Scientific Article

Remineralization of caries-like lesions of enamel with acidulated calcifying fluids: a polarized light microscopic study

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Abstract

The purpose of this in vitro study was to evaluate the effects of acidulated calcifying fluids (CF) on remineralization of caries-like lesions of enamel. Lesions were created in sound enamel using an acidified gel. Central longitudinal sections were taken from each tooth (N = 20) following lesion formation to serve as control lesions (CL). Each tooth was sectioned into quarters and each quarter was assigned to one of four groups: 1) 1 mM calcium CF at pH 7.0 (CF1); 2) 1 mM calcium CF at pH 5.0 (ACF1); 3) 3 mM calcium CF at pH 7.0 (CF3); 4) 3 mM calcium CF at pH 5.0 (ACF3). Tooth quarters were treated with assigned CFs (HAP [Ca/P = 1.63], pH 7.0 or 5.0, 0.05 mM Fl) for ten 60-sec periods. Longitudinal sections were prepared and imbibed in water for polarized light study. Mean lesion depths were determined and compared (ANOVA and DMR). With 1 mM calcium CFs, mean lesion depths were 187 μm for CL, 154 μm for CF1 and 133 μm for ACF1. With 3 mM calcium CFs, mean lesion depths were 192 μm for CL, 172 for CF3 and 149 for ACF3. Acidulated (22%, 29%) and nonacidulated (10%, 18%) CFs resulted in significant reductions ($P < 0.05$) in lesion depths when compared with control lesions. Acidulated CFs (13%, 14%) resulted in significant lesion depth reductions ($P < 0.05$) when compared with nonacidulated CFs. The acidulated 1 mM calcium CF produced the greatest degree of remineralization. Acidulation of CFs enhanced the degree of remineralization over that attained by nonacidulated CFs. This improvement in remineralizing ability of acidulated calcifying fluids may be due to creation of a more reactive enamel surface. (Pediatr Dent 18:205–9, 1996)

Dental caries within the pediatric and adolescent population continues to be a disease of considerable clinical importance.[1–3] Although a recent NIDR caries prevalence survey[1] indicated that slightly less than 50% of children and adolescents were caries-free, these results are somewhat misleading.[2] In fact, 45% of 10-year-old children and 72% of 14-year-old adolescents have experienced caries.[1,2] By age 17 years, dental caries has occurred in almost 85% of these late adolescents.[1,2] The caries prevalence data from the NIDR survey[1] represent only clinically detectable caries and do not include caries that would be diagnosed by radiographic examination. Furthermore, it is well known that enamel caries may exist for a considerable length of time prior to either radiographic or clinical detection.[4] The prevalence of such undetectable white spot lesions within the pediatric, adolescent or adult populations is not known. This information reinforces the need to develop innovative techniques and alter existing regimens for prevention and remineralization of both clinically detectable enamel lesions with intact surfaces and clinically undetectable white spot lesions.

A variety of calcifying fluids, synthetic salivas, and oral fluids have been developed in the past to remineralize enamel caries.[5–17] Unfortunately, remineralization of naturally occurring white spot lesions and caries-like lesions of enamel requires numerous exposures to the treatment fluid for considerable time periods. Recently, acid-etching of caries-like lesions of enamel prior to treatment with calcifying fluids has been shown to enhance remineralization, using a regimen that would appear to be clinically applicable.[7] With this encouraging information, it may be possible to facilitate remineralization by acidulating calcifying fluids, thereby eliminating the need for the acid-etch step prior to calcifying fluid treatment.

The purpose of this in vitro study was to evaluate the effects of acidulation of synthetic calcifying fluids containing 1 mM and 3 mM calcium on remineralization of caries-like lesions of enamel using polarized light microscopic techniques.

Methods and materials

Twenty macroscopically caries-free human molar teeth were chosen for this laboratory study. Following a fluoride-free prophylaxis, the specimens were coated

with an acid-resistant varnish except for two windows of sound enamel on both buccal and lingual surfaces. The specimens were then exposed to a dialyzed-reconstituted acidified gel[18] (1.0 mM calcium, 0.6 mM phosphate, 0.05 mM fluoride at pH 4.75 ± 0.02) to create caries-like lesions in the exposed enamel windows. After 10 weeks, central longitudinal sections were prepared from each specimen to serve as control lesions prior to experimental treatment. After obtaining central longitudinal sections, the teeth were sectioned into quarters and specific quarters from each tooth were assigned to a treatment group:

1. 1 mM calcium calcifying fluid (CF) at pH 7.0 (mesiolingual)
2. 1 mM calcium CF at pH 5.0 (mesiobuccal)
3. 3 mM calcium CF at pH 7.0 (distolingual)
4. 3 mM calcium CF at pH 5.0 (distobuccal).

After tooth quarter preparation, a fluoride-free toothbrush prophylaxis was performed to remove residual acidified gel from the specimens. Acid-resistant varnish was applied to the cut faces of the specimens and adjacent to the enamel windows, leaving two windows with caries-like enamel lesions per tooth quarter exposed. The 1 mM and 3 mM calcium calcifying fluids at pH 7.00 ± 0.02 and pH 5.00 ± 0.02 were prepared from hydroxyapatite (Ca:P ratio = 1.63), with addition of sodium chloride to adjust ionic strength and 0.05 mM (1 ppm) fluoride.[12, 13, 17] The specimens underwent 10 separate 60-sec exposures to the assigned calcifying fluid (2 ml/specimen). After each exposure period, the specimens were rinsed with deionized distilled water for 30 min. After the final treatment and following the water rinse, longitudinal sections from each tooth quarter were taken, imbibed in water, and examined using polarized light microscopic techniques. Photomicrographs of the lesions were projected onto a digitized tablet and five measurements were made along the advancing front of the body of the lesion to determine mean body of the lesion depth. In addition, mean surface zone depth was obtained by taking five measurements along the inner aspect of the surface zone. Data from 40 paired lesions from each of the experimental and control groups were available for statistical analysis. Comparisons were made among the mean depths for each group using ANOVA and Duncan's multiple range test for a paired design with an alpha level of P 0.05.

TABLE. REMINERALIZATION OF CARIES-LIKE LESIONS OF ENAMEL: EFFECT OF ACIDULATED CALCIFYING FLUIDS

	Surface Zone Depth (Mean ± SD)	*Body of Lesion Depth (Mean ± SD)*	*Reduction in Lesion Depth*
1 mM calcium calcifying fluids			
Control lesions	22 ± 5 µm	187 ± 19 µm^{g,h}	18%
Calcifying fluid at pH 7.0	19 ± 4 µm^{a,e}	154 ± 15 µm^{g,i,m}	29% / 14%
Calcifying fluid at pH 5.0	26 ± 4 µm^{a,f}	133 ± 12 µm^{h,i,n}	10% / 11%
3 mM calcium calcifying fluids			
Control lesions	18 ± 6 µm^{b,c}	192 ± 17 µm^{j,k}	10%
Calcifying fluid at pH 7.0	29 ± 5 µm^{b,d,e}	172 ± 13 µm^{j,l,m}	22% / 13%
Calcifying fluid at pH 5.0	37 ± 7 µm^{c,d,f}	149 ± 15 µm^{k,l,n}	

ANOVA & DMR-paired sample results: means with same letters significantly different ($P < 0.05$).

Results

With 1 mM calcifying fluids, lesion depths for both nonacidulated (pH 7.0) and acidulated (pH 5.0) CFs (Table, Fig 1) were significantly reduced ($P < 0.05$) when compared with that of the paired control lesions. The body of the lesion depth was reduced by 18% with the nonacidulated CF and by 29% with the acidulated CF. Acidulation of the 1 mM calcium CF resulted in a further 14% reduction in body of the lesion depth when compared with paired lesions treated with the nonacidulated CF ($P < 0.05$). The mean surface zone depth with the acidulated CF was increased by 18% ($P > 0.05$) and 37% ($P < 0.05$) when compared with paired control lesions and nonacidulated CF- treated lesions, respectively.

With 3 mM calcifying fluids, acidulation resulted in a 22% reduction ($P < 0.05$) in mean body of the lesion depth when compared with that for paired control lesions (Table, Fig 2). In contrast, remineralization of the lesions with the nonacidulated CF produced a 10% decrease in lesion depth when compared with control lesions ($P < 0.05$). The body of the lesion depth was reduced by 13% with acidulation of the 3 mM calcium CF ($P < 0.05$) when compared with the nonacidulated CF. Surface zones from lesions treated with either nonacidulated (61%) or acidulated (105%) CFs had significant ($P < 0.05$) depth increases when compared with paired control lesions. Acidulation of the 3 mM calcium CF resulted in a further 28% increase in surface zone depth when compared with nonacidulated CF ($P < 0.05$).

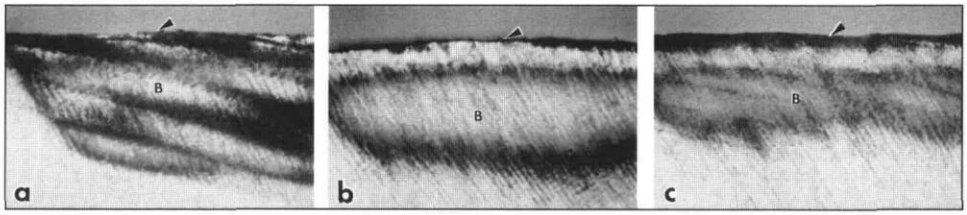

Fig 1: Remineralization of caries-like lesions of enamel with 1 mm calcium calcifying fluid (arrow = surface zone; B = body of lesion; water imbibition, polarized light microscopy; original magnification 250x).
a. Representative paired lesion from control group.
b. Representative paired lesion remineralized with nonacidulated (pH 7.0) calcifying fluid.
c. Representative paired lesion remineralized with acidulated (pH 5.0) calcifying fluid.

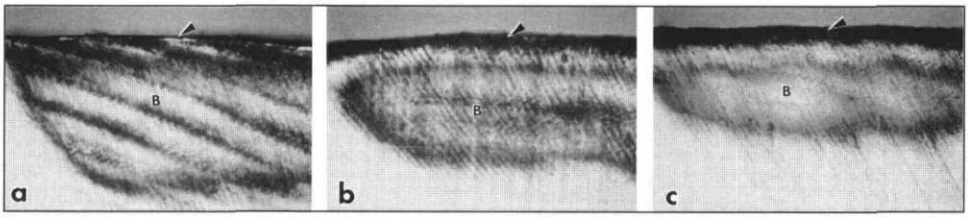

Fig 2: Remineralization of caries-like lesions of enamel with 3 mm calcium calcifying fluid (arrow = surface zone; B = body of lesion; water imbibition, polarized light microscopy; original magnification 250x).
a. Representative paired lesion from control group.
b. Representative paired lesion remineralized with nonacidulated (pH 7.0) calcifying fluid.
c. Representative paired lesion remineralized with acidulated (pH 5.0) calcifying fluid.

When comparisons were made between the 1 mM calcium CFs and the 3 mM calcium CFs, certain differences were noted (Table 1). The nonacidulated and acidulated 1 mM calcium CFs possessed significant reductions (10 and 11%, $P < 0.05$) in body of the lesion depths when compared with their corresponding 3 mM calcium CFs. For all calcifying fluids, the greatest reduction in body of the lesion depth was in with those lesions treated with the acidulated 1 mM calcium CF. In contrast, surface zone depth was increased significantly (42 and 53%, $P < 0.05$) with the 3 mM calcium CFs. In addition, the greatest increase in surface zone depth was seen with the acidulated 3 mM calcium CF.

Discussion

Dental scientists initially explored the effects of dilute acid on prevention and restoration of dental caries during the mid 1950s and early 1960s.[19–22] Currently, the acid-etch technique is a well-established clinical procedure used to retain preventive and restorative resin materials; while acidulated phosphate fluoride gels and rinses are the preferred professionally applied topical fluoride agent for caries prevention in most dental practices. Both the acid-etch technique and acidulation of sodium fluoride with phosphoric acid depend upon creating a certain degree of enamel dissolution to produce the desired effects.[19–28] The action of the acidic environment of etching solutions and acidulated phosphate fluoride results in removal of superficial organic pellicle and debris, and exposure of a more reactive, somewhat porous enamel surface.[19–24] It is well known that the acid-etch technique allows for creation of characteristic etching patterns and microporosities that extend into the enamel to depths of 50–100 μm; thereby allowing resin material to penetrate into the etched enamel.[23–25]

In contrast, the acidulation of topical fluoride agents provides a means for enhanced and rapid substitution of hydroxyl groups for fluorine ions.[20–22, 26–28] The incorporation of fluoride into hydroxyapatite produces a fluoridated hydroxyapatite, which has a significantly increased solubility coefficient and is able to resist demineralization by organic acids produced by dental plaque to a greater extent than hydroxyapatite.

The current in vitro investigation took advantage of the beneficial effects of acidulation to enhance remineralization of caries-like lesions of enamel by synthetic calcifying fluids. Although significant remineralization may be achieved with calcifying fluids at neutral pH,[7, 8, 10–17] our study demonstrated the advantage of acidulation of calcifying fluids. While the nonacidulated calcifying fluids produced reductions in lesion depths of 18% for the 1 mM calcium CF and 10% for the 3 mM calcium CF, acidulation of these remineralizing agents resulted in significantly greater lesion depth reductions (29% for 1 mM calcium CF and 22% for 3 mM calcium CF). This degree of remineralization was realized while maintaining intact negatively birefringent (pore volume < 5%) surface zones. In fact, the surface zone depths were substantially increased following treatment with acidulated calcifying fluids when compared with nonacidulated calcifying fluids. The findings in our study compare favorably with those previously reported[7] when acid etching of caries-like lesions of enamel preceded remineralization with calcifying fluids. In that particular in vitro study,[7] the combination of acid etching with calcifying fluid treatment resulted in a lesion depth reductions of 34% for 1 mM calcium CF and 24% for 3 mM calcium CF. Surface zones from the etched, remineralized lesions were increased by more than 80% with both 1 mM calcium and 3 mM calcium CFs. Both this and previous in vitro studies[7] utilized the

same experimental design with ten separate, 60-sec exposures to the calcifying fluids. The substitution of acidulation of the calcifying fluids for acid etching the lesions prior to calcifying fluid treatment appears to have a similar effect on remineralization of caries-like enamel lesions.

The pattern of enamel caries remineralization and mechanism of mineral deposition depend on the saturation levels of the mineral phases composing the calcifying fluid.[10,13,14,17] Prior analysis of the 3 mM calcium CF has indicated certain mineral phases are supersaturated and include hydroxyapatite, fluorapatite, octacalcium phosphate, and tricalcium phosphate. Because of these supersaturated mineral phases in the 3 mM calcium CF, rapid precipitation of newly formed crystals — termed crystal nucleation — within the pore structure of enamel caries is the mechanism involved in remineralization. Remineralization by nucleation results in the formation and deposition of small diameter (50 to 75 nm) crystals within the surface zone and superficial aspect of the body of the lesion. Due to the rapid deposition of crystals, the more superficial aspect of the lesion is remineralized, and access to the underlying pore structure of the enamel lesion is obstructed. With the 1 mM calcium CF, remineralization of enamel lesions involves a more prolonged deposition of mineral phases on existing crystals within the lesion, termed crystal growth. This results in a more gradual "plugging" of porosities within the lesion and allows access to the more deeply situated regions of the lesion. Remineralization of enamel lesions with the 1 mM calcium CF by crystal growth produces crystal diameters of 50–150 nm within the lesion. This phenomenon occurs because only hydroxyapatite and fluorapatite are supersaturated in this remineralizing fluid. The mechanisms of caries remineralization for the 1 mM calcium CF by crystal growth and 3 mM calcium CF by crystal nucleation provide an explanation for the differences in remineralization pattern between these calcifying fluids. With the 3 mM calcium CF, the more superficial aspect of the lesion tends to be affected; whereas, with the 1 mM calcium CF, the entire lesion depth may be affected. This difference results in a greater reduction in lesion depth for 1 mM calcium CF than for 3 mM calcium CF, but a greater increase in surface zone depth for 3 mM calcium CF than for 1 mM calcium CF. Both remineralizing fluids may provide significant protection against caries progression.

Remineralization may be enhanced significantly by acidulation of synthetic calcifying fluids while maintaining an intact surface over the enamel lesion. Although our study was carried out in a laboratory setting, the experimental regimen and treatment times may be transferred easily to the clinical setting. Perhaps in the near future, clinical investigations into remineralization of clinically detectable white spot lesions may be realized. Just as important is the clinical adaptation of calcifying fluids for prophylaxis against the formation of clinically detectable lesions, and the reversal of clinically undetectable white spot lesions and hypocalcified enamel.

Conclusions

The conclusions from this in vitro polarized light microscopic study that evaluated the effects of acidulated calcifying fluids on remineralization of caries-like lesions of enamel are:

1. Remineralization of caries-like lesions of enamel may be accomplished with both acidulated and nonacidulated calcifying fluids using a series of relatively short treatment periods.
2. The greatest lesion depth reduction was realized with the acidulated calcifying fluid containing 1 mM calcium; however the greatest increase in surface zone depth was found with the acidulated calcifying fluid containing 3 mM calcium.
3. Acidulation of calcifying fluids enhanced the degree of remineralization over that attained by nonacidulated calcifying fluids. This improvement in remineralizing ability of acidulated calcifying fluids may be due to the removal of superficial organic pellicle and debris, and the creation of a more reactive enamel surface.

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Mild illness no reason to delay childhood immunizations

RESEARCHERS SAY MISSED VACCINATIONS CONTRIBUTE TO DISEASE OUTBREAKS

There is no reason to delay vaccinating mildly ill children for measles, mumps and other ailments, according to an article in a recent issue of *The Journal of the American Medical Association.*

Gail E. King, MD, MPH, Centers for Disease Control and Prevention, and colleagues studied 386 children, aged 15–23 months, at six county health department immunization clinics in the Atlanta area from February 1992 to April 1993. They examined the response to the measles-mumps-rubella vaccine among children with and without illness.

The researchers write: "Vaccinations that are missed because of mild illness are an important cause of under-vaccination among preschool and children in high-risk populations and have contributed to the occurrence of disease outbreaks. Reducing all missed opportunities to vaccinate is one of the standards for pediatric immunization practices ... (and) is also an important component in the strategy for reaching the U.S. disease reduction and immunization goals for the year 1996."

The American Academy of Pediatrics has long advised physicians not to view mild illness as a reason to delay vaccinations, but the "recommendation has not been universally followed, delaying vaccinations for some children," according to the researchers, who point to concerns among many physicians of vaccine failure and increased side effects when vaccines are administered during an acute illness.

In the current study, 157 of the 386 children had one or more mild illnesses including upper respiratory tract infection, ear infection, and diarrhea.

The researchers found seroconversion (development of antibodies) rates to measles, mumps and rubella antigens slightly, but not significantly, higher for children with mild illness compared to children who were well at the time of immunization. For instance, the seroconversion rate for measles was 99% for children with a mild illness compared to 97% for well children. And, multiple symptoms in mildly ill children were not found to increase the likelihood of vaccine failure.

Overall seroconversion rates were 98% for measles, 83% for mumps and 98% for rubella.

The researchers conclude: "Mild illness or a history of recent mild illness is not associated with reduced seroconversion to MMR or with increased adverse events, and such illnesses are not associated with a reduced level of antibody response to MMR vaccine."