IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 8 Ver. 2 (August. 2018), PP 86-89
www.iosrjournals.org

Comparison of Pathologic Effects of Hyaluronic Acid And Collagenase on Wound Healing of Rabbit Ear Cartilage Tissue

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Abstract
Objective: *We aimed to compare the effects of hyaluronic acid and collagenase on wound healing process of the rabbit ear cartilage tissue.*
Study Design: *Experimental animal study.*
Methods: *Eighteen New Zealand white male rabbits aged approximately 4 months and weighing from 2500 to 3000 g (mean 2800 g) were included in the experiments and randomly divided into three groups: control, hyaluronic acid and collagenase, each containing 6 rats. The wound was induced by scalpel and freer elevetor in both ears of each rabbit on which either hyaluronic acid, collagenase, or nothing(control group) was applied. Pathologic evaluations were performed 15 and 30 days after wound induction, and included the assesment of cartilage viability, inflammation, fibrosis, and glycosaminoglycan level.*
Results:*The scores for cartilage viability, fibrosis, inflammation and glycosaminoglycan did not show significant difference between the specimens obtained at 15 days and 30 days after wound induction (p>0.05 for all). There was also no significant difference between control, hyaluronic acid and collagenase groups in terms of mean scores of cartilage viability, fibrosis, inflammation and glycosaminoglycan in 15 days and 30 days (p>0.05 for all).*
Conclusion: *The findings of the present experimental study indicate that neither hyaluronic acid nor collagenase has any promoting effect on wound healing of ear cartilage tissue. However, further larger scale studies are needed to reach a definitive conclusion on the effect of hyaluronic acid or collagenase on wound healing process of ear cartilage tissue and to evaluate their use in clinical treatment of ear injuries.*
Keywords: *hyaluronic acid, collagenase, cartilage, ear, wound healing*
Level of Evidence: *NA*

Date of Submission: 25-07-2018 Date of acceptance: 10-08-2018

I. Introduction

Local application of collagenase or hyaluronic acid is a common and effective non-invasive technique used clinically to promote the healing process of various wounds.[1,2] It has been well-known that both collagenase and hyaluronic acid play an important role in wound healing by stimulating tissue regeneration and reducing formation of fibrin and exudate.[3] Hyaluronic acid is an endogenous substance and key component of extracellular matrix, which increases during the process of wound healing.[4]In vitro and clinical studies proved that hyaluronic acid and its derivatives activate fibroblast proliferation and new vessel formation during tissue repair and remodelling process.[4-6]Collagenase is an enzymatic debriding agent, which has also been shown to be effective in promoting wound healing.[7]

Although the effectiveness and safety of both hyaluronic acid and collagenase is well-documented, there are limited studies in literature comparing the effect of two products on the wound healing process of the cartilage tissue, particularly ear cartilage, which is a common site of injury.[7,8]

Therefore, in this study, we aimed to compare the effects of hyaluronic acid and collagenase on wound healing in short- and long-term in the rabbit ear cartilage tissue.

II. Materials And Methods

The animal experiments and procedures were performed in accordance with national guidelines for the use and care of laboratory animals, and were approved by the Bağcılar Reserch and Education Hospital Ethics Committee for Animal Research (date 18.5.2015.No.2015/66.). Eighteen New Zealand white male rabbits aged approximately 4 months and weighing from 2500 to 3000 kg (mean 2800 kg) were included in the experiments and randomly divided into three groups: control, hyaluronic acid and collagenase, each containing 6 rats. The wound was induced by scalpel and freer elevetorin both ears of each rabbit on which either hyaluronic acid, collagenase, or nothing(control group) was applied. Pathologic evaluations were performed 15 and 30 days after wound induction.

Histopathologic evaluation

After proper anesthesia, ear specimens were optained from each rabbit and fixed immediately in 10% buffered formaldehyde solution. They were then sampled in 2 mm transverse sections, and dehydrated and paraffinized in tissue processing machine (Leica ASP300 S, Wetzlar, Germany). Each sprecimen was embedded in paraffin blocks by Thermo Shendon Histocentre 3, from which three 4 µm sections were obtained by Thermo Rotary microtome. Each of three sections was stained with either hematoxylin and eosin (Sakura Tissue-Tek Film System) or Masson trichrome (Bio-Optica 04-010802 kit) or alcian blue (Bio-Optica 04-160802 alcian blue pH 2.5 kit). Histopathologic cartilage viability was evaluated by a pathologist at all areas of hematoxylin-eosin stained sections under Olympus CX41 Japan light microscope and scored between 0 and 6: 0 for common bone formation, 1 for cartilage resorption with multifocal bone formation, 2 for cartilage resorption with focal bone formation, 3 for common cartilage resorption, 4 for multifocal cartilage resorption, 5 for focal cartilage resorption, and 6 for normal viable cartilage tissue. According to severity, inflammation and fibrosis around cartilage were scored from 0 to 3 (none, mild, moderate, and severe). In the alcian blue stained sections, glycosaminoglycan content of cartilage tissue was graded as 3, 2, 1, or 0 for no, mild, moderate or severe decrease in glycosaminoglycan level, respectively.

Statistical methods

Study data were summarized using descriptive statistics, i.e. mean, median, standard deviation, minimum-maximum, frequency and percentage. For analysis of non-normally distributed data, Kruskal-Wallis and Wilcoxon signed rank tests were used for unpaired and paired (repeated measurements) data, respectively. Statistical analysis was performed with SPSS software (Statistical Package for Social Sciences, version 12.0, SPSS Inc., Chicago, Illinois, USA). Statistical level of significance was set to $p<0.05$.

III. Results

The distribution of scores for cartilage viability, fibrosis, inflammation and glycosaminoglycan did not show significant difference between the specimens obtained at 15 days (Figure 1,2,3) and 30 days (Figure 4,5,6) after wound induction ($p>0.05$ for all, Table 1). There was also no significant difference between control, hyaluronic acid and collagenase groups in terms of mean scores of cartilage viability, fibrosis, inflammation and glycosaminoglycan in 15 days and 30 days ($p>0.05$ for all, Table 2).

IV. Discussion

In this experimental study, we evaluated the effect of hyaluronic acid and collagenase application in comparison to control group on the wound healing process of ear cartilage tissue at 15 and 30 days after the wound induction. Our findings indicated that wound healing parameters did not show significant difference with time—between 15 days and 30 days. Furthermore, there was no remarkable difference between hyaluronic acid and collagenase in terms of their effects on wound healing process of ear cartilage including cartilage viability, fibrosis, inflammation, and glycosaminoglycan accumulation.

Collagenase with or without hyaluronic acid is commonly used in clinical practice to promote the healing process of various wounds.[3,4,9-11]Both collagenase and hyaluronic acid have modulatory effects on all stages of wound healing, which are inflammation, proliferation, and remodelling.[3,12,13]Jila et al.[7]studied the effect of collagenase on ischemic wound healing in ear tissue of rabbits and found that collagenase is superior to semi-occlusive dressing or petrolatum ointment in histological parameters of reepitalization and granulation tissue formation during wound healing. Riley and Herman[14] reported that collagenase promotes the cellular response to wound healing in vitro and in vivo.

While some mechanisms of healing of cartilage tissue have been determined, the complete biological processes and pathways underlying wound healing of cartilage tissue including articular cartilage and ear need to be elucidated.[8,15,16] In the present study, we evaluated and scored inflammation, and cartilage viability, fibrosis, and glycosaminoglycan accumulation, which indicate remodelling and proliferation of cartilage tissue.[17] Among these parameters, inflammation and fibrosis are common to healing process of all tissues, while cartilage viability and glycosaminoglycan accumulation are specific indicators of cartilage regenaration.[18] Cartilage viability assessment included histopathological evaluation of cartilage resorption and bone formation.

The main limitation of the study was its limited sample size and experimental design, which precludes us from reaching a definitive conclusion. However, this is the first study comparing the wound healing effects of collagenase and hyaluronic acid on the histopathology of ear cartilage tissue.

V. Conclusion

In conclusion, the findings of the present experimental study indicate that neither hyaluronic acid nor collagenase has any promoting effect on wound healing of ear cartilage tissue. However, further larger scale studies are needed to reach a definitive conclusion on the effect of hyaluronic acid or collagenase on wound healing process of ear cartilage tissue and to evaluate their use in clinical treatment of ear injuries.

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Figure Legends:

Figure 1: Control group 15.day: Fibrosis (arrow) and focal ossification (arrow) in cartilage resorbtion area. Hematoxylin Eosin stain X40

Figure 2: Hyaluronic acid 15. Day: mild fibrosis(star)in cartilage resorbtion area.Hematoxylin Eosin x40)

Figure 3: Collogenase group 15.day: Moderate fibrosis (star) and focal ossification(arrow) in cartilage resorbtion area. Hematoxylin Eosin X100

Figure 4: Control group 30.day: Glycosaminoglycan mild decrease(score 2) in cartilage tissue Alcian Blue x100

Figure 5: Hyaluronic acid 30.day: Moderate fibrosis(score 2) in resorbtion area of cartilage. Masson_trichrome X40.

Figure 6: Collogenase group 30.day: Ossification in cartilage resorbtion area. Hematoxylin Eosin x100

Table 1. Distribution of rabbit ear specimens at 15 and 30 days with respect to cartilage viability, fibrosis, inflammation and glycosaminoglycan scores

Cartilage viability score	Total (n=18)		Control (n=6)		Hyaluronic acid (n=6)		Collagenase (n=6)	
	15 days	30 days	15 days	30 days	15 days	30 days	15 days	30 days
Cartilage resorption with multifocal bone formation	1 (5.6)	-	1(16.7)	-	-	-	-	-
Cartilage resorption with focal bone formation	3 (16.7)	10 (55.6)	2(33.3)	4 (66.7)	-	2 (33.3)	1(16.7)	4 (66.7)
Common cartilage resorption	7 (38.9)	4 (22.2)	2(33.3)	1 (16.7)	3(50)	2 (33.3)	2(33.3)	1 (16.7)
Multifocal cartilage resorption	7 (38.9)	4 (22.2)	1(16.7)	1 (16.7)	3(50)	2 (33.3)	3(33.3)	1 (16.7)
P*			1.000		0.334		0.059	
Fibrosis score								
Mild	8 (44.4)	7 (38.9)	1(16.7)	1 (16.7)	4(66.7)	4 (66.7)	3(66.7)	2 (33.3)
Moderate	9 (50.0)	11 (61.1)	4(66.7)	5 (83.3)	2(33.3)	2 (33.3)	3(66.7)	3 (66.7)
Severe	1 (5.6)	-	1(16.7)	-	-	-	-	-
P*			0.655		1.000		0.317	
Inflammation score								
Mild	13 (72.2)	9 (50.0)	5(83.3)	2(33.3)	4(66.7)	4(66.7)	4	3(50.0)
Moderate	5 (27.8)	7 (38.9)	1(16.7)	4(66.7)	2(33.3)	2(33.3)	2	1(16.7)
Severe	-	2 (11.1)	6(100)	-	-	-	-	2(33.3)
P*			0.180		1.000		0.334	
Glycosaminoglycan score								
Mild	11(61.1)	9(50.0)	3	4(66.7)	4	3(50.0)	4	2(33.3)
Moderate	7(38.9)	9(50.0)	3	2(33.3)	2	3(50.0)	2	4(66.7)
P*			0.564		0.655		0.317	

* Wilcoxon test
Data are presented as number of specimens (%).

Table 2. The mean cartilage viability, fibrosis, inflammation and glycosaminoglycan scores of rabbit ear specimens at 15 and 30 days in study groups

	Control (n=6)	Hyaluronic acid (n=6)	Collagenase (n=6)	P*
15 days				
Cartilage viability	2.5±1.0 2.5 (1-4)	3.5±0.5 3.5 (3-4)	3.3±0.8 3.5 (2-4	0.159
Fibrosis	2.0±0.6 2 (1-3)	1.3±0.5 1 (1-2)	1.5±0.5 1.5 (1-2)	0.160
Inflammation	1.2±0.4 1 (1-2)	1.3±0.5 1 (1-2)	1.3±0.5 1 (1-2)	0.770
Glycosaminoglycan	1.5±0.5 1.5 (1-2)	1.7±0.5 2 (1-2)	1.7±0.5 2 (1-2)	0.802
30 days				
Cartilage viability	2.5±0.8 2 (2-4)	3.0±0.9 3 (2-4)	2.5±0.8 2 (2-4)	0.458
Fibrosis	1.8±0.4 2 (1-2)	1.3±0.5 1 (1-2)	1.7±0.5 2 (1-2)	0.213
Inflammation	1.7±0.5 2 (1-2)	1.3±0.5 1 (1-2)	1.8±1.0 1.5 (1-3)	0.521
Glycosaminoglycan	1.7±0.5 2 (1-2)	1.5±0.5 1.5 (1-2)	1.3±0.5 1 (1-2)	0.533

* Kruskall-Wallis test
Data are presented as mean±standard deviation, median (min-max).

Altun Hüseyin, "Comparison of Pathologic Effects of Hyaluronic Acid And Collagenase on Wound Healing of Rabbit Ear Cartilage Tissue."IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 17, no. 8, 2018, pp 86-89.