Effect of Defective Collagen Synthesis on Epithelial Implant Interface: Lathyritic Model in Dogs. An Experimental Preliminary Study

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Peri-implant mucosa is composed of 2 compartments: a marginal junctional epithelium and a zone of connective tissue attachment. Both structures consist mainly of collagen. Lathyrism is characterized by defective collagen synthesis due to inhibition of lysyl oxidase, an enzyme that is essential for interfibrillar collagen cross-linking. The lathyritic agent beta-amino-propionitrile (β-APN) is considered a suitable agent to disrupt the connective tissue metabolism. Therefore, the purpose of this study was to assess the effect of defective connective tissue metabolism on epithelial implant interface by using β-APN created chronic lathyrism in the canine model. Two 1-year-old male dogs were included in this study. A β-APN dosage of 5 mg/0.4 mL/volume 100 g/body weight was given to the test dog for 10 months, until lathyritic symptoms developed. After this, the mandibular premolar teeth (p2, p3, p4) of both dogs were atraumatically extracted, and the investigators waited 3 months before implants were placed. In the test dog, 3 implants were placed in the left mandible, and 2 implants were placed in the right mandible. In the control dog, 2 implants were placed in the left mandibular premolar site. The dogs were sacrificed 10 months after healing. Peri-implant tissues obtained from the dogs were examined histomorphologically and histopathologically. Bone to implant contact (BIC) values and bone volumes (BV) were lower in the lathyritic group compared to the control group; however, no statistical significance was found. Significant histologic and histomorphometric changes were observed in peri-implant bone, connective tissue, and peri-implant mucosal width between test and control implants. Defective collagen metabolism such as lathyrism may negatively influence the interface between implant and surrounding soft tissue attachment.

Key Words: peri-implant mucosa, collagen crosslinking, defective collagen synthesis

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INTRODUCTION

There is an epithelial implant interface, which is composed of collagen fibers in the supra-alveolar region at the ridge of peri-implant bone, that forms a parallel unit with the abutment portion of the titanium body. This region is generally accepted as a structure that is collagen rich but cell poor and similar to scar tissue. This transmucosal passage constitutes an effective barrier between the oral environment and the peri-implant mucosa. 1

Abrahamsson et al2 reported that this compartment of connective tissue was not homogeneous. They claimed that while the density of collagen was high in the peripheral layers of this zone, a narrow region closer to the implant surface appeared to be richer in cells.2

The clinical syndrome of lathyrism was described by Stockman in 1917, who reproduced the disease in frogs, guinea pigs, rabbits, and monkeys.3 Lathyrism was successfully induced in the rat, an animal previously thought to be resistant, using oral administration of a crystalline substance isolated from Lathyrus odoratus (sweet pea).4–6 The active chemical ingredient, beta-(gamma-L-glutamyl)-aminoproprionitrile (β-APN) was first isolated in 1954.7,8 It is well known today that lathyrism tissues contain an unusually high amount of collagen.4,6,9–14 It was identified that lathyrism occurred after the digestion of Lathyrus odoratus, especially under famine conditions in certain parts of Bangladesh, Ethiopia, Spain, Russia, and India.15,16

Early symptoms of lathyrism include walking difficulties, muscle cramps, and leg weakness. Subsequently, it produces irreversible spastic paralysis and even causes death due to failure of the muscles responsible for respiration. The pathologic effects of experimental lathyrism were mostly observed on cartilage, bone, and elastic connective tissues that consist of high amounts of collagen.10 Damage to the vascular system (angiolathyrism) and bone development in children (osteolathyrism) have also been described.17

β-APN is an excellent molecule for the study of connective tissue metabolism in lathyrism. A high dosage of β-APN caused acute intoxication and severe systemic lesions and often resulted in high mortality rate.18 Therefore, it is difficult to observe the long-term effects of lathyrism. Acute lathyrism has failed to mimic the complex pathogenic mechanism of chronic periodontitis.9 Hence, it was thought that the experimental model of chronic lathyrism developed by Bouissou and co-workers10 in 1970 might provide valuable information about the effect of low doses of β-APN on the periodontium. The periodontium was considered an excellent model in terms of studying the effects of systemic and local factors on a highly specialized connective tissue.4,6,9,10 This is because periodontium has a rapid turnover of collagen, especially during growth and development, and is exposed to masticatory stress.

Periodontal disease is an inflammatory disease of periodontal tissues that leads to the loss of tooth-supporting tissues, such as the alveolar bone.11 Alveolar bone resorption is an important feature of periodontal disease. However, it was suggested that a decrease in the bone mineral content of the skeleton might aggravate periodontal disease.12–14,19

The organic matrix of cortical bone consists of fibrous protein collagen. Collagen forms 90% of the organic matrix of bone and has a major function in calcification.20,21 Bone demineralization is characterized by the activity of osteoclasts. During the resorptive phase of bone metabolism, the inorganic constituents, collagen fibers, and organic substances are lost.22 The effect of lathyrogens on the bone metabolism or calcium homeostasis is not well understood, though their effects on the organic portion of the bone are well known.23 Osteolathyrism, a connective tissue disease, is characterized by decreased intramolecular and intermolec-
ular cross-linking of collagen molecules.\textsuperscript{9,10,18,24} Lathyrogens inhibit lysyl oxidase, which catalyzes the conversion of lysine and hydroxylysine into an aldehyde group and the formation of collagen cross-linking.\textsuperscript{9,25,26}

Dental implants have proved to be a successful treatment modality; however, a significant number of implants fail due to improper healing or lack of osseointegration.\textsuperscript{27} It would be of importance to identify subjects at risk to minimize these complications. Currently, no information is available with regard to how defective connective tissue disorders (eg, individuals with lathyrism and Marfan syndrome) influence implant healing. Therefore, the aim of this study was to examine the effect of a chronic lathyism model on the connective tissues of the peri-implant region through histomorphologic and histopathologic analyses.

**Materials and Methods**

The chemical $\beta$-APN was used to induce the chronic lathyism model. Two male shepherd dogs, each 12 months old, were used for the study. During this preliminary experimental study, dogs were maintained under similar conditions. This study was carried out in the Surgical Research Center of Ondokuz Mayis University, Faculty of Medicine after obtaining approval from the appropriate ethics committee. The $\beta$-APN dosage, 5 mg/0.4 mL/volume 100 g/body weight, was given to the test dog for 10 months. Lathyism symptoms developed at the 19th week. All periapical radiographs and films were taken (55 kVp, 10 mA, Ardet, Italy) before and after tooth extractions. Similarly, radiographs were taken 3 months after implant placement to assess bone healing. The dogs were anesthetized using intramuscular injections of xylazine HCl (Rampun 2%, Bayer) 2.2 mg/kg of body weight, ketamine HCl (Ketalar, Parke Davis, Istanbul, Turkey) 10 mg/kg of body weight, and local injection of prilocaine HCl (Citanest, Octopressin 3%, Astra Zeneca, London, UK) $\beta$-

The test dog was observed daily for clinical symptoms. Nineteen weeks after starting $\beta$-APN, chronic lathyism developed. Mandibular premolar teeth (p2, p3, p4) of both dogs wereatraumatically extracted and dental root form implants were placed 3 month after healing. Three implants (Zimmer Dental, Tapered Swiss Plus MTXTM implant system, Aseptico AED-707, Carlsbad, Calif) with an endosseous length of 8 mm and a diameter of 3.7 mm were placed in the left mandible, and 2 implants were placed in the right mandible of the test dog. In the control dog, 2 implants were placed in the left mandibular premolar site. The implants were 4 mm apart from each other, and the distance between the implants and the natural tooth was 10 mm. Sutures were removed 10 days postoperatively. Postoperative care included soft brushes and professional mechanical plaque removal 3 times a week. After the experimental period of 10 months the dogs were sacrificed using pentobarbital. The tissues surrounding the implants were harvested for histomorphologic and histopathologic examinations.

**Determining the peri-implant mucosa width from histologic views**

Biologic width was calculated using standardized magnification of the histologic images. The distance between gingival margin and the most coronal point of the bone and implant interface was defined as biologic width and calculated using Adobe Photoshop 6.0 (San Jose, Calif).

**Probing pocket depth measurements around the implants**

The pocket depth measurements of the implant sites in the test and control groups were carried out using a periodontal probe (UNC-15, Hu-Friedy, Chicago, Ill) 2 and 3 months after implant surgery. Probing was performed at 6 sites surrounding the implant (mesiobuccal, buccal, distobuccal,
mesiolingual, lingual, and distolingual). The adjacent peri-implant mucosa was used as a reference for measuring the probing pocket depth.

**The preparation of histologic cross sections**

The histologic and histomorphometric examinations were made using the IAS 2000 (Delta system, Rome, Italy) program equipped with image analysis. By calculating the histomorphometric percentages in all cross sections, the bone-implant contact ratio (BIC) and the bone volume ratio (BV) were calculated. The parameters of the linear implant surface, total implant surface, and the ratio of total implant surface that was directly touching the mineralized bone matrix were calculated using the IAS 2000 program. The mesial and distal sites were measured using Adobe Photoshop 6.0 on implants from the first contact point to the bone and implant mucosa margin. The biologic widths measured from magnified images were thus compared.

**Statistical analysis**

The statistical analyses of the data (bone contact ratio, bone volume) were evaluated by Mann-Whitney U test, and the data from standard histologic views and probing depths were evaluated by analysis of variance (ANOVA). All statistical analyses were determined by SPSS (SPSS.11.0 for Windows, SPSS, Chicago, Ill).

**Results**

During the study period, no postoperative complications were observed in both test and control groups. The mean probing depth in the experimental group was significantly higher than the control group (4.08 ± 0.35 mm vs 2.0 ± 0.55 mm, respectively; *P* < .05). Radiographically, the β-APN treated group had more bone loss when compared to the healthy control group.

**Histomorphometric values**

Although BIC values and bone volumes were lower in the β-APN treated group when compared to the control group, no statistical significance was found between the 2 implants (*P* > .05) (Table 1).

**Observational histologic results: control implants**

Implants showed a good amount of bone apposition in the crestal compact bone, while the apical region only had a small amount of immature woven bone. This new composite bone consisted of newly woven and lamellar bone. At the cortical level, new primary osteons were visible. In the cancellous region, new bony trabeculae were formed on the implant surface made of composite bone. Bone remodeling processes were visible with the coupling activity of bone formation and resorption. The crestal bone was well preserved, and no infrabony pocket was present in the supracrestal peri-implant soft tissue. The supracrestal collagen fibers were dense and well organized, running parallel and perpendicular to the implant abutment. Few vessels and a light inflammatory infiltrate were visible (Figures 1a,b and 3b).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Bone-implant contact (BIC) ratios and bone volume (BV) ratios of the implants in the control and beta-aminoproprionitrile treated test group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratios</td>
<td>Test Group</td>
</tr>
<tr>
<td>BIC, %</td>
<td>50.87 ± 19.44</td>
</tr>
<tr>
<td>BV, %</td>
<td>50.47 ± 10.08</td>
</tr>
</tbody>
</table>
A high bone to implant apposition rate was present in both crestal and apical cancellous areas. A continuous layer of bone surrounded almost the entire implant length. The bone quality and stage of maturation were similar to the control implants. At the crestal level, an infrabony pocket was always present with active bone resorption, and in one implant a large bone resorption was evident (Figure 2a,b,c). In this group, the supracrestal collagen fibers were loose or almost absent, and pocket epithelium was present (Figures 2d and 3a). A disruption of the continuity of the pocket epithelium was evident. Numerous large, rounded empty bodies, similar to adipocytic cells were penetrated into the subepithelial connective tissues. A heavy inflammatory infiltrate was present in the peri-implant connective tissues, which could explain the crestal bone resorption.

**Control and β-APN treated teeth**

Basically, no difference was found between the 2 groups. The periodontal ligament fiber orientation and space, type of cells, and the amount of blood vessels that existed in the periodontal space were all similar in both groups. The attachment apparatus was well preserved in all the cases at the level of the cemento-enamel junction. No inflammatory infiltrate was present in any of the evaluated teeth.
FIGURE 2. Beta-aminopropionitrile treated experimental group. (a) Toluidine blue basic fuchsin, magnification ×8. Active bone resorption (blue arrow) and gap were visible at the neck of implant. Fatty cells or adipose tissues were seen at connective tissue region (red arrow). (b) Buccolingual section, magnification ×8. Pathologic gaps were observed (black arrows). (c) Buccolingual section, (toluidine blue basic fuchsin, magnification ×8). Active bone resorption was visible in lingual region.
There was a statistically significant correlation between 2 groups of histologic peri-implant mucosal width ($P \leq .01$) (Table 2).

DISCUSSION

Dental implants are surrounded by 3 different tissues: epithelium, fibrocollagenous soft connective tissue, and bone. Although the success of dental implants is high today, there are still failures, some of which might be related to an absence of gingival connective tissue attachment to the implant.\(^\text{27}\)

So far, there is no record of what changes occur in mucosal width of peri-implants, when collagenous cross binding is obstructed. In a case reported by Straub et al,\(^\text{28}\) the relationship between severe periodontitis and Marfan syndrome, a disease of the connective tissue, had been examined, and it was found that the connective tissue abnormalities might play a significant role in increasing the inflammatory destruction of periodontal tissues. The clinical symptoms of Marfan syndrome resembled those of lathyrism.\(^\text{29}\) Again, no literature was found on the effects of...
lathyrism on dental implants. Hence, how the dysfunctional connective tissue may affect implant osseointegration is of interest. As a result, the observations obtained from this preliminary study might provide some insight into determining the clinical problems that may be encountered during implant healing for individuals who have a connective tissue disease such as lathyrism.

In this study, β-APN was used to create lathyrism, a disease with a connective tissue disorder component, in dogs. β-APN has been used as a lathyrogenic agent especially in rats, in experimental studies conducted to evaluate both an experimental periodontitis model and fibroblastic and osteoblastic activity.9,30–33 The onset of the lathyritic effect was reported to occur between 2 weeks and 3 months.17,30–33 In our study, β-APN (5 mg/10.4 mL volume/100 g body weight/day) was applied to the test group dog for 10 months. The disease was confirmed through clinical symptoms such as exhaustion, lack of appetite, drop in mobility, and decreased alkaline phosphatase level at 3 months. In the literature, the onset of lathyrinic effects might occur at different time points.9,31 This might be due to differences in the animal model, weight, age, type of lathyrogenic agent used, and the β-APN dosage applied. A dog model was chosen for this study because of its similarities to humans.

The probing depth found 3 months after placement of the implant in the test group dog was statistically deeper than that of the second month (P < .05). This might be due to the effect of the lathyrogenic agent on the collagen fiber cross-linking surrounding the implant. This caused functional deformation of the soft tissue surrounding the implant, hence leading to deeper periodontal probing. Literature has shown that probing depth in healthy peri-implant mucosa varies between 0.5 and 2 mm around the implant, while in severe mucositis probing depth increases up to 4 mm, and in peri-implantitis it may reach 6 mm.34 In the present study, probing depths around control implants were 2.0 ± 0.6 mm; however, in the test group it measured 4.1 ± 0.4 mm with a statistically significant difference (P < .05), in agreement with published data.34

It has been shown that the rich connective tissue of fibroblasts forms a soft tissue barrier around implants, where fibroblasts found both parallel and vertically oriented on the implant surface play a significant role in fiber formation and orientation.35,36 In our study, β-APN affected the fibroblastic activity, which may have caused the deterioration of the supracrestal connective tissue around the implant, which resulted in the increase in probing pocket depth. Moreover, in the histologic examination of the present study, the test group’s connective tissue fibers surrounding implants were found less than that in the control group. This finding further supported our hypothesis.

The mechanical properties of the implant bone connection and the determination of the degree of osseointegration was mostly evaluated by histomorphometric analysis.37 In this study the BIC rate and the density rate of the bone indicating the values were recorded. Data from this study showed BIC values of the test group implants were lower than those of the control group; however, the difference was not significant (P > .05). This was in agreement with the findings of Novaes et al38 and Balatsouka et al39 who studied the

<table>
<thead>
<tr>
<th>Region</th>
<th>Control Implants (Ratio ± SD), mm</th>
<th>Test Implants (Ratio ± SD), mm</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesial</td>
<td>4.48 ± 0.88</td>
<td>9.17 ± 1.5</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Distal</td>
<td>4.73 ± 1.65</td>
<td>10.65 ± 4.27</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

TABLE 2

Biostatistical analysis of histologic peri-implant mucosal width
osseointegration of oral implants in infected sides and in patients who smoked, respectively. As far as we are concerned, this was the first study being performed in lathyritic dogs.

Results from this study revealed that the supracrestal collagen fibers around test implants were loose or completely absent. The peri-implant sulcus had a dense infiltration of inflammatory cells. Broad and round bodies resembling fat cells covering the subepithelial connective tissue were observed. In the connective tissue around the implant, a dense inflammatory infiltrate existed. These findings were not present in the control group. A possible hypothesis to explain the inflammatory infiltrate and bone loss in the test group implants is the lack of connective tissue sealing due to the absence of collagen cross-linking caused by the lathyritic agent. These mechanisms could be compared to the collagen disruption induced by the periodontal pathogenic bacteria producing collagenase.

In the histopathologic findings of this study, the bone type and bone quality around implants were similar between the control and test implants. Infected cell infiltration, resorption in alveolar bone, and organizational deformation of the periodontal ligament had been reported in studies conducted on lathyritic rats. However, there was no study examining implant osseointegration in these test animals. In our study, no differences in the fiber organization and periodontal ligament distance were observed on the control and test dog’s teeth.

For the test implants, at the crestal level an infrabony pocket was observed, which represents active bone resorption. Additionally, there was disorientation of supracrestal collagen fibers and the occurrence of pocket epithelium, which presents a threat to the long-term osseointegration of the implant.

In this preliminary experimental study, β-APN was found to influence peri-implant subepithelial connective tissue, but no effect was shown on bone structure. Poor collagen content around peri-implant subepithelial connective tissue might lead to early stage implant failure. In conclusion, lathyrism is a potentially threatening factor that may cause failure of dental implants.

Our results suggested that when performing dental implant surgery, special care must be taken for patients presenting with impaired collagen synthesis. Future studies with expanded control of confounding factors and intervention studies may add to the understanding of this disease and its implications for implant dentistry.

**ABBREVIATIONS**

β-APN: beta-aminoproprionitrile
BIC: bone to implant contact
BV: bone volume

**ACKNOWLEDGMENTS**

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