Research article

Histological evaluation of camel colostrum extracts effects as a new biological therapy on auricular cartilage defect healing.

Amir I. Towfik

Arabian Horses Center, University of Al-Qadisiyah, Al-Qadisiyah, Iraq

Corresponding Author Email: amir.towfik@qu.edu.iq

(Received 17/7/2017, Accepted 23/10/2017)

Abstract

The aim of the present study was to demonstrate the effect of camel colostrum extracts on auricular cartilage defect healing. Iraqi camel colostrum were collected post-parturition at 0, 6 hours, double centrifuged, filtrated and refrigerated until use. Fifteen albino rats were divided randomly into 3 groups; (TG1) treated with colostrum collected directly after birth, (TG2) treated with colostrum collected 6 hours post-parturition, and (TG3) treated with normal saline. Under routine surgical approach, holes were done in their ear cartilages. The diameter of the holes were measured daily for one week. Biopsies were taken at 1, 2, 3 weeks for histological evaluation of cartilages healing. (TG1) showed the superiority of histological evaluation by complete healing between the two edges of chondrocytes in the site of defect, profuse fibrosis with formation of new blood vessels and mild infiltration of inflammatory cells. The results demonstrated that, the camel colostrum extracts had a beneficial therapeutic effects on cartilage defects, which might regarded as a new biological therapy.

Key words: Camel, Colostrum, Extracts, Cartilage, Defect, Healing.

Introduction

One of the most difficult challenges in reconstructive surgery remains the total or partial reconstruction of the external ear (1,2). Camel is great gift of our God. Dromedary camel colostrum is a nutrient biological fluid produce immediately after giving birth- rich with immune and growth factors. Colostrum is differ from mature milk by its nutritional contain and immunological composition. In the matter of fact, camel colostrum contains more protein, non-protein nitrogen, vitamins, minerals, and ash than milk (3). IgG1, IgG2, and IgG3 are the main sub-classes' immunoglobulins which are presented in camel colostrum (4). The sub-classes IgG2 and IgG3 are devoid of light chains with a molecular mass of 42 and 45 KDa respectively. The biological activity of the protein composition of camel colostrum may be due to this feature (5). Topical use of many growth factors earn the

Food and Drug Administration (FDA) clearance or approval and are commercially available (6). A lot of growth factors have been implicated in angiogenesis, including vascular endothelial growth factor (VEGF), angiopoietin (Ang), platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF) (7,8). Auricular cartilage is an elastic cartilaginous tissue that include two discrete zones: a central cartilaginous region (or chondrium) and an outer fibrous region, termed the perichondrium. The central, cartilaginous region is rich in collagen II and large aggregating proteoglycans as well as abundant elastic fibers (9). The perichondrium surround the central cartilaginous region, it is a thin fibrous tissue layer may be essential for the growth and maintenance of elastic cartilage (10, 11, 12). Elastic and hyaline cartilages have a poor intrinsic repair capacity when

damage once occur (13, 14). For durable repair of cartilage lesions, an important condition is the integration of wound edges or the integration of repair tissue with the surrounding host cartilage (13). Although many publications demonstrate the effect of growth factors on chondrocytes proliferation and matrix production in vitro, we have little knowledge about the mechanism of regulating cartilage wound healing by the growth factors (15). The aim of the present study is to evaluate histological the effect of camel colostrum extract on auricular cartilage defects healing.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 426

The study was performed at Veterinary Medicine Collage, University of Al-Qadisiyah from January 2017 to March 2017.

Experimental design:

Iraqi camel (*Camelus dromedaries*) colostrum 30 ml. were collected post-parturition at 0, 6 hours in sterile containers and sent directly in icebox to the laboratory. The colostrum samples were double centrifuged at (3000 rpm for 10 min). The supernatants were infiltrated by microfiltration system (Vacuum Membrane Filter Funnel Apparatus, Shaoxing Warmer Lab Equipment CO.LTD, Shanghai, China)

with microfiltration membrane 0.22 μ m, and the extracts were refrigerated at 4°C until use. Fifteen Albino rats (*Rattus norvegicus*) with mean weight 154.3 $\pm$ 2.06 gm were obtained from the animal holding unit of the department of Physiology of Veterinary Medicine Collage/University of Al-Qadisiyah, and they were divided randomly and equally into 3 groups, under routine surgical approach with general anesthesia by chloroform, 5mm in diameter holes were done using a biopsy punch in the right elastic ear cartilages of all the experimental rats, the treatment groups were as follow: treatment group(TG1) treated with colostrum collected directly after birth, treatment group(TG2) treated with colostrum collected with 6 hours post-parturition, and control group(TG3), treated with normal saline. All groups treated with three drops topically, daily for one week. The diameter of the holes were measured daily for one week.

Statistical analysis:

The results were analyzed by using one-way ANOVA program; the variances were regarded significant at $P \leq 0.05$.

Histological evaluation:

Biopsies were taken at 1, 2, 3 weeks, preserved in formalin 10% for histopathological evaluation of cartilage healing. Smears were stained with hematoxylin-eosin (H&E) stains and examined under light microscope x10 and x40.

Results

The daily measurement of ear cartilages diameter holes for one week shows gradual decrease in all groups. At the 7th day, the mean values of TG1, TG2, and TG3 are

1.8 $\pm$ 0.119 mm. 3.5 $\pm$ 0.375 mm. and 3.6 $\pm$ 0.234 mm. respectively. Only the diameters of TG1 were significant variance at $P \leq 0.05$ from the 3rd day until the 7th day.

Table (1): The mean values $\pm$ SE of the ear holes diameters (mm.) of the treatment groups

Groups	1 st day	2 nd day	3 rd day	4 th day	5 th day	6 th day	7 th day
TG1	5 $\pm$ 0.000 A	4.5 $\pm$ 0.119 A	4.2 $\pm$ 0.118 B	4 $\pm$ 0.221 B	3.6 $\pm$ 0.181 B	2.9 $\pm$ 0.188 B	1.8 $\pm$ 0.119 B
TG2	5 $\pm$ 0.000 A	4.8 $\pm$ 0.120 A	4.6 $\pm$ 0.098 A	4.5 $\pm$ 0.000 A	4.1 $\pm$ 0.097 A	3.7 $\pm$ 0.123 A	3.5 $\pm$ 0.375 A
TG3	5 $\pm$ 0.000 A	5 $\pm$ 0.000 A	4.8 $\pm$ 0.121 A	4.3 $\pm$ 0.121 A	4.1 $\pm$ 0.098 A	3.8 $\pm$ 0.120 A	3.6 $\pm$ 0.234 A

* Different litters mean significant variance at $P \leq 0.05$.

Ear cartilage defect after one week of treatment in (TG1) show profuse fibrosis, infiltration of inflammatory cells and hemorrhage (hemostasis).

Figure (1): Ear cartilage defect after one week of treatment in (TG1) show profuse fibrosis (a), infiltration of inflammatory cells (b) and hemorrhage (hemostasis) (c) (X 10. H&E. stain).

Ear cartilage defect after one week of treatment in (TG2) show profuse granulation tissue, severe infiltration of inflammatory cells with severe hemorrhage and marked vacuolation of chondrocytes.

Figure (2): Ear cartilage defect after one week of treatment in (TG2) show profuse granulation tissue (a), severe infiltration of inflammatory cells (b) with severe hemorrhage(c) and marked vacuolation of chondrocytes (d) (X10.H&E.stain).

Ear cartilage defect after one week of treatment in (TG3) show mild fibrosis, severe

infiltration of inflammatory cells with presence of hemorrhage and marked vacuolation of chondrocytes in the edges of defect.

Figure (3): Ear cartilage defect after one week of treatment in (TG3) show mild fibrosis (a), severe infiltration of inflammatory cells with presence of hemorrhage (b) and marked vacuolation of chondrocytes in the edges of defect(c) (X10.H&E.stain).

Ear cartilage defect after two weeks of treatment in (TG1) show mild healing between the two edges of chondrocytes in the edges of the defect, mild infiltration of inflammatory cells mainly macrophages and marked fibroblasts.

Figure (4): Ear cartilage defect after two weeks of treatment in (TG1) show mild healing between the two edges of chondrocytes in the edges of the defect(a), mild infiltration of inflammatory cells mainly macrophages(b) and marked fibroblasts(c) (X40.H&E.stain).

Ear cartilage defect after two weeks of treatment in (TG2) show the chondrocytes

are ruptured and vacuolated with deposition of minerals at within these ruptured cells.

Figure(5): Ear cartilage defect after two weeks of treatment in (TG2) show the chondrocytes are ruptured and vacuolated(a) with deposition of minerals (b) at within these ruptured cells (X40.H&E.stain).

Ear cartilage defect after two weeks of treatment in (TG3) show fibrosis with severe infiltration of inflammatory cells mainly macrophages in the site of defect.

Figure (6): Ear cartilage defect after two weeks of treatment in (TG3) show fibrosis with severe infiltration of inflammatory cells mainly macrophages in the site of defect (X40. H & E-stain).

Ear cartilage defect after three weeks of treatment in (TG1) show complete healing between the two edges of chondrocytes in the site of defect, profuse fibrosis with formation of new blood vessels and mild infiltration of inflammatory cells.

Figure(7): Ear cartilage defect after three weeks of treatment in (TG1) show complete healing between the two edges of chondrocytes in the site of defect(a), profuse fibrosis(b) with formation of new blood vessels(c) and mild infiltration of inflammatory cells (X10.H&E stain).

Ear cartilage defect after three weeks of treatment in (TG2) show mild healing between the two edges of chondrocytes, mild fibrosis with mild formation of new blood vessels and moderate infiltration of inflammatory cells.

Figure (8): Ear cartilage defect after three weeks of treatment in (TG2) show mild healing between the two edges of chondrocytes (a), mild fibrosis(b) with mild formation of new blood vessels(c) and moderate infiltration of inflammatory cells(d) (X10.H&E. stain).

Ear cartilage defect after three weeks of treatment in (TG3) show severe infiltration of inflammatory cells mainly macrophages and marked severe vacuolation of chondrocytes.

Figure (9): Ear cartilage defect after three weeks of treatment in (TG3) show severe infiltration of inflammatory cells mainly macrophages (a) and marked severe vacuolation of chondrocytes(b) (X40.H & E. stain).

Discussion

The regenerative medicine is regarded now as a medical revolution that the world heading toward prepare and use a commercial biological therapies for cure many diseases for which antibiotics are failed. Camel is the desert ship in our country; it is one among the animals mentioned in Quran as a miracle of the God (16). The nutrients, immunoglobulins and growth factors that present in the camel colostrum are very essential for regeneration of defect tissues, especially the bioactive factors (3). For all these reasons, we design this project to evaluate histologically the effect of camel colostrum extract as a biological treatment for healing of auricular cartilage defects. In vitro effects of the growth factors on chondrocytes and matrix proliferation suggest that the temporal release of endogenous growth factors may indicates an autocrine and/or paracrine stimulation of chondrocytes metabolism following acute cartilage injury (17). In case of articular cartilage, there is a relation between wounding, proteoglycans releasing, and chondrocytes metabolism, which is difficult to study because of the influence of loading on proteoglycans (18), so we choose the auricular cartilage. Table-1 shows the gradual decrease of cartilage holes diameter, which indicate to the accelerated proliferation of the chondrocytes of the auricular elastic cartilage so TG1 diameter holes were significant variance from 3rd day

until the 7th day. This result is clear when we compare it with diameter holes of TG3. According to histo-pathological examination of fig.-7, our results showed a complete healing of the cartilage in three weeks with formation of new blood vessels and profuse fibrosis due to the high peak of IgG1 and IgG2 in the colostrum specially IgG2, which collected directly after birth while the colostrum which is collected 6 hours post-birth may contain less levels of growth factors. In addition, we believe that, the colostrum, which is collected directly after birth, may have a rich content of lactoferrin and bioactive molecules such as antioxidant and antihypertensive peptides (19). These bioactive factors are responsible for the acceleration of chondrocytes proliferation. In addition, this research shows the easy administration of a new therapy by topical application as few drops only without any special device. The results indicate clearly that camel colostrum extract can be depended as a new biological useful therapy for cartilage defects.

Conclusions

Our research provides the opportunity to apply the growth factors topically but still we need more studies about the real effects and the mechanism of the bioactive factors, which represent in the camel colostrum. Our results are very useful for veterinarians and medical companies.

References

- 1-Shieh SJ, Terada S, Vacanti JP. Tissue engineering auricular reconstruction in vitro and in vivo studies. *Biomaterials* (2004); 25: 1545-1557.
- 2-Yanaga H, Imai K, Koga M, Yanaga K. Cell-engineered human elastic chondrocytes regenerate natural scaffold in vitro and neocartilage with neoperichondrium in the human body post-transplantation. *Tissue Eng. Pt A18*: (2012); 2020-2029.
- 3-Zeineb J, Nadia O, Isabelle A, Touhami k, Pascal D, Halima El-H . Camel colostrum: Nutritional composition and improvement of the antimicrobial. *Emir. J. Food Agric.* (2015); 27(4): 384-389.
- 4-Azwai SM, Carter SD, Woldehiwet Z. Immunoglobulins of Camel (*Camelus dromedarius*) colostrum. *J. Comp. Pathol.* (1996); 114:273-282.
- 5-Hamers-Casterman C, Atahouch T, Muyldermans S, Robinson G, Hamers C, Songa EB, Bendahman N, Hamers R. Naturally-occurring antibodies devoid of light chains. *Nature.* (1993); 363:446-448.
- 6-Mao AS, Mooney DJ. Regenerative medicine: Current therapies and future directions. *PNAS* (2015); 112 (47) 14452-14459.
- 7-Darland DC, D'Amore PA. Blood vessel maturation: Vascular development comes of age. *J Clin Invest* (1999); 103(2):157-158.
- 8-Conway EM, Collen D, Carmeliet P. Molecular mechanisms of blood vessel growth. *Cardiovasc Res* (2001); 49(3):507-521.
- 9-Naumann A, Dennis JE, Aigner J, Coticchia J, Arnold J, et al. Tissue engineering of autologous cartilage grafts in three-dimensional in vitro macroaggregate culture system. *Tissue Eng* (2004); 10: 1695-1706.
- 10-Cao Y, Vacanti JP, Paige KT, Upton J, Vacanti CA. Transplantation of chondrocytes utilizing a polymer-cell construct to produce tissue-engineered cartilage in the shape of a human ear. *Plast Reconstr Surg* (1997); 100: 297-302.
- 11-Hellingman CA, Verwiel ETP, Slagt I, Koevoet W, Poubelon RML, et al. . Differences in cartilage-forming capacity of expanded human chondrocytes from ear, nose, and their gene expression profiles. *Cell Transplant* (2011); 20: 925-940.
- 12-Madsen K, Moskalewski S, Mark von der K, Friberg U. Synthesis of proteoglycans, collagen, and elastin by cultures of rabbit auricular chondrocytes—relation to age of the donor. *DevBiol*(1983); 96: 63-73.
- 13-Ahsan T, Sah R. Biomechanics of integrative cartilage repair. *Osteoarthritis Cart.* (1999) 7:29-40.
- 14-Verwoerd-Verhoef HL, Ten Koppel PG, Van Osch GJ, Meeuwis CA, Verwoerd CD Wound healing of cartilage structures in the head and neck region. *Int. J. Pediatr. Otorhinolaryngol* (1998); 43:241-51.
- 15-Boumediene K, Conrozier T, Mathieu P, Richard M, Marcelli C, Vignon E, Pujol JP. Decrease of cartilage transforming growth factor-beta receptor II expression in the rabbit experimental osteoarthritis-potential role in cartilage breakdown. *Osteoarthritis Cart* (1998); 6:146-9.
- 16-Deuraseh, N The urine of camels. Treatment of diseases in AL-Tibb Al-Nabawi. *Int. Med. J.*, (2005) 4 (1): 25-28.
- 17-Bos PK, Van Osch GJVM, Frenzt DA, Verhaar JAN, Verwoerd-Verhoef HL Growth factor expression in cartilage wound healing:temporal and spatial immunolocalization in a rabbit auricular cartilage wound model. *Osteoarthritis and Cart.* (2001); 9:382-389.
- 18-Sah RL, Kim YJ, Doong JY, Grodzinsky AJ, Plaas AH, Sandy JD Biosynthetic response of cartilage explants to dynamic compression. *J. Orthop. Res.* (1989); 7:619-36.
- 19-Conesa C L, Sanchez C, Rota MD, Pérez M. Calvo S. Farnaud RW Ewans. Isolation of lactoferrin from milk of different species: Calorimetric and antimicrobial studies. *Comp. Biochem. Physiol. B.* (2008); 150:131-139.