Effects of Daidzein on the Production of Type I Collagen and Matrix Metalloproteinase1 by Stretched Human Periodontal Ligament Cells

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Abstract
Purpose: Relapse is clinical problems in orthodontic treatment, and the cause is not clear (1). The release of accumulated mechanical tension on the collagen fibers in the periodontal ligament (PDL) is thought to be one of the factors associated with relapse. Daidzein is extracted from soybeans and which is involved in collagen turnover of skin. However, little is known about the effects of daidzein on the collagen metabolism in human PDL(hPDL) cells. Then, we investigated the effects of daidzein on the release and the expression of collagen type I(COL-I) and matrix metalloproteinase(MMP)-1 in stretched hPDL cells.

Materials and Methods: hPDL cells were subjected to tension (10%) using cell stretch-chambers, after 12 hours, the cells were treated daidzein (50μg/ml) for 48 h. The release and gene expression of COL-I and MMP1 in stretched hPDL cells treated with daidzein were examined using enzyme-linked immunosorbent assays and a real-time PCR.

Results: The daidzein + TF group increased the gene expression of COL-I and MMP1 in comparison to the corresponding TF group in a time-dependent manner for up to 24 h. The release of COL-I and MMP1 from the daidzein + TF group were increased in comparison to the corresponding TF group in a time-dependent manner for up to 48 h. The relative COL-I and MMP1 mRNA expression in treatment of daidzein were significantly higher than in the not treatment of daidzein group.

Conclusion: The results in this study showed that daidzein regulates the collagen metabolism in stretched hPDL cells via the release and expression of COL-I and MMP1.

Keywords: periodontal ligament, daidzein, collagen type I, matrix metalloproteinase, relapse.

Introduction
Relapse is one of major clinical problems in orthodontic treatment, and the cause is not clear (1). The tension on periodontal tissues would therefore remain at a certain level when the orthodontic appliance is removed, eventually resulting in relapse. This is thought to be one of the factors associated with relapse. The trans-septal fibers in the PDL connect from the cementum to the adjacent cementum and from the cementum to the gingival papillae (2). The trans-septal fibers in the PDL consist of collagen and oxtalan fibers. These fibers play an important role in stabilizing the tooth position and are involve with relapse after orthodontic tooth movement (3–4). The PDL is composed of an abundance of types I and III collagen (COL-I and III), which are degraded by matrix metalloproteinase (MMP) (5). The precise regulation of the gene expression of MMP in relation to the collagen gene expression is critical for tissue repair and homeostasis as its dysregulated expression might lead to pathological events (6). COL-I and MMP1 are increased on both the tension and compression sides during orthodontic tooth movement (7). These reports suggest, we thought that extension of PDL after tooth movement is one of the important factors related to relapse.

Daidzein is a compound with one isoflavone that is extracted from soybeans. The structure of daidzein
resembles that of the female hormone estrogen. Daidzein has been shown to stimulate skin collagen synthesis in cultured skin fibroblasts via the TGF-β/smab signaling pathway in vitro\(^8\). In addition, daidzein is modulated by solar ultraviolet light-induced matrix metalloproteinase (MMP)1 in normal human dermal fibroblasts\(^8\). Little is known about the preventive effects of daidzein against relapse in patients who have undergone orthodontic treatment.

In the present study, we investigated the effects of daidzein on the release and expression of COL-I and MMP1 in stretched hPDL cells in vitro.

**Materials and Methods**

**Cell culture**

The hPDL cells were prepared according to a modification of the method described by Somerman et al.\(^9\). Informed consent was obtained from the patients or guardians prior to the extraction of hPDL cells. hPDL tissues were then taken from the roots of premolars extracted from healthy young volunteers (age: 12-20 years) during the course of orthodontic treatment. The study protocol was reviewed by the Ethics Committee of Nihon University School of Dentistry at Matsudo (approval No. EC15-002).

The hPDL cells culture performed according to the method of Takano et al.\(^10\). hPDL cells at passages 6-9 were used for all of the experiments.

**The treatment of hPDL cells with daidzein after the application of tension**

We used a STREX-chamber (STREX Co, Osaka, Japan) as stretch-chamber in order to simulate orthodontic movement. This was based on the method of Takano et al.\(^10\). We used the stretch-chambers tension (10%) to the hPDL (Fig. 1). Long et al. previously reported that 10% tension can be effectively applied by the utilized method in order to create tension stress\(^11\). Before starting the experiment, the hPDL cells (7 × 10⁴ cells/well) were transferred to the stretch-chamber. Confluent-stage cells were subjected to tension (10%) for 12 hours in culture medium containing 1% FCS, after which they were treated with daidzein (50µg/ml) and incubated for 48 hours in the presence of it. The groups were divided 3 groups as follow: the tension force group (without treatment of daidzein + without tension force), the daidzein group (treatment of daidzein + without tension force), and the daidzein + TF group (treatment of daidzein+ with tension force).

The cells in confluent were to determine the time-course of the effects, the hPDL cells were treated with daidzein (50µg/ml), which was added to the supernatant for the indicated times (0-48 h). After incubation, the conditioned culture media were collected for enzyme-linked immunosorbent assays (ELISA), and total RNA was extracted for a real-time PCR.

**The ELISA**

The levels of COL-I and MMP1 supernatant proteins were measured using a COL-I ELISA Kit (ACEL, Kanagawa, Japan) and MMP1 ELISA Kit (R&D System, MN, USA),
The PCR primers were designed with reference to the respective cDNA sequences as Table 1.

<table>
<thead>
<tr>
<th>COL-I</th>
<th>Fw: 5’-CCCCGGTTTCAGAGACAACCTTC-3’</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rv: 5’TCCACATGCTTTATTCCAGCAATC-3’</td>
</tr>
<tr>
<td>MMP1</td>
<td>Fw: 5’-ACAACGTGCAATTGGGCTTG-3’</td>
</tr>
<tr>
<td></td>
<td>Rv: 5’-CTGTCCCTGAACAGCCAGTACTTA-3’</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Fw: 5’-GCACCCTCAAGGCTGAGAA-3’</td>
</tr>
<tr>
<td></td>
<td>Rv: 5’-TGGTGAAGACGCGTGAAC-3’</td>
</tr>
</tbody>
</table>

A: COL-I

B: MMP1

Fig. 2. Time course effect of daidzein on mRNA expression of COL-I and MMP1 in stretched hPDL cells. The COL-I and MMP1 mRNA expression the daidzein + TF group was significantly increased, as compared with the TF groups (*p<0.05,** p<0.01).

according to the manufacturers’ protocols.

The real-time polymerase chain reaction (PCR)

The real-time PCR was also performed according to the method of Takano et al. (10). We used the primer sequences designed by Takano et al. (10) (Table 1).

Statistical analysis

Statistical analysis was performed using the JMP software (SAS, Inc., 2012). The results were analyzed by the Mann–Whitney test as a nonparametric method. P values of <0.05 were considered statistically significant.

Results

Effects of daidzein by collagen metabolism of PDL

The daidzein + TF group increased the gene expression of COL-I and MMP1 in comparison to the corresponding TF group in a time-dependent manner for up to 24 h (Fig. 2). The release of COL-I and MMP1 from the daidzein + TF group were increased in a time-dependent manner for up to 48 h. The release of COL-I and MMP1 in daidzein + TF group were significantly higher than in the TF group (Fig. 3).

Further, the gene expression of COL-I and MMP1 in compared the stretched culture hPDL cells treated with daidzein with the stationary culture hPDL cells treated with daidzein. The daidzein + TF group of the relative COL-I and
MMP1 mRNA expression was 3.16 and 3.87, which was 1.25 and 1.3 times that of daidzein group. In spite of culture, the relative COL-I and MMP1 mRNA expression in treatment of daidzein + TF group were significantly higher than in the TF group (*p<0.05).

Fig. 3. Time course effect of daidzein on protein level of COL-I and MMP1 in stretched hPDL cells. The release of COL-I and MMP-1 in daidzein + TF group were significantly higher than in the TF group (*p<0.05).

Fig. 4. The gene expression of COL-I and MMP1 in compared the stretched culture hPDL cells treated with daidzein with the stationary culture hPDL cells treated with daidzein. The relative COL-I and MMP1 mRNA expression in treatment of daidzein were significantly higher than in the not treatment of daidzein group (*p<0.05, **p<0.01).

(**p<0.005, **p<0.01)**

Discussion

One of factors in the relapse is involved in the memory of PDL fibers[1–3]. In the present study, we used an *in vitro* model which hPDL cells were subjected to tension (elongation by 10%) as assuming tooth rotation during orthodontic movement. We previously reported that relaxin regulated the collagen metabolism in stretched hPDL cells (10% elongation), and suggested that it may be for preventing orthodontic relapse (10). As the 10% elongation of hPDL cells can reproduce the tension that causes rotation during orthodontic tooth movement, 10% stretching was applied in the present study.

Zhao et al. (8) reported that daidzein increased the expression of type I procollagen in mouse skin fibroblasts, meanwhile, the expression of MMP1, and MMP2 was significantly inhibited. Gopaul et al. (12) showed that equol, a plant- and intestinal flora-derived isoflavonoid molecule, significantly stimulated the expression of COL-I and COL-
III in human skin fibroblasts, while MMPs were significantly decreased in comparison to control values. These findings support our results that daidzein increased the production of COL-I by skin fibroblasts, while negating the finding that daidzein increased the production of MMP1 by the cells. Considering the contradictory results with regard to MMP1, Svoboda et al. (13) reported that the collagen turnover of PDL cells is faster than that of skin fibroblasts.

The results showed the value of the release and expression of COL-I and MMP1, and the value of daidzein + TF group were higher than daidzein group in hPDL cells in vitro. Thus, daidzein may stimulate collagen metabolism in stretched hPDL cells through the increasing of release and expression of COL-I and MMP1. Further studies are necessary to investigate the prevent effects of daidzein on relapse after orthodontic rotation movement in vitro. In the near future, daidzein may be expected as a prevent medicine of relapse after orthodontic rotation movement.

The PDL is composed of COL-I as well as other types of collagen, such as COL-III, V, VI, XII, and XIV, which are expressed during experimental tooth movement (14-16). However, tissue inhibitors of metalloproteinases (TIMPs) also play important roles in collagen metabolism. A recent study found that daidzein strongly suppressed the gene expression of TIMP-1 in angiotensin II-induced abdominal aortic aneurysm mice (17). Therefore, additional studies are necessary to examine the effects of daidzein on the modulation of other types collagens and the MMP/TIMP complex in hPDL cells.

Conclusions
Daidzein regulates the collagen metabolism in stretched hPDL cells through increasing of the release and expression of COL-I and MMP1.

Conflict of interest
We declare that there are no conflict of interest in this paper.

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References


