Argon laser irradiation and acidulated phosphate fluoride treatment in caries-like lesion formation in enamel: an in vitro study

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Abstract

The purpose of this in vitro study was to determine the combined effects of argon laser irradiation (ArI) and acidulated phosphate fluoride treatment (APF) on caries-like lesion formation in human enamel. Each specimen was divided into tooth quarters with each quarter assigned to one of four groups: 1) control; 2) ArI Only; 3) ArI before APF treatment; 4) APF treatment before ArI. After a fluoride-free prophylaxis, acid-resistant varnish was applied to the tooth quarters, leaving sound enamel windows exposed on buccal and lingual surfaces. Argon laser irradiation was at 2 watts for 10s (100 J/cm^2). APF treatment was with a 1.23% APF gel for 4 min. Lesions were created in sound enamel windows with an acidified gel. After lesion formation, sections were obtained and imbibed with water for polarized light study. Body of the lesion depths were determined and compared among the four groups. Lesion depths were: 195 $\pm$ 23 μm for control; 129 $\pm$ 17 μm for ArI only; 96 $\pm$ 14 μm for ArI before APF; and 88 $\pm$ 11 μm for APF before ArI. Significant differences ($P < 0.05$) were found between the control group and all treatment groups, and between the ArI only group and both combined APF and ArI groups. Significant difference ($P > 0.05$) was not found between the ArI before APF and the APF before ArI groups. Laser irradiation alone reduced lesion depth by 34% compared with control lesions. When ArI was combined with APF treatment, lesion depth decreased by more than 50% compared with control lesions, and by 26 to 32% when compared with lased-only lesions. Combining ArI and APF treatment significantly enhances the resistance of sound enamel to an in vitro cariogenic challenge. (Pediatr Dent 17:31–35, 1995)

Although recent epidemiologic studies have reported a decline in dental caries in the pediatric and adolescent population in the United States, dental caries represents the most prevalent disease process occurring during childhood and adolescence, with only about 15% of 17-year-olds remaining caries free.[1] Especially disconcerting is the fact that approximately one-sixth of this population accounts for two-thirds of the total caries experience.[1] These facts emphasize the need for optimizing current caries-preventive regimens and introducing innovative preventive techniques. The role of systemic and topical fluoride in the decline of caries is well known.[2–4] Fluoride has become the mainstay in the battle against caries, with more than 60% of children residing in communities with optimal water fluoridation.[2,3] In addition to professionally applied topical fluorides, fluoridated dentifrices and mouth rinses are readily available without prescription.

More recently, the role of lasers in caries prevention has been explored. Several different types of lasers have been shown to reduce enamel solubility and dissolution rates substantially, as well as affect caries-like lesion formation.[5–15] Laser irradiation of sound enamel at relatively low energy levels results in significant resistance to a cariogenic challenge,[5–15] and avoids thermal temperatures[16] that would harm both hard and soft tissue, including the dental pulp. It would appear that laser irradiation of enamel in combination with topical fluoride application may provide a synergistic effect on caries resistance in enamel and provide further benefit over either treatment modality alone.

The purpose of this in vitro study was to determine the combined effect of argon laser irradiation (ArI) and acidulated phosphate fluoride (APF) treatment on caries-like lesion formation in human dental enamel using polarized light techniques.

Methods and materials

Twenty caries-free human molar teeth were selected for this in vitro study. The buccal and lingual surfaces were examined with a binocular, dissecting microscope at a magnification of 16x to ensure that these surfaces were free of clinically detectable white spot lesions. The specimens were sectioned into tooth quarters, then quarters from each tooth were assigned to one of four treatment groups: 1) control (no treatment); 2) ArI only; 3) ArI before APF treatment; and 4) APF treatment

TABLE. ARGON LASER IRRADIATION AND ACIDULATED PHOSPHATE FLUORIDE TREATMENT: EFFECT ON CARIES-LIKE LESION FORMATION IN ENAMEL

	Surface Zone Depth (Mean ± SD)	*Body of Lesion Depth (Mean ± SD)*	*Reduction in Body of Lesion Depth*
Control lesions	8 ± 3 μm[a–c]	195 ± 23 μm[d–f]	34%
Argon laser irradiation	16 5 μm[a]	129 17 μm[d, g, h]	51% 55% 26%
Argon laser irradiation before APF treatment	18 4 μm[b]	96 14 μm[e, g]	32%
APF treatment before argon laser irradiation	21 ± 5 μm[c]	88 ± 11 μm[f, h]	8%

ANOVA and DMR-paired sample results: means with same letters are significantly different at $P < 0.05$.

before ArI. Following a fluoride-free prophylaxis, an acid-resistant varnish was placed, leaving two windows of sound enamel exposed on the buccal and lingual surfaces. Argon laser irradiation (HGM Corporation, Salt Lake City, UT) of the sound enamel windows was at 2 watts for 10 sec (100 Joules/cm^2) using a 320-μm bare fiber with an effective spot size of 5 mm. APF treatment was with a 1.23% APF gel (Oral-B Laboratories, Redwood City CA) for 4 min. After the fluoride treatment, the APF gel was removed by a copious, air-water spray rinse followed by a 24-hr deionized-distilled water rinse. Caries-like lesions were created in the sound enamel windows using a dialyzed-reconstituted acidified gelatin gel containing 1.0 mM calcium, 0.6 mM phosphate and < 0.05 mM fluoride at pH 4.25 ± 0.02.[17] Following a 10-week exposure period to the artificial caries medium, longitudinal sections were prepared from the tooth quarters for polarized light evaluation. The sections were imbibed with water and photomicrographs were taken. Mean surface zone and body of the lesion depths were obtained using a digitized tablet and taking five measurements along the inner aspect of the surface zone and body of the lesion. Forty paired lesions from each of the four treatment groups were available for statistical analyses. Because of the paired research design, the surface zone and body of the lesion depths from the treatment and control groups were compared using analysis of variance and Duncan's multiple range analysis for paired samples, thereby limiting tooth-to-tooth variability in the statistical analysis.

Results

ArI alone and combinations of argon laser and topical fluoride treatment resulted in significant reductions ($P < 0.05$) in body of the lesion depths when compared with those for paired control lesions (Table). Comparison of lesion depths between the control group (195 μm) and ArI-only group (129 μm) indicated a 34% reduction in lesion depth when ArI was carried out prior to lesion formation ($P < 0.05$). When ArI was combined with APF treatment either before (96 μm) or after (88 μm) laser irradiation, lesion depth was 51 to 55% less than that of the control group ($P < 0.05$). Combining laser irradiation and fluoride treatment resulted in a 26 to 32% reduction in lesion depth compared with ArI alone ($P < 0.05$). While there was a slight reduction (8%) in lesion depth when fluoride treatment preceded rather than followed laser irradiation, this was not statistically significant ($P > 0.05$). Mean surface zone depths for all treatment groups (Table) were significantly increased over that for the control group ($P < 0.05$). The increase in surface zone depth for the treatment groups ranged from 2 to 2.5 times. While there was a trend toward increased surface zone depths in the combined laser and fluoride treatment groups when compared with laser irradiation alone, no statistically significant differences were found ($P > 0.05$).

These findings regarding the statistical analyses among groups for surface zone and body of the lesion depths may be readily identified with the histopathologic appearances of the representative paired lesions from the same specimen (Fig 1). The body of the lesion dramatically decreases in depth from the representative control lesion (236 μm) to the argon lased lesion (139 μm) to the lesion treated with fluoride prior to laser irradiation (97 μm). Not only has the lesion depth been affected, but also certain qualitative differences in the degree of birefringence within the body of the lesion also are noticeable. With the combined laser irradiation and fluoride treatment groups (Figs 1C and 1D), the body of the lesion shows a qualitatively decreased degree of positive birefringence (pore volume > 5%) and an increase in pseudoisotropy (pore volume = 5%). In contrast, the control lesion (Fig 1A) shows a relatively high degree of positive birefringence with partial loss of the typical striae of Retzius and prism markings within the body of the lesion.

The histopathologic appearance of the surface zone is also considerably different when the combined laser irradiation and fluoride treatment groups are compared with their paired control lesion. The control lesion (Fig 1A) shows a relatively thin, somewhat discontinuous, but intact surface zone. This surface zone is composed of areas of negatively birefringent (pore volume < 5%) enamel, but interrupted by areas of pseudoisotropy (pore volume = 5%). In contrast, the paired lesion that was treated with fluoride prior to laser irradiation (Fig 1D) possesses an intact continuous, negatively bire-

Fig 1A–1D. Effect of argon laser irradiation and acidulated phosphate fluoride treatment on caries-like lesion formation in enamel. (Arrow = surface zone; B = Body of lesion; spacebar = 100 μm; water imbibition, polarized light microscopy).

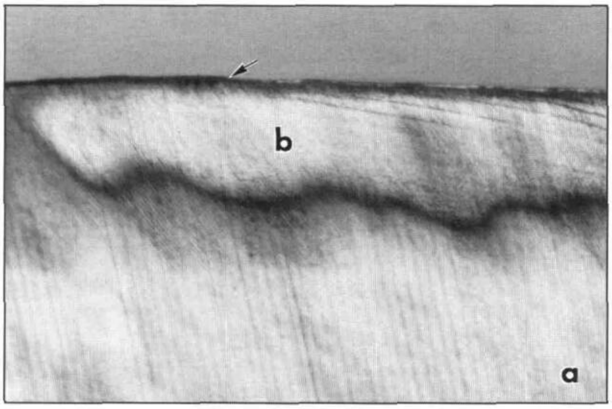

Fig 1A. Representative paired lesion from control group.

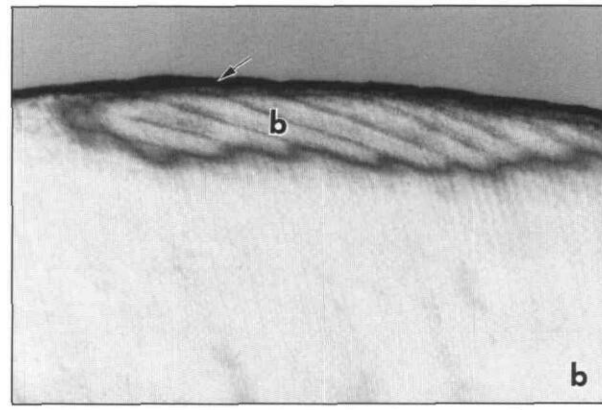

Fig 1B. Representative paired lesion from argon laser irradiation only group.

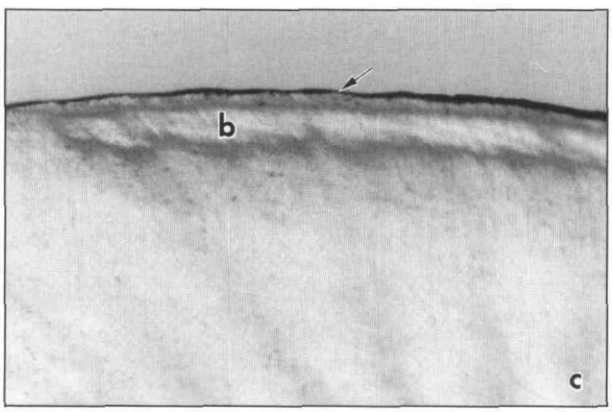

Fig 1C. Representative paired lesion from argon laser irradiation before acidulated phosphate fluoride treatment group.

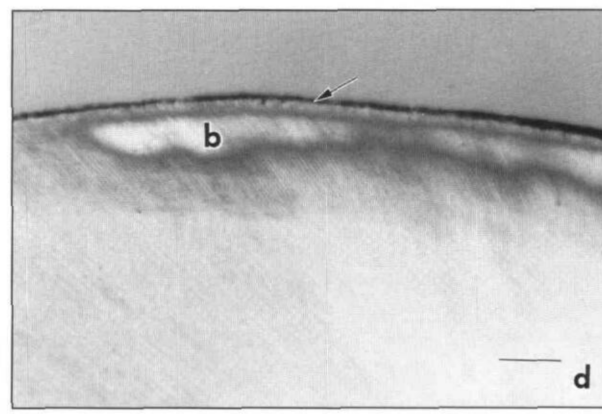

Fig 1D. Representative paired lesion from acidulated phosphate fluoride treatment before argon laser irradiation group.

fringent (pore volume < 5%) surface zone of considerably increased depth overlying the positively birefringent (pore volume > 5%) body of the lesion. These differences in the histopathologic appearances among the control and treatment groups are worth noting, considering that these paired lesions were created in tooth quarters from the same extracted human molar tooth under identical artificial caries conditions.

Discussion

Although initial laser research in dentistry carried out during the 1960s was directed toward removing and ablating caries,[18, 19] laser applications in current dental practices are predominantly concerned with managing periodontal and soft tissue disease.[20] With the refinement of laser technology, a number of laser types have been introduced that emit different wavelengths and have varying energy (fluence) levels.[12, 20] With this technology has come the ability to precisely control the energy emitted, collimate the beam to a specific diameter, and focus the beam to a specific depth.[20, 21] With the dentition, this can avoid thermal damage to the adjacent supporting soft tissue and dental pulp. Because of these advances, a renewed interest in laser applications to dental hard tissues has emerged. Some of these applications include: creating enamel and dentinal surfaces suitable for adhesive resin bonding; both bonding and debonding of orthodontic brackets; fusing hydroxyapatite to pits and fissures, thereby acting as a "biological" sealant; providing a biological apical seal in teeth undergoing root canal therapy; and polymerizing dental materials with the argon laser.[6, 12, 20–22]

The finding that has been most intriguing is the effect of laser irradiation on enamel solubility and in vitro caries formation. In the current study, ArI alone resulted in a 34% reduction in the body of the lesion depth and a two-fold increase in surface zone depth compared with control lesions. Similar findings have been reported for argon laser-irradiated enamel and root surfaces exposed to an in vitro caries challenge.[5–14, 23] In fact, laser irradiation of enamel has been shown to reduce the threshold pH for enamel dissolution by 0.72 pH units, which corresponds to an almost five-fold increase in the amount of acetic acid normally required to initiate enamel dissolution.[10, 11] Effectively, the dissolution threshold is lowered from pH 5.50 for nontreated enamel to 4.78 for lased enamel. In addition, the enamel solubility at pH 4.5 following laser treatment is reduced by five-fold for lased enamel when compared with nontreated enamel.[10,11]

Several mechanisms for improved caries resistance following laser irradiation have been proposed. These mechanisms may:

1. Decrease carbonate, water, and organic content of the mineral phases in enamel and dentin, resulting in a reduction in lattice strain of hydroxyapatite and decreased enamel solubility
2. Create a microsieve or micropore system within the mineral structure forming enamel, dentin, and cementum, which may allow for reprecipitation or entrapment of calcium, phosphate, and fluoride released during caries formation
3. Decrease the permeability of mineral structure due to protein denaturation with resultant protein expansion and reduce micropores within the mineral, thereby decreasing access of cariogenic acids into the underlying enamel
4. Affect micro-organisms within plaque (only at high energy)
5. Increase the uptake of fluoride, phosphate and calcium by the lased mineral phases.[5–15]

Although the exact mechanism of caries resistance for argon-lased enamel is not known, the most likely mechanism is creation of a microsieve network with entrapment and reprecipitation of mineral phases released during the demineralization process.[6,8,12]

The combination of APF treatment with ArI provided more caries resistance than ArI alone. The decrease in lesion depth ranged from 26 to 32%, depending upon whether laser treatment followed or preceded fluoride treatment. Similar results also have been reported recently with regard to the effects of combined laser and fluoride treatment on in vitro root surface caries.[14,23] The combination of fluoride and laser irradiation have been shown to have a synergistic effect on caries formation.[10,11] The addition of less than 0.1 ppm fluoride to demineralizing fluids results in a significant decrease in enamel solubility for both sound and lased enamel.[10,11] In the presence of fluoride, the threshold for sound enamel dissolution is reduced from pH 5.50 to 5.14. Lased enamel in the presence of fluoride has an enamel dissolution threshold that is reduced from pH 4.78 to 4.31. Particularly interesting is the fact that lased enamel exposed to low levels of fluoride has a solubility that is six-fold less than that for nonlased enamel exposed to the same fluoride level.

Within this study, a 1.23% acidulated phosphate fluoride gel was utilized either before or after laser irradiation and resulted in significant reductions in lesion depths. This fluoride treatment provides 12,300 ppm fluoride and results in both labile- and bound-fluoride retention within enamel.[24] Typically, fluoride agents at this concentration produce surface coatings that may:

1. Act as diffusion barriers
2. Reduce enamel solubility in acidic conditions
3. Act as reservoirs for fluoride-rich reaction products such as calcium fluoride
4. Desorb proteins and micro-organisms from the enamel surface.[25–29]

Enamel caries has been shown to have an increased affinity for fluoride over that for sound enamel.[25,30,31] This increased fluoride uptake may result in replenishment of fluoride within the lesion and also may act as a reservoir for fluoride release during demineralization. As noted previously, even low concentrations of fluoride will result in decreased enamel solubility.[10,11,25,30,31] If fluoride is available either in dental plaque or within the superficial layers of enamel, demineralization of enamel will be affected. The release of even small quantities of fluoride from enamel during a cariogenic attack will result in rapid reprecipitation of mineral phases and will necessitate significantly lower pHs for demineralization to occur. The fact that laser irradiation also has been shown to enhance fluoride uptake may also be of benefit. From the findings in this study, it is apparent that the combination of fluoride treatment and laser irradiation provides an additional degree of caries-resistance over ArI alone.

This combined regimen of laser irradiation and fluoride treatment may be of clinical significance in preventing dental caries and treating existing hypomineralized enamel and white spot lesions. Because it is possible to collimate the laser beam to a selected diameter and focus the beam to a specific depth, it may be possible to utilize this combined preventive protocol for sound enamel and enamel caries at interproximal sites, as well as on smooth surfaces and in pits and fissures. Application of a topical fluoride agent to early enamel lesions either preceding or following laser irradiation may provide a means for arresting or reversing the caries process.

Conclusions

The following conclusions may be drawn from this in vitro study:

1. ArI (100 J/cm^2) alone resulted in a significant reduction in lesion depth when compared with nontreated sound enamel.
2. The combination of ArI and APF (1.23%) produced an enhanced degree of caries resistance when compared with ArI alone.

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