Original Article

The Effects of Q-switched Nd : YAG Laser Irradiation on
Demineralization in Early Enamel Caries Lesions

Hisazumi IKEDA, Daisuke INABA*, Kentaro MINAMI*,
Yoshiko SOMEYA*, Masami YONEMITSU*, Tsugio INOKUCHI
and Okuji TAKAGI**

Second Department of Oral and Maxillofacial Surgery,
Nagasaki University School of Dentistry
(Chief: Prof. Tsugio INOKUCHI)

*Department of Preventive Dentistry,
Iwate Medical University School of Dentistry
(Chief: Prof. Masami YONEMITSU)

**Department of Preventive Dentistry,
Nagasaki University School of Dentistry
(Chief: Prof. Okuji TAKAGI)

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エナメル質初期齲蝕の脱灰におよぼす Q スイッチ Nd : YAG レーザー照射の効果

池田久住 稲葉大輔* 南健太郎*
染谷美子* 米満正美* 井口次夫
高木興氏**

長崎大学歯学部口腔外科第二講座
(主任: 井口次夫教授)

*岩手医科大学歯学部予防歯科学講座
(主任: 米満正美教授)

**長崎大学歯学部予防歯科学講座
(主任: 高木興氏教授)

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要旨: 本研究はレーザー照射の脱灰エナメル質におよぼす影響を *in vitro* と *in situ* で検討する目的で実施した。人工的に脱灰したエナメル質に Q-switched Nd : YAG 第 2 高調波 (532nm; 100 J/cm²) を照射した。続いて、非照射の対照を含む試料を、pH 5 の酸性ゲルに 3 週間浸漬、または上顎第一大臼歯の頬側面に接着して 16 名の成人口腔内に 1 か月間接触させた。エナメル質表層のミネラル分布はマイクロラジオグラフィ (TMR) と画像定量法により評価した。paired t-test の結果、いずれの実験においてもミネラルパラメータ値はレーザー照射と非照射試料の間において $p=0.05$ で有意な違いを示さなかった。結論として、

今回の結果はエナメル質病巣のさらなる脱灰進行が使用したレーザーによっては効果的に抑制されないことを示唆した。

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Key words=Q-switched Nd:YAG laser/Enamel/Caries preventive effect

キーワード=Q スイッチ Nd:YAG レーザー, エナメル質, 齲蝕予防効果

Introduction

Dental caries is a dynamic process characterized by alternations of de- and remineralization¹⁾. Caries prevention is, therefore, fundamentally achieved when the positive mineral balance exceeds the rate of demineralization over an extended period. In this phase, physicochemical properties of tooth surfaces and subsurface regions are important factors to define susceptibility and resistance to caries.

Recently, laser has been introduced as a new technology for dental treatments^{2,3)} and some types of laser, e.g. CO₂ and Nd:YAG lasers, were suggested to reveal caries preventive effects for intact enamel by improving its acid resistance⁴⁻⁷⁾. Our previous studies also indicated that irradiation of second harmonic wave of Q-switched Nd:YAG laser significantly reduced Ca dissolution rate of human enamel *in vitro*^{8,9)}.

The acid resistance of enamel surfaces has generally been assessed by a Ca dissolution rate^{4,7-9)}. From viewpoint of cariology, however, one drawback of the Ca dissolution test is that an acid etching procedure usually employed does not simulate caries attack but erosion of enamel. In addition, it should be noted that caries prevention is essential rather for the most caries susceptible regions in enamel where surfaces are frequently demineralized by dental plaque. In this sense, recovery or arrest of incipient caries with subsurface lesions is primary clinically.

The aim of this study was to assess microradiographically whether irradiation of Q-switched Nd:YAG laser protects demineralized enamel from lesion formation under presence of dental plaque *in situ* and a caries simulation system

using a viscous acidic gel which is known to produce caries-like lesions successfully by acting as dental plaque^{10,11)}.

Materials and Methods

1. Sample preparation

Sound human premolars extracted from patients aged 11-14 years old (mean 11.7 years old) for orthodontic reasons were used. After removal of the roots, enamel blocks were cut from buccal or lingual side of the crowns using a water-cooled diamond-coated saw. The blocks were completely coated with an impression compound (Pericompound, Shofu, Japan) except for the original enamel surfaces. Subsequently, the enamel surfaces were slightly abraded to expose fresh enamel surfaces of about 15 mm² on a wet abrasive paper (grit 800). The samples were divided into 2 treatment groups; 24 samples for *in vitro* evaluation and 32 samples for assessment *in situ*. In the *in situ* group, an U-shaped groove (about 300 μ m in width and about 500 μ m in depth) was formed at the central part using a water-cooled blade (Isomet, Buehler, U.S.A.) to simulate a fissure structure and to enhance dental plaque accumulation. Subsequently, all the enamel samples were immersed in a 0.1M lactic acid gel (pH 4.5) containing 1wt% carboxymethylcellulose (CMC) at 37°C for 12h to produce artificial early caries lesions.

2. Laser irradiation

The second harmonic wave of a Q-switched Nd:YAG laser (Quanta-Ray DCR-3, Spectra-Physics, U.S.A.) with a wavelength of 532 nm was used in this study. Enamel surfaces of 18 samples in *in vitro* group and 16 samples in *in situ* group were painted with a black India ink and lased at a fluency of 1 J/pulse/cm² and a

frequency of 10Hz for 10 s (100 shots) resulting in the total energy dose of 100 J/cm². This condition was employed because of ability to add acid resistance to enamel *in vitro*^{8,9)}. The laser beam was guided by a quartz prism and projected vertically to the enamel target plane.

The beam intensities were measured using a power meter (30A-P, Ophir Optics, Israel). The residual samples were used as unlased controls in each assessment.

3. *In vitro* experiment

Lased (n=12) and unlased controls (n=12) were again immersed in a 0.1M lactic acid gel containing 6 wt% CMC (pH=5) at 37°C for 3 w. The gel volume was always 100 ml per 6 samples.

4. Intraoral experiment

Sixteen adults aged 21-28 y (mean±SD=23.2±3.6, 8 males and 8 females) participated in the intraoral experiments. It was confirmed that they were free from xerostomia and chronic diseases under medication. The lased and unlased samples (n=16, each) were attached on the sound buccal surfaces of right and left maxillary first molars respectively using a non-fluoridated light-cured resin for 1 month. During the experimental period, the samples were kept from brushing to accumulate dental plaque in the grooves and the participants used non-fluoridated toothpaste to brush the other teeth. In addition, any fluoride agents were not applied, but diet was not controlled peculiarly to maintain natural caries susceptibility of each individual.

5. Microradiography

After the *in vitro* or intraoral experiments, a thin planoparallel sections of about 80 μm thickness were cut transversally from the central part of the sample. Each section was microradiographed together with a reference aluminium step wedge (30 μm×10 steps) under a standardized condition. For theoretical background of the transversal microradiography (TMR), we refer to de Josselin de Jong, *et al.*¹¹⁾. Mineral distributions in the bottom of the grooves were assessed by the computer-assisted videodensitometry (CAV) and mineral distribu-

Fig. 1 Schematic mineral distribution of enamel lesion and mineral parameters.

Vs=Sound mineral level (=89 vol%)

Vs 95%=95% level of Vs (=85 vol%)

D0=Outer surface position (Depth zero)

ld=Lesion depth in μm

ΔZ=Mineral loss value in vol% · μm

tion analyzer program (MDA) previously described in detail by Inaba, *et al.*¹²⁻¹⁴⁾. As shown in Fig. 1, a mineral profile was computed from the videodensitometric data and the two mineral parameters of interest, namely the lesion depth l_d (μm) and the mineral loss value ΔZ (vol% · μm) were measured¹⁴⁾.

Results

The histogram of the l_d and ΔZ values were represented in Figs. 2a and 2b. The l_d values of the unlased control and lased samples were 104 ± 38 and 82 ± 34 μm (mean±SD), respectively from *in vitro* experiment, and 41 ± 18 and 34 ± 24 μm from *in situ* study. The ΔZ values were $4,256 \pm 1,142$ and $4,077 \pm 1,904$ vol% · μm (*in vitro*) and $1,769 \pm 1,031$ and $1,684 \pm 1,171$ vol% · μm (*in situ*), accordingly. Although the lased group showed 21% lower l_d value compared with the unlased samples *in vitro*, no statistically significant difference was detected between unlased and lased samples for each mineral parameter (paired t-test) both in *in vitro* and *in situ* cases. In microradiographic images, no visual evidence of remineralization was observed for all the samples.

Fig. 2 The lesion depth l_d (a) and mineral loss values ΔZ (b) of enamel samples after caries challenge using a lactic acid gel (*in vitro*) or intraoral experiments (*in situ*). Bar=SD.

Discussion

In contrast to the results from Ca dissolution test, the same dose of Q-switched Nd:YAG laser irradiation failed to protect the underlying enamel lesions from further demineralization by caries challenge both *in situ* and *in vitro*. The disparity between our previous and present results indicates that the lased enamel surface is acid resistant but is not an effective barrier to inhibit penetration of hydrogen ions into a lesion body. To this point, Kinney *et al.* demonstrated a remarkable lesion progression in lased dentin when an acidic gel was applied as a caries attack¹⁵⁾.

Integrating our studies on the Q-switched Nd:

YAG laser, it is implied that the acid resistance of enamel surfaces determined by the Ca dissolution test does not always stand for clinical caries inhibition of underlying enamel. Thus, it should be attentive that acid resistance and caries resistance are not similar concepts fundamentally. Therefore, a reasonable illustration of lased enamel (or lesions) could be a mineralized tissue coated with a penetrable acid resistant layer.

The increased acid resistance of enamel surfaces by laser irradiation has been explained from presence of a fused (or melted) layer formed by the thermal effects of lasers^{4,8)}. On the other hand, thermal effects of laser irradiation and subsequent cooling produce microcracks in enamel surfaces^{5,6)} and this may be responsible for enhanced permeability of outer ions resulting in progressed subsurface demineralization. Moreover, it was revealed that laser irradiation at a dose of about 100 J/cm² alters only the outermost enamel surfaces up to 1–5 μm at most^{4,5)} by electron microscopic investigations or within 100 nm¹⁶⁾ in depth from crystallographical study. The fact that laser irradiation decreases water, carbonate and organic components from enamel sizably¹⁷⁾ may attenuate its structure and rather contribute to enhance caries susceptibility.

Although these findings were assured mainly in lased sound enamel, such changes might be heightened in enamel lesions resulting in enhancement of ion permeability and progression of demineralization in underlying enamel. As observed in our previous SEM study, there is also a possibility that the second harmonic wave of the Q-switched Nd:YAG laser influences demineralized enamel destructively because of its greater amplitude of vibration¹⁷⁾. Recent TEM study on the Nd:YAG laser appended a new evidence that lased demineralized enamel showed a significantly inferior crystallinity in outermost layers up to 50–60 nm in depth compared with underlying areas¹⁸⁾.

The loss of organic components can be interpreted from another view point. Diffusion of Ca ions dissolved from subsurface enamel may

be trapped in microscopic voids created by such morphological changes¹⁸⁾. But, it would be reasonable to consider that this alternation can conversely augment outer hydrogen ions to penetrate faster into subsurface enamel due to differences in elemental diffusion rates. The other is that such component damages may be capable of promoting remineralization by increased enamel permeability. However, our study suggested that irradiation of Nd: YAG laser had neither promotive nor inhibitory effects on subsequent remineralization of enamel lesions *in vitro*¹³⁾. Therefore, it is adequate to understand that subsurface demineralization of enamel can be rather stimulated by projection of Nd: YAG laser even if outermost enamel surfaces obtain acid resistance in part.

Relating to experimental methods, it should be noted that caries prevention is not achieved by a single factor related to a physical property of enamel. Actual caries formation or arrest is regulated by dynamic interaction between enamel and oral environment such as diet, dental plaque and saliva¹⁾. Therefore, an intraoral experiment should be encouraged to examine whether a laser therapy can prevent dental caries effectively.

Conclusions

In conclusion, it was suggested that irradiation of the second harmonic wave of Q-switched Nd: YAG laser to the surface of incipient enamel lesion may not contribute to protection of its subsurface demineralization. Since caries inhibitory effects of laser irradiation are still needed to be examined from view point of cariology, further intraoral evaluation should be required to establish optimized laser treatments for practical caries prevention.

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