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Original Article

Effect of rinse with calcium enriched milk on plaque fluid

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Previous research has shown that rinsing the mouth with milk significantly diminished the pH in dental plaque fluid; however, the degree of saturation with respect to the dental enamel (DS) was not significantly decreased because of an increase in the calcium ion concentration in plaque fluid. The aim of this study was to investigate the cariostatic effect of adding calcium to milk on the DS value of the plaque fluid after rinsing. Plaque samples were collected from 8 Japanese male dental students. Prior to plaque collection, all subjects refrained from practicing oral hygiene for 48 hr and fasted overnight. Supragingival plaque samples were collected from one side of the mouth of each subject, and then collected from the other side, following a 30-second rinse with 15 mL of calcium-enriched milk, which was prepared by adding calcium carbonate to ordinary milk, and a 10-minute waiting period. The samples were cleared by centrifugation, and the plaque fluid was analyzed for inorganic ions and pH, using an ion chromatograph and pH microelectrode, respectively. The calcium ion concentration of the milk was 6.4 mM, which was about 36% higher than that of ordinary milk. The pH decreased significantly ($p<5\%$) from 6.4 to 6.1 following the rinse with calcium enriched milk, as tested by the paired t-test. The decrease in pH might have caused a reduction of the DS value; however, it was compensated for by a significant ($p<0.5\%$) increase in the calcium ion concentration of plaque fluid.

Key words: plaque fluid, milk, calcium, rinse

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1. Introduction

Milk is a very common and popular drink that can be drunk with meals or snacks without needing to be concerned much about dental caries, unlike the case of beverages sweetened with sugar. Positive effects of milk on cariogenesis have been reported by many investigators. McDougall[1] showed that exposure of the surface of artificially demineralized dental enamel lesions to milk for 50 hours resulted in remineralization of the demineralized enamel. Mor and McDougall[2] indicated that milk rinse was least acidogenic among rinses containing 5% sucrose and 5% lactose. Bowen and Pearson[3] suggested that milk may have modest cariostatic properties when ingested with a cariogenic challenge. This cariostatic effect of milk is partly attributable to its high calcium content. To find a method of improving the cariostatic nature of dental plaque, we investigated the effect of milk on the inorganic composition of dental plaque fluid.

Our previous study showed that milk rinsing significantly diminished the pH in plaque fluid; however, the DS did not show a significant decrease[4]. This finding could be explained by the increased concentration of calcium ion in plaque fluid. We surmised that a further increase of calcium ion concentration in the plaque fluid might lead to an increased DS value. The present study was carried out to know whether the DS value can be

increased through an enhanced calcium concentration resulting from rinsing with calcium-added milk.

Materials and methods

Dental plaque samples were collected from 8 Japanese male dental students. Prior to plaque collection, all subjects refrained from practicing oral hygiene for 48 hr and fasted overnight. Supragingival plaque samples were collected from one side of the mouth of each subject with a dental excavator paying attention not to carve the tooth enamel surface. Plaque samples were collected in the same fashion from the other side of the same mouth, following a 30-second rinse with 15 ml of calcium-enriched milk and a 10-minute waiting period. The calcium-enriched milk was prepared by stirring 200 ml of ordinary milk (Meiji 3.5 milk, Meiji Milk Co, Chiba、Japan) with an excessive amount of calcium carbonate powder (0.5 g, food additive grade, Wako Chemical ltd. Tokyo, Japan) for 10 minutes. After leaving the stirred milk undisturbed for at least 30 minutes, 15 ml of the milk upper layer was used for the study.

The collected plaque was stored in an ultrafiltration tube (pore size: 0.45 μm, Milipore, USA) and kept at ice temperature until it was centrifuged at 5000 g for 30 min at 4℃. The isolated dental plaque fluid was kept in mineral oil to prevent evaporation until it was used for analysis. Then 1 μl of the plaque fluid was used for pH measurement under 100% humidity with a microelectrode (Microelectrodes, Inc., Londonderry, New Hampshire, USA). Another 1 μl was diluted 101 times and analyzed for inorganic ions by ion chromatography (Dionex Corp., Sunnyvale, CA, USA). Inorganic cations were analyzed on an IonPac CS12 column (Dionex) and a CSRS-I cation micro-membrane suppressor (Dionex) with the eluent of 20 mM methane sulfonic acid. An IonPac AS4A-SC column (Dionex) and an ASRS-I anion micro-membrane suppressor (Dionex) were used to analyze inorganic anions. The eluent was a mixed solution of 1.8 mM sodium carbonate and 1.7 mM sodium hydrogencarbonate.

The DS value was calculated according to the definition of Moreno and Margolis[5] with the following equation,

$$DS = \left[\frac{(Ca^{2+})^5 (OH^-)(PO_4^{3-})^3}{K_{EN}} \right]^{1/9},$$

where parentheses indicate activities of the enclosed ions, and K_{EN} is the solubility product constant for enamel, which is reported to be 5.5×10^{-55} (Moreno and Margolis[5]). The statistical significance was tested using the paired t-test.

Results

Table 1 shows the comparison of inorganic ion concentrations in ordinary milk and the calcium-enriched milk. The calcium-enriched milk was prepared by saturating ordinary milk with an excessive amount of calcium carbonate. Its calcium ion concentration was 6.4 mM, which was about 36% higher than that of original milk. The phosphate ion concentration also increased by 20%, the potassium ion concentration increased by 19%, but the ammonium ion could not be detected in the calcium-enriched milk.

All of our data on pH and inorganic ion concentration in the dental plaque fluid both before and after the milk rinse are summarized in Table 2. The mean pH value decreased significantly from 6.4 to 6.1 following the calcium-enriched milk rinse. In contrast, calcium ion and phosphate ion concentrations were increased significantly by the rinse, from 1.5 to 2.8 mM and 7.7 to 9.4 mM respectively. No significant changes were observed in the other inorganic ion concentrations. In spite of the significant decrease in pH of the plaque fluid, the DS did not decrease significantly.

Discussion

Additives to milk should not adversely affect the taste of the milk. We chose the taste of calcium carbonate because of its tasteless nature compared with other calcium compounds such as calcium chloride and calcium phosphate. As a result, the calcium ion concentration in milk could not be increased very much. Unsavory milk, however, cannot be used even though it were effective, because it would not be accepted by children and thus could not improve their oral environment.

The mean pH value decreased significantly from 6.4 to 6.1 following the calcium-enriched milk rinse. This result is consistent with the research findings of Rugg-Gunn et al.[6], Mor and McDougall[2], and our previous study[4], and can be attributed to the fermentation of lactose in the milk. Although the decrease of pH should result in a decreased DS value, that decrease

Table 1. Comparison of each ion concentration in ordinary milk and calcium-enriched milk

	Ordinary milk	Calcium-enriched milk
pH	6.8	6.8
Ca (mM)	4.7	6.4
P (mM)	7.5	9.0
Mg (mM)	2.0	2.1
NH_4 (mM)	1.9	ND
Na (mM)	15.9	16.8
K (mM)	29.5	35.1
Cl (mM)	25.9	28.6
DS(EN)	7.6	9.2

DS(EN): Degree of saturation with respect to enamel
Each milk was ultrafiltrated before analysis.
ND: Not detected

Table 2. Effect of calcium-enriched milk rinse on the inorganic ion concentration in the plaque fluid

	Before rinse	After rinse	Significance
pH	6.4 ± 0.4	6.1 ± 0.4	$p<0.5\%$
Ca (mM)	1.5 ± 0.7	2.8 ± 0.9	$p<0.5\%$
P (mM)	7.7 ± 2.7	9.4 ± 2.5	$p<5\%$
Mg (mM)	1.4 ± 0.4	1.6 ± 0.2	NS
NH_4 (mM)	19.0 ± 3.4	17.5 ± 2.1	NS
Na (mM)	14.6 ± 5.5	13.9 ± 4.3	NS
K (mM)	41.0 ± 8.3	45.0 ± 6.7	NS
Cl (mM)	19.3 ± 4.8	18.2 ± 3.0	NS
DS(EN)	2.2 ± 0.8	2.0 ± 1.0	NS

DS(EN): Degree of saturation with respect to enamel
NS: Not significant

was not actually significant. This can be explained by the increase of calcium ion and phosphate ion concentrations in the dental plaque fluid. The increased calcium and phosphate ion concentrations worked favorably to keep the saturation level of the plaque fluid fairly constant. Nevertheless, the results of this study indicate that a 36% increase of calcium ion concentration in milk could not be effective enough to increase the DS value of the plaque fluid after the rinse with milk.

The effect of the added calcium in the milk on the increase of calcium ion concentration in the plaque fluid is compared with the data from our previous study[4] and shown in Table 3. The increase of calcium ion concentration following the rinse with ordinary milk was 0.8 ± 0.5 mM; with the calcium-added milk it was 1.3 ± 0.7 mM. Although the increase was larger when calcium enriched milk was used, the difference was not statistically significant. Neither was there any significant difference in the changes of pH and DS between these two kinds of milk rinse. Conversely, the phosphate concentration was increased significantly by the calcium enriched milk rinse, which reflects the increase of phosphate concentration in the calcium-enriched milk itself. This increase, however, could not be rationally explained.

The calcium ion concentration in the plaque fluid was increased by the milk rinse in these two studies. Two reasons can be pointed out for the increase of calcium. One is the decrease of pH and the other is the diffusion of calcium ions from the milk to plaque. Edgar et al.[7] reported that an increase in plaque fluid calcium concentration of monkeys after a sucrose rinse was accompanied by a simultaneous fall in pH. Rankine et al.[8] also reported an inverse relationship between pH and calcium concentration in human plaque. These results are supported by the data on plaque fluid by Margolis and Moreno[9] and Margolis et al[10]. This increase of calcium following acid production was attributed to the release of calcium from the surface of microorganisms in the plaque as shown by Rose et al.[11]

Table 3. Comparison of the effect of rinse on pH, calcium, phosphate, and DS between ordinary milk and calcium-enriched milk

	ΔpH	ΔCalcium(mM)	ΔPhosphate(mM)	ΔDS
Ordinary milk	-0.3 ± 0.3	0.8 ± 0.5	0.1 ± 0.9	-0.2 ± 0.8
Calcium-enriched milk	-0.4 ± 0.4	1.3 ± 0.7	1.7 ± 1.4	-0.2 ± 1.1

Δ: Changes in the value after the rinse
DS : Degree of saturation with respect to enamel

The increase of calcium concentration in plaque by diffusion from outside of the plaque has been reported by many investigators. Pearce et al.[12] and Pearce[13] reported whole calcium in plaque was increased significantly by the use of a mouth rinse containing calcium, phosphate, fluoride, urea, and monofluorophosphate. Van der Hoeven et al.[14] also showed a significant calcium increase in plaque following a rinse with a calcium lactate solution. Jenkins and Hargreaves[15] showed a highly significant rise in plaque calcium levels after subjects consumed cheese that is rich in calcium content. In contrast, the reports on calcium ion concentration in plaque are not very decisive. Schamshula et al.[16] showed a small but significant increase in water extractable calcium from plaque one minute after a rinse with a supersaturated calcium phosphate solution containing urea and monofluorophosphate. Rankine et al.[17] found no significant increase in the calcium ion in plaque fluid after subjects ate chewable candy supplemented with dicalcium phosphate dihydrate or calcium lactate.

It is ionized calcium that influences the saturation level of the plaque fluid. In the experiment we have conducted, we found that a much higher increase of ionized calcium was necessary in the plaque fluid in order to increase the DS value in the plaque fluid. However, we expect that can be achieved by further increasing the calcium ion concentration of the milk through changing the kind of calcium compound added to one that has a higher solubility and ionizing constant, with some modification of taste by food additives, as necessary. On the other hand, there are some kinds of mineral water with high calcium concentrations. They might provide a viable alternative to milk. Further study on this point is now under way.

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