the test beverages.

Hence, it is concluded that modification of the test beverages with low concentrations of calcium, phosphate and fluoride is able to reduce the erosive potential of the drinks. However, with these low concentrations enamel dissolution could not be completely prevented.

Acknowledgement

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