Localisation of Decorin in Leptin–Treated Traumatic Oral Ulcer in Rats

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Abstract

**Background:** The oral mucosa is mainly exposed to injury by trauma or pathologic diseases. Leptin is a hormone known to have many physiological roles that affect the cell function and, acts as wound healing accelerator. The aim of the present study is to evaluate the effect of porcine recombinant leptin on induced traumatic oral ulcer healing by means of immunohistochemical localization of Decorin.

**Materials and methods:** Forty-eight male albino rats aged between 2-3 months were used in this study. They were maintained under control conditions of temperature, drinking and food consumption. They were subjected to traumatic ulcer by surgical blade on the right side of the buccal mucosa by surgical blade (no.15), with diameter of (8 mm). The animals were divided into two groups: control group: the ulcer treated with sterilized distilled water; the experimental group: the ulcer treated with 10µl of 1µg/ml recombinant leptin. The rats were sacrificed at 3, 7, and 10 days. Immunohistochemical methods were used to detect the expression of decorin in both control and study groups.

**Results:** The present study showed that the recombinant leptin treatment increased expression of decorin in ulcer area from the 3rd day of ulceration by epithelial cell, endothelial cells, fibroblast cells, with highly significant differences in comparison with control group.

**Conclusion:** Leptin accelerated the healing process in oral mucosa ulcer by increase the expression of decorin in early healing period than control.

**Key words:** leptin, oral mucosa, ulcer, Decorin

INTRODUCTION

Any wound healing is a series of biological processes, include migration, adhesion, proliferation, and differentiation of several cell types. All these activities are triggered by chemo-attraction of the cells; polypeptide mediators bind to their cell-surface receptors, integrins bind to extracellular matrix components, and different growth factors regulate different cell functions. The process ending with the formation and maturation of a new extracellular matrix (ECM). The extracellular matrix (ECM) contains a collection of molecules that regulate both structural integrity and function of the cell. The most extensively studied member of the ECM class is decorin which is a small leucine-rich proteoglycan (SLRP). Decorin interacts with a variety of different ligands including; other ECM constituents, cellular receptors, growth factors, proteases, and other signaling molecules; to regulates different cellular processes including angiogenesis, innate immunity, inflammation, wound healing, and isolates the growth factors. Leptin, a 16 kDa anti-obesity hormone produced predominantly by adipose tissues and secreted into the blood stream as a free, or as a protein. In addition to its influences on the body weight homeostasis, it also exhibit a variety of physiological actions such as lactation, bone formation, angiogenesis, and wound healing. The multi-functionality of leptin and it plays a variety of physiological roles not only as a systemic hormone but also as a local growth factor.

MATERIALS AND METHODS

Forty-eight albino rats weighting (200-270 gm), aged (2-3) months were used in this study. They maintained under control conditions of temperature, drinking and food consumption. All experimental procedures were carried out in accordance with the animal experimentation ethical principles of the Biotechnological Research Center at Al-Nahrain University.

**Induction of oral ulcer**

Ulceration of the oral mucosa of each rats in this study were done by the following steps: First the animal anesthetized via intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The mucosal ulceration with 8 mm diameter was performed on the right side of the buccal mucosa by abrasiun with a surgical scalpel blade (no.15). The control group (24 rats): the ulcers treated with 10 µl of sterilized distilled water. While the Experimental group (24 rats): the ulcers treated with 10 µL of 1 µg/ml recombinant leptin protein from Abcam company UK (ab646). Then the animals were sacrificed according to 3 healing intervals into 3, 7, and 10 days (16 rats from both groups in each period). Then the specimens from each rats were taken and prepared for histological (H&E stain) and for immunohistochemical localization of decorin by using of Anti-Decorin antibody, Rabbit polyclonal, (ab175404), and detection kit (ab80436) from Abcam company UK.

**Determination of immunohistochemical results for decorin**

Under light microscope at x40, a five fields were chosen from epithelium area, and another five fields were chosen from connective tissue from each tissue section, captured by digital camera and the images evaluated imported to computer. The evalation of staining results was achieved by applying Aperio positive pixel count algorithms program (from Aperio Image Scope software v11.1.2.760 (Aperio Technologies Inc, USA)), we neglected the weak positive reading in yellow color. The average of mean positive percentages for each five area were obtained and considered as the value of expression of Decorin per slide.

**RESULT:**

**Decorin expression**

At 3rd day in control group, weak positive reactivity to decorin was seen in suprabasal layer of the new front epithelium and stromal cells of lamina properia(Fig.1A&B). In the study group, strong positive membranous expression for decorin were seen in spinous layer of the new epithelium and in collagen fibers and stromal cells of lamina properia (Fig.1C&D).

At 7th day in the control group, moderate positive expression for decorin was detected in the spinous and granulomas layers of new epithelium as well as in fibroblast cells, endothelial cells, and in collagen fibers of lamina properia (Fig.2A&B). In the study group, strong positive reaction to the decorin can be observed in spinous and granulomas layers of epithelium and in endothelial cells, fibroblasts and collagen fibers of lamina properia (Fig 2C&D).

At 10th day in the control group, strong reactivity to the decorin was oberviously seen in the epithelium. The lamina properia also showed strong expression in the collagen fibers and endothelial
cells (Fig. 3A&B). In the study group, weak positive expression was seen in the granulosum layer of epithelium tissue only. In the lamina propria it was limited to the collagen fibers and fibroblast (Fig. 3 C&D).

**Statistical analysis of immunohistochemical result**

The mean difference between control and study group was illustrated in Table-1, which showed highly significant differences between study and control group in the expression of decorin in epithelial cells at 3rd day and 10th day, and significant at 7th day. For the lamina propria, the result showed significant differences between study and control group in the expression of decorin in all healing periods.

Table-2 showed that there were highly significant differences between epithelium and lamina proeria in expression of decorin in control group at 3rd and 10th day, and significant at 7th day. For study group the result revealed highly significant differences between epithelium and lamina proeria in expression of decorin at 3rd day and significant differences between them in 7th and 10th days.

Regarding to the duration differences in each control and study group, the ANOVA test was used as shown in Table-3. For control group both the epithelium and lamina proeria showed highly significant differences. For study group, epithelium showed highly significant differences between duration, while in lamina proeria showed non-significant difference.

**Figure 1 A:** Decorin expression at 3rd day in epithelium of control group x40.

**Figure 1 B:** Decorin expression at 3rd day in lamina preria of control group x40.

**Figure 1 C:** Decorin expression at 3rd day in study group in epithelium tissue x40.

**Figure 1 D:** Decorin expression at 3rd day in study group in lamina properia x40.

**Figure 2 A:** Decorin expression at 7th day in control group in epithelium tissue x40.
Figure 2B: Decorin expression at 7th day in control group in lamina propria x40.

Figure 2C: Decorin expression at 7th day in study group in epithelium tissue x40.

Figure 2D: Decorin expression at 7th day in study group in lamina propria X40.

Figure 3A: Decorin expression at 10th day in control group in epithelium tissue X40.

Figure 3B: Decorin expression at 10th day in control group in lamina propria.

Figure 3C: Decorin expression at 10th day in study group in epithelium tissue.
positive reaction of decorin in epithelial at 3rd day was strong in group. Our result disagree with previous studies (27, 28) who
Decorin is a member of small-leucine rich proteoglycan, linked to glycosaminoglycan chain (GAG), either chondroitin sulfate (CS) in inflammation, cell differentiation, proliferation, adhesion, decorin can regulates different cellular processes such as
Figure3.D: Decorin expression at 10th day in study group in lamina prperia.

Table 1: Groups’ comparison for Positive cells expressed decorin in both epithelium and lamina properia at each duration

<table>
<thead>
<tr>
<th>Day</th>
<th>Site</th>
<th>Control Mean</th>
<th>Control SD</th>
<th>Study Mean</th>
<th>Study SD</th>
<th>T-test</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>3rd</td>
<td>Epithelium</td>
<td>8.375</td>
<td>4.519</td>
<td>35.65</td>
<td>10.35</td>
<td>7.184</td>
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<tr>
<td></td>
<td>Lamina properia</td>
<td>11.68</td>
<td>4.709</td>
<td>28.67</td>
<td>10.92</td>
<td>3.343</td>
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<tr>
<td>7th</td>
<td>Epithelium</td>
<td>23.51</td>
<td>4.230</td>
<td>36.98</td>
<td>9.368</td>
<td>4.133</td>
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<td></td>
<td>Lamina properia</td>
<td>19.981</td>
<td>7.230</td>
<td>34.015</td>
<td>7.659</td>
<td>3.505</td>
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<td>10th</td>
<td>Epithelium</td>
<td>36.731</td>
<td>9.304</td>
<td>15.256</td>
<td>9.552</td>
<td>5.343</td>
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<tr>
<td></td>
<td>Lamina properia</td>
<td>29.771</td>
<td>7.992</td>
<td>36.51</td>
<td>5.776</td>
<td>1.939</td>
<td>0.094</td>
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Table 2: Sites’ comparisons for Positive cells expressed decorin in both groups at each duration

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>Mean different</th>
<th>SE</th>
<th>T-test</th>
<th>P-value</th>
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<td>5.836</td>
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<tr>
<td>10th</td>
<td>Control</td>
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<td>4.290</td>
<td>5.588</td>
<td>0.001</td>
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<td>Study</td>
<td>1.907</td>
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<td>1.909</td>
<td>0.094</td>
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Table 3: ANOVA test for duration differences in epithelium and lamina properia in both groups

<table>
<thead>
<tr>
<th>Marker</th>
<th>Groups</th>
<th>F-test</th>
<th>P-value</th>
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<td>Decorin</td>
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<td>Lamina properia</td>
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<td>Study</td>
<td>12.308</td>
<td>&lt;0.001</td>
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<td></td>
<td>Lamina properia</td>
<td>1.900</td>
<td>0.174NS</td>
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DISCUSSION:
Decorin is a member of small-leucine rich proteoglycan, linked to glycosaminoglycan chain (GAG), either chondroitin sulfate (CS) or dermatan sulfate(DS), this linking gave decorin the ability to bind to other ECM molecules and to several growth factors decorin can regulates different cellular processes such as

CONCLUSION:
According to our knowledge the present study is the first one that evaluated the effect of topical application of leptin on abrasion mucosal ulcer healing , and assessment the expression of decorin in trumatic ulcer healing. Although previous study reported that decorin expression in incisional wound cant be detected until day 7th. We observed in our study that decorin expression detected at 3rd day in the study group treated with leptin.
REFERENCES


