

Hyaluronic Acid and Wound Healing

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ABSTRACT - Background. We developed an experimental model of ethanol-induced dermatotoxicity and hepatocytotoxicity using normal human keratinocytes and normal human hepatocytes that preserve inducible cytochrome p450 activities. The original work was described in several articles. The objective of this study was to determine whether hyaluronic acid attenuates skin necrosis, and to further clarify its uses in wound repair in humans, animal models and *in vitro* studies. **Methods.** We performed a systematic review of the literature using the terms “hyaluronic acid” and “wound healing”. PubMed was searched for studies published during the period 2010-2014. **Results.** Hyaluronic acid is used in tissue regeneration alone or in combination with herbal or Western medicine. Scaffolds made up of hyaluronic acid were used to embed basic fibroblast growth factor. **Conclusion.** Hyaluronic acid extracts are safe and efficacious products to be used in skin repair.

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INTRODUCTION

Hyaluronic acid (HA) is a polysaccharide that belongs to the glycosaminoglycan family and consists of a basic unit of two sugars, glucuronic acid and N-acetyl-glucosamine. HA usually exists as a high molecular mass in the synovial fluid that surrounds joints, cartilage, and tissues of the eye and skin. Previously, our group was able to reduce alcohol-induced fibrogenesis *in vitro* in human normal hepatocytes (1) and to induce *in vitro* healing of human epithelial cells (2) using a preparation of HA obtained from human umbilical cord excision. The present work reviews in depth some of the mechanisms of tissue damage and repair in which HA plays an important role.

TISSUE REGENERATION

Recent studies using HA for tissue regeneration are discussed in Table 1. HA has recently been used in several *in vitro* studies (2-5). Our group has used HA jellies at concentrations of 2%, 4% and 8% that were formulated at the Placental Histotherapy Center in Cuba from residual umbilical cords of human placentas obtained according to the National

Recollection Program. We showed that HA 2% and 4% has a protective dose-dependent effect on skin cells exposed to ethanol. HA protected against ethanol-induced cytotoxicity by a mechanism that involves the prevention of apoptosis, and this is mediated by decreased inflammation. Cells exposed to the same amount of ethanol in the presence of HA 8% were not protected against toxicity. We believe this to be the result of an inability of this highly viscous gel to penetrate into the cells (2). In a separate study, Wu et al. (5) show that high molecular weight (HMW)-HA (1525 kDa) has superior cytoprotective effects compared to low molecular weight (LMW)-HA (127 kDa). HMW-HA 0.3% facilitates cell migration and wound healing (5). HMW-HA also promotes wound healing and cell survival in a rat heart ischemia-reperfusion model, while LMW-HA has no protective effects (4). Another recent *in vitro* study showed that amniotic fluid is associated with re-epithelialisation, mediated by the presence of HA (3).

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HA was further used in animal models (6-13). HA was used for its wound healing potential in two full-thickness surgical wound models. HA facilitated re-epithelialization, led to the formation of soft tissue with good elasticity, and increased microvascular density (6, 7). HA also showed promising results for connective tissue repair in terms of tendon and cartilage repair in two rabbit models (8, 9). Bone repair is further shown in rat and rabbit models (10, 11). Zanchetta et al. (11) found that histological and overall appearance of the healing bone were superior in untreated hole made in the left side of the parietal bone compared to HA-treated hole made in the right side. Better results obtained in the control site could be partly explained by the transport of the treatment components in blood, thereby spreading them evenly and suggesting systemic activity (11).

The use of chitosan-hyaluronan/silk fibroin cardiac patches led to cardiac repair in a rat model of myocardial infarction. Blood vessel-like structures were widely distributed in myocardial infarction regions of the left ventricle in the treatment group, yet were absent under the control condition. This further led to higher secretion of paracrine factors in myocardial infarction regions of the left ventricle in the treatment group compared to control (12). In another rat model, artificial tears containing HA and high potassium ion concentration promoted corneal epithelial wound

healing in a mechanical scraping model but not in an alkali burn model (13).

HA was used in humans in several studies (14-21). The skin re-epithelialization potential of *Avena rhealba* extract® and HA was shown in a small sample of volunteers in which skin lesions were produced with erbium-YAG laser (14). Different gel formulations of HA were used in burn patients (15, 16), and for the treatment of persistent ulcers (17-19). Four cases of upper limb deep wounds treated with HA, bacterial fermented sodium hyaluronate salt (0.2% w/w), and bacterial collagenase obtained from non-pathogenic *Vibrio alginolyticus* are described in a case series. Aside from wound healing, this treatment led to satisfactory aesthetic results, and optimal recovery of the affected limb functionality (22).

In humans, platelet rich plasma and HA were used to treat postoperative wound dehiscence and tendon exposure after surgery in patients with Morton's neuroma, an entrapment neuropathy of the plantar digital nerve (21). Elsewhere, the application of HA-containing gels in early wound healing after scaling and root planting was tested in individuals with chronic periodontitis. HA led to significantly higher reduction in probing depth, as well as the number of pockets with probing depth ≥ 5 mm, compared to scaling and root planting alone (20).

Table 1: Tissue Regeneration Studies

| Ref | Study type and Model Used | Formulation Containing HA and the HA Level | Efficacy Findings |
|-----|---|--|--|
| 2 | Human A431 epidermoid skin cells and mouse fibroblasts exposed to ethanol (50 and 100 mM) | HA (2%, 4% and 8%) | HA reduced ethanol-induced cytotoxicity in a dose-dependent manner between 2% and 4%; 8% HA had no effect; HA decreases production of the pro-inflammatory cytokine TNF- α ; HA prevented ethanol-induced apoptosis |
| 3 | Deep dermal wounds produced <i>in vitro</i> in human skin | Amniotic fluid rich in nutrients, growth factors and HA | Amniotic fluid (50%) led to similar epithelialization as fetal bovine serum (10%); superior epithelialization vs. cell culture media; HA degradation prevented epithelialization |
| 4 | Rat cardiomyocyte cell line H9C2 treated with hydrogen peroxide as a heart ischemia-reperfusion model | LMW-HA: low molecular weight HA (100 kDa); HMW-HA: high molecular weight HA (1000 kDa) | HMW-HA (0.3%) modulates cell survival and promotes wound healing; HMW-HA (0.3%) significantly facilitates cell migration via cytoskeletal rearrangement (restores activity of migration-associated cytoskeletal proteins); No effect of LMW-HA |
| 5 | Alkali-injured human corneal epithelial cells (HCE-2 cells treated with NaOH) | HMW-HA group: high molecular weight HA (1525 kDa); LMW-HA group: low molecular weight HA (127 kDa) | HMW-HA increased cell viability in dose-dependent manner (24 h incubation after exposure for 1 min to 0.012 N NaOH) |

Table 1: Tissue Regeneration Studies (Cont`d)

| Ref | Study type and Model Used | Formulation Containing HA and the HA Level | Efficacy Findings |
|-----|---|---|--|
| 6 | Full-thickness pieces of rabbit skin | HA1 group: porcine acellular dermal matrix + HA (0.3 mL exogenous HA, about 0.17 mg/cm ²) + thin skin autograft; HA2 group: porcine acellular dermal matrix + HA (0.6 mL exogenous HA, about 0.34 mg/cm ²) + thin skin autograft; PADM group: porcine acellular dermal matrix + thin skin autograft; TS group: thin skin autograft; NS group: normal skin | Skin grafts grew well in all groups; HA1 and HA2: soft with good elasticity; PADM: quite hard with poor elasticity; TS: thin and susceptible to tearing; NS: best elasticity among all the groups; Collagen I and III content on day 28: higher in HA1 and HA2 than rest; higher in HA1 than HA2; Microvascular density at day 14: higher in HA1 and HA2 than other groups; higher in HA2 than HA1 |
| 8 | Rabbit superficial digital flexor tendon rupture; full thickness tendon transection and surgical repair | NaH group: exogenous sodium hyaluronate injected subcutaneously over the lesion; Control group: saline injection | Time-dependent decrease in the diameter of the injured tendon: superior in NaH vs. control; Treatment was effective in restoring morphological and biomechanical properties of lacerated superficial digital flexor tendon rupture |
| 9 | Rabbit knee articular defect (induced articular cartilage defect) | Treatment: bilateral knee arthrotomies, chondral defects, microfracture + intraarticular HA; Control: saline | Higher potential for healing in the experimental group, with thicker and more organized repair tissue in treatment vs. control |
| 10 | Anterior wall of the maxillary sinus removed in rabbit 4 mm circumferential wound on both the nasal and the sinus side | Rapid-gelling HA hydrogel or preformed HA hydrogel filled randomly into the right or left sinus; Blank control or Merogel control into the other sinus | HA preserved neo-ostium opening (prevented ostial stenosis); Degree of lymphocyte or plasmacyte infiltration similar between groups; Preformed HA hydrogel led to lower acute inflammation and heterophile infiltration; Rapid-gelling HA reduced fibrosis and osteogenesis; HA hydrogel promoted wound healing |
| 7 | Full-thickness surgical wound in rats Full-thickness skin defect in diabetic mice | EGF dressing: HMW-HA spongy sheet (upper layer) + arginine, magnesium ascorbyl phosphate and EGF (lower layer); Control: epidermal growth factor-free dressing | Substantially facilitate epithelialization, granulation tissue formation and angiogenesis in rat; EGF dressing superior to EGF-free dressing; Improved wound condition and decreased wound size, and facilitated epithelialization, granulation tissue formation and angiogenesis in mouse; EGF dressing superior to EGF-free dressing |
| 11 | Rat critical size defect model (hole made in parietal bone) | Right hole (treatment): HA, chondroitin 6 sulphate, dermatan sulphate and 2.5% saline solution; Left hole (control): untreated | Substantial periosteal macroscopic neo-angiogenesis at both sites |
| 12 | Myocardial infarction induced by cryoinjury in rat | Treatment group: chitosan-HA/silk fibroin cardiac patches implanted in left ventricle; Control group: no patches | Reduced the dilation of the inner diameter of left ventricle in treatment (p<0.05); Increased wall thickness of left ventricle in treatment (p<0.05); Improved the fractional shortening of left ventricle of hearts (p<0.05) |
| 13 | Epithelial defects on the corneas in rat: mechanical scraping and alkali burns | Artificial tears containing HA (0.3% or 0.15%) + high-K ion concentration; PBS | Mechanical scraping model: smaller areas of fluorescein staining in the eyes in HA + high-K artificial tears vs. PBS by 36 h; Alkali burn model: no significant effect of HA + high-K artificial tears vs. PBS by 36 h |
| 14 | RTC (n=21); skin de-epidermised by Er-YAG laser in healthy volunteers | Group A: <i>Avena rhealba</i> extract® + HA; Group B: reference product (panthenol and madecassoside); Group C: reference product (resveratrol-copper); Group D untreated control | Laser wound completely healed after 9 days in groups A and B, 12 days in group C, and 16 days in group D; Healing generally slow between days 1 and 6, faster thereafter; Safety profiles of treatments were favorable and comparable |
| 15 | Cohort (n=60); partial thickness burns (average 3% total body surface area); | Zinc-HA gel | On average, wound size reduced by 50% by day 5; Full epithelialization in 93.3% of sample by day 21; Resolution of pain in 91.7% of sample by day 10; No infections |