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

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Abstract

Back ground: The oral mucosa mainly exposed to injury by trauma or pathologic diseases, Leptin is a hormone known to has many physiological roles that effects the cell function and, acts as wound healing accelerator. The aim of the present study is to evaluate the effect of topical application of recombinant leptin on induced traumatic oral ulcer healing by mean of immunohistochemical localization of Decorin.

Materials and methods: Forty eight male Albino rats age between 2-3 months with body weight between (200.45-270.53g),were subjected for 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 divided into two groups ;control group : the ulcer treated with sterilized distal water, the experimental group: the ulcer treated with 10µl of 1 µg/ml *recommbenant leptin*.The rats were sacrificed at 3,7,10 days. Immunohistochemical methods were used to detect the expression of decorin in both control and study groups .

Results: The present study showed that *the recompenant leptin* treatment increased expression of decorin in ulcer area from the 3rd day of ulceration by epithelial cell, endothelial cells, fbroblast 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 [1,2,3] .

The extracellular matrix (ECM) contains a collection of molecules that regulate both structural integrity and function of the cell [4]. The most extensively studied member of the ECM class is decorin which is a small leucine-rich proteoglycan (SLRP) [5] . Decorin interact with a variety of different ligands including; other ECM constituents, cellular receptors, growth factors, proteases, and other signaling molecules; to regulates different cellular processes [6,7] including angiogenesis [8,9] innate immunity [10], inflammation [11,12] , fibrosis[13] ,wound healing [14] . Decorin has ability to activate or inhibit receptor signaling [15], and isolates the growth factors [16].

Leptin, is 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 [17]. Its also exhibit a different physiological actions such as hematopoiesis [18], bone formation [19], angiogenesis [20], and wound healing [21]. 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 [22].

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 Biotechnical 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 anesthetize via intrapretoneal injuction of ketamine (50 mg/kg) and xylazine (5 mg/kg) [23]. The mucosal ulceration with 8 mm diameter was performed on the right side of the buccal mucosa by abarasion with a surgical scalpel blade (no.15) [24]. The control group(24 rats): the ulcers treated with 10µl of sterilized distal water. While the Experimental group(24 rats):the ulcers treated with 10µL of 1µg/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 periods). Then the specimens from each rats were taken and prepared for histological (H&Estain) 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 evalution 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 spinosum 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, moderat positive expression for decorin was detected in the spinosum and granulosum 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 spinosum and granulosum layers of epithelium and in endothelial cells, fibroblasts and collagen fibers of lamina properia (Fig2 C&D).

At 10th day in the control group, strong reactivity to the decorin was obeviously 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 properia 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 illusetrated 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 properia ,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 difference 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 properia 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 properia showed highly significant differences.For study group, epithelium showed highly significant differences between duration ,while in lamina properia showed non - significant difference.

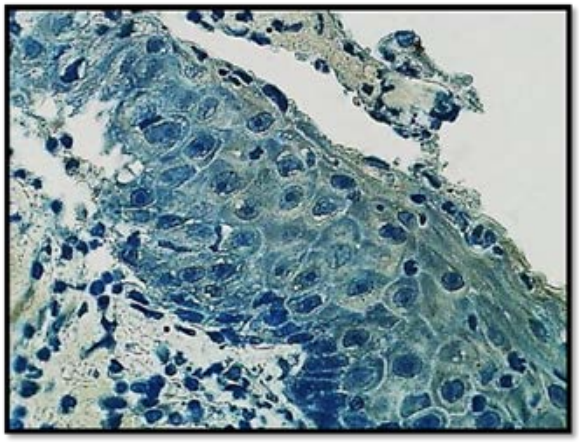

Figure1 A: Decorin expression at 3rd day in epithelium of control group x40.

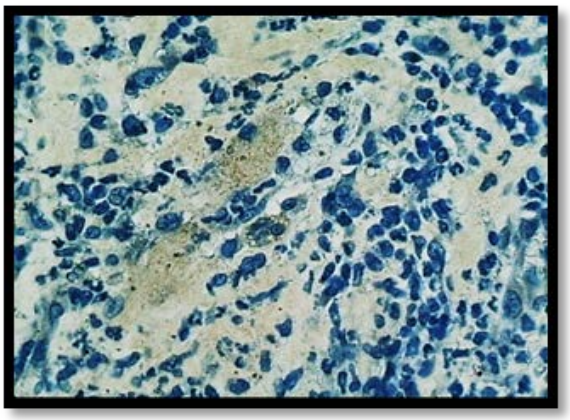

Figure1B: Decorin expression at 3rd day in lamina prperia of control group x40.

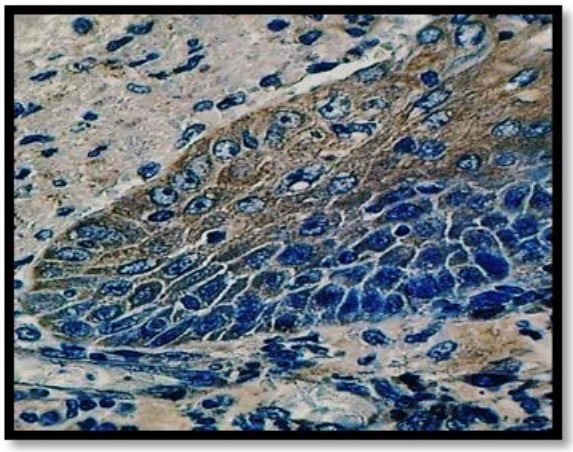

Figure 1 C : Decorin expression at 3rd day in study group in epithelium tissue x40

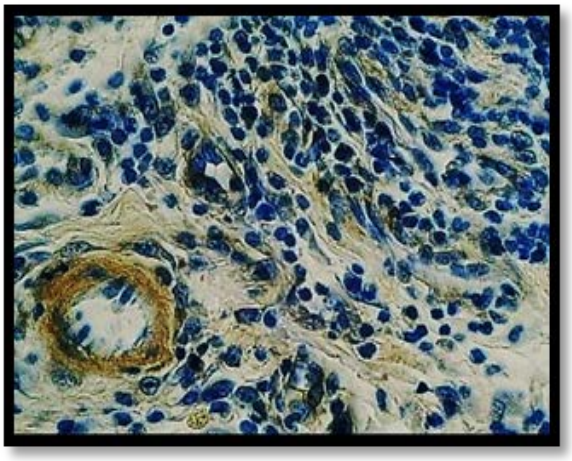

Figure1D: Decorin expression at 3rd day in study group in lamina properia x40.

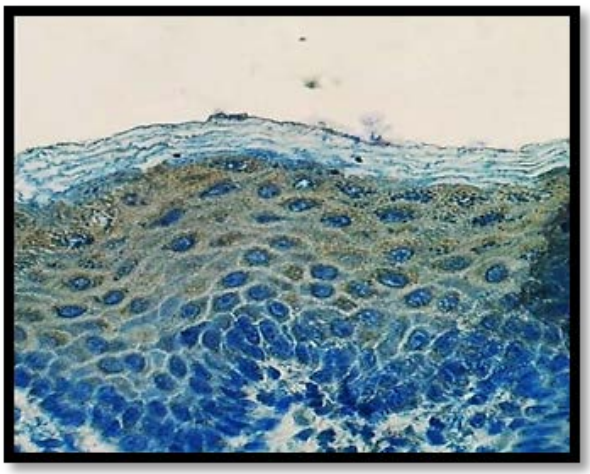

Figure 2.A: Decorin expression at 7th day in control group in epithelium tissue x40.

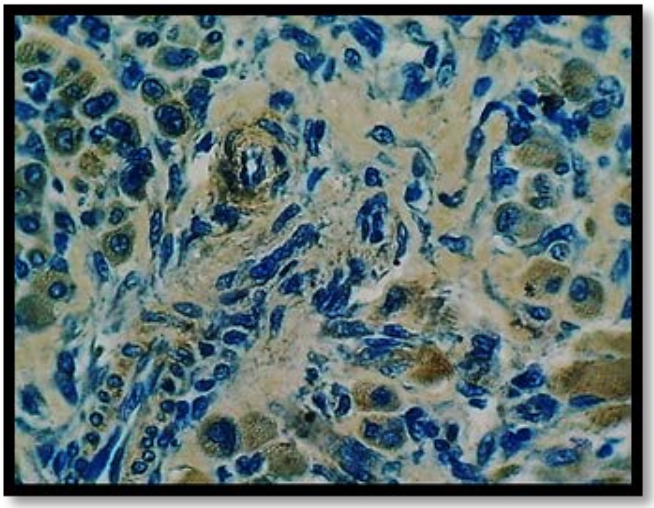

Figure 2B: Decorin expression at 7th day in control group in lamina prperia x40.

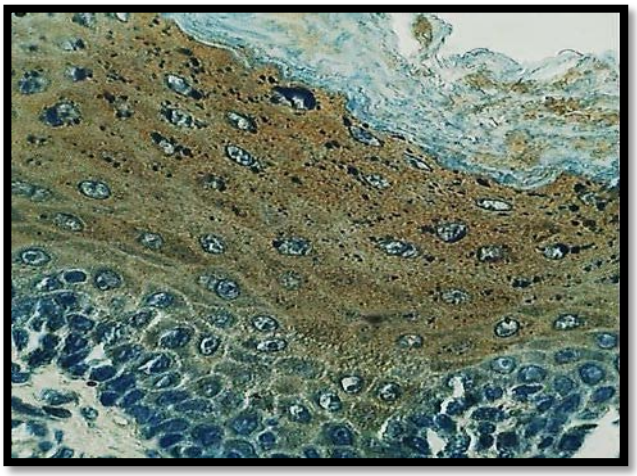

Figure3.A: Decorin expression at 10th day in control group in epithelium tissue X40

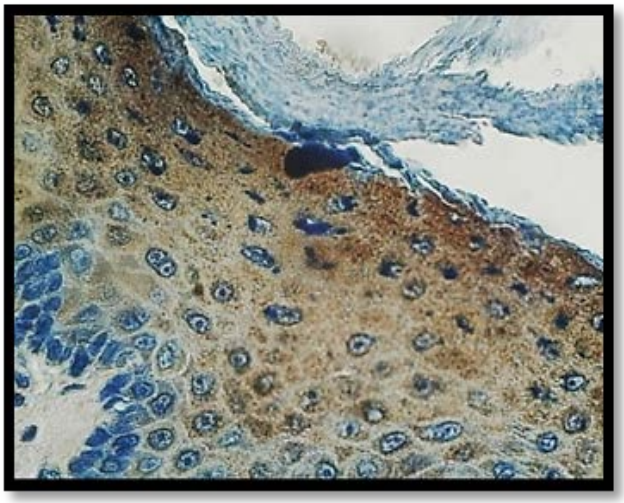

Figure 2.C: Decorin expression at 7th day in study group in epithelium tissue x40.

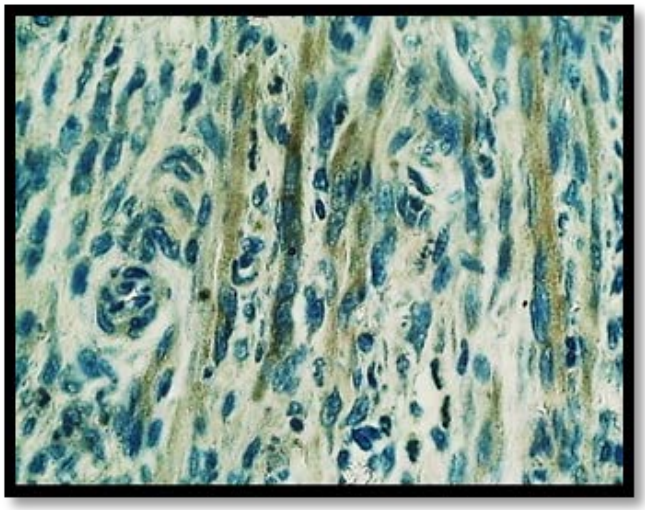

Figure3.B: Decorin expression at 10th day in control group in lamina properia.

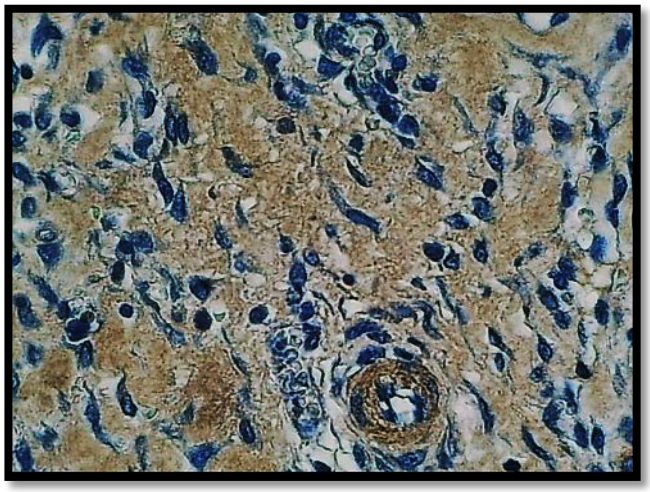

Figure 2.D: Decorin expression at 7th day in study group in lamina properia X40.

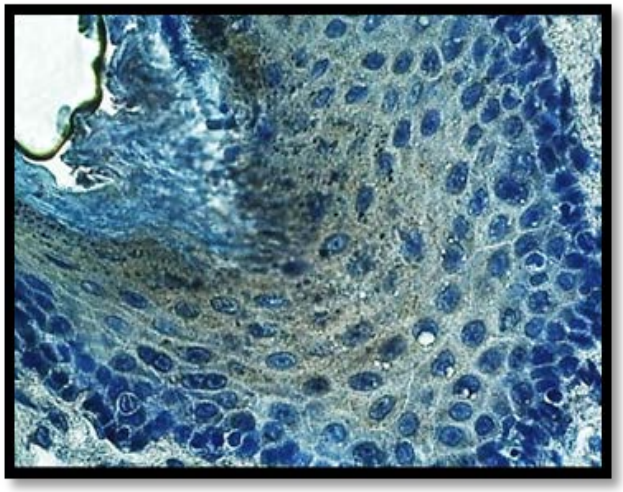

Figure3.C: Decorin expression at 10th day in study group in epithelium tissue.

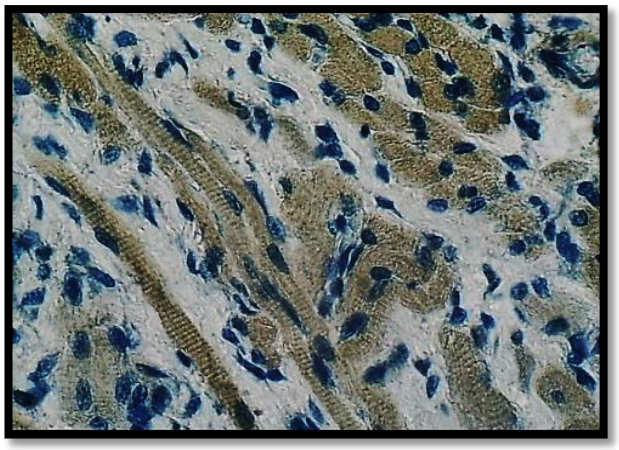

Figure3.D: Decorin expression at 10th day in study group in lamina prperia.

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

Day	Site	Control		Study		T-test	P-value
		Mean	SD	Mean	SD		
3rd	Epithelium	8.375	4.519	35.65	10.35	7.184	0.000 HS
	Lamina properia	11.68	4.709	28.67	10.92	3.343	0.012 S
7th	Epithelium	23.51	4.230	36.98	9.368	4.133	0.004 S
	Lamina properia	19.981	7.230	34.015	7.059	3.505	0.010 S
10th	Epithelium	36.731	9.304	15.256	9.552	5.343	0.001 HS
	Lamina properia	29.771	7.992	36.51	5.776	1.939	0.094 S

Table 2:Sites' comparisons for Positive cells expressed decorin in both groups at each duration

Day	Group	Mean different	SE	T-test	P-value
3rd	Control	-20.09	3.443	5.836	0.001 HS
	Study	21.863	1.350	16.195	0.000 HS
7th	Control	-12.427	3.763	3.304	0.013 S
	Study	7.543	2.773	2.719	0.030 S
10th	Control	23.975	4.290	5.588	0.001 HS
	Study	1.907	0.999	1.909	0.094 S

Table 3: ANOVA test for duration differences in epithelium and lamina properia in both groups

Marker	Groups		F-test	P-value
Decorin	Control	Epithelium	61.892	P<0.001HS
		Lamina prperia	14.214	P<0.001HS
	Study	Epithelium	12.308	P<0.001HS
		Lamina properia	1.900	0.174NS

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 inflammation, cell differentiation , proliferation, adhesion, migration, and fibrillogenesis [25,26]. In the present study the positive reaction of decorin in epithelial at 3rd day was strong in the study group with highly significant difference than control group. Our result disagree with previous studies [27, 28] who reported that the decorin was not detected in incisional skin wound until 7th day. While At 10th day the decorin expression in the study group showed lowest mean positive percentage, and the expression restricted to the suprabasal layers only**,** this agree with previous study done by **[29]**, while the control group recorded a gradual elevation in the mean positive percentages of decorin expression in epithelial cells.

The decorin expressed where there is active proliferation and migration in keratinocytes which was seen in study group that treated with leptin at 3rd day more obvious than control group ,and then its expression decreased and become less than control group with high significant difference as the keratinocytes reach to full maturity and nearly full thickness of epithelium. This result agree with[30] who found that the DS - decorin is a pivotal for keratinocyte proliferation and differentiation, suggesting that DS of decorin is playing an important role in wound healing .Järveläinen *et al*., 2006.[15] who demonstrated that the wound healing was delayed in decorin null mice because of impairment of keratinocyte differentiation. Decorin expression was detected in the the lamina properia in; inflammatory cells , extracellular collagen matrix, fibroblast cells, and endothelial cells at 3rd day in leptin-treated group with significant differences in comparing with the control group who showed primarily weak, and mainly abscente expression at 3rd day. Again our result disagree with Oksala *et al*.,1995 and Alian *et al*.,2000 both reported that the decorin was not detected in incisional skin wound until 7th day. While At 10th day decorin expression in connective tissue was restricted to the collagen fibers in the reticular area in agreement with Fleischmajer *et al*., 1991, and showed the highly significant difference in comparison with 3rd and 7th day.

Although decorin prevent macrophages proliferation by inhibits the macrophage colony- stimulating factor (M-CSF) , decorin enhance the macrophages adhesion , spreading and protect them from apoptosis, to allow the destruction of extravascular bacteria and bacterial elimination [31]. Decorin can increasing wound strength by promoting the accumulation of fibroblast cells in the wound associated with the increasing collagen deposition and formation of a mature extracellular matrix [31]. In an experimental study on mice the result showed that mice with decorin deficient exhibit extremely fragile skin that tears easily. In the histological examination there was irregular profiles and abnormal fibril fusion patterns **[32]**.

In angiogenesis decorin can promoting angiogenesis by control collagen type I fibril formation which provide a template for vascular tube formation **[33]**. Also decorin can involvement with formation of new blood vessles associated with inflammation [34] by prevent the endothelial cells apoptosis associated with inflammation **[35]**. Another roles of decorin in angiogenesis is by upregulates the vascular endothelial growth factor (VEGF) expression by activation of VEGF transcription via EGf-R signal transducer and activator of transcription 3 (Stat3), and enhanced angiogenesis. Decorin activates the proteolytic enzymes MMP2 and 9 which to degrade gelatins and collagens in basement membrane, to eliminate the matrix surrounding endothelial cells, and increasing shedding the matrix-bound VEGF, making it available to interact with the VEGF receptor and activate new blood vessles formation [36].

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.

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