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Systemic administration of high-molecular weight hyaluronan stimulates wound healing in genetically diabetic mice

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ABSTRACT

Hyaluronic acid (HA), an essential component of the extracellular matrix, is an efficient space filler that maintains hydration, serves as a substrate for assembly of proteoglycans and is involved in wound healing. Although numerous pieces of evidence demonstrate beneficial effects in promoting wound healing in diabetes, a systemic approach has never been tested. We used an incisional wound healing model in genetically diabetic mice to test the effects of systemic injection of HA. Diabetic ($n=56$) and normoglycemic ($n=56$) mice were subjected to incision and randomized (8 groups of 7 animals each) to receive HA at different doses, 7.5, 15 and 30 mg/kg/i.p., or vehicle (0.9% NaCl solution) for 12 days. At the end of the experiment animals were sacrificed and skin wounds were excised for histological, biochemical and molecular analysis. Histology revealed that the most effective dose to improve wound repair and angiogenesis in diabetic mice was 30 mg/kg. Furthermore HA injection (30 mg/kg) improved the altered healing pattern in diabetic animals, increased skin remodeling proteins TGF-β and transglutaminase-II and restored the altered expression of cyclin B1/Cdc2 complex. Evaluation of skin from diabetic animals injected with HA revealed also an increase in HA content, suggesting that systemic injection may be able to restore the reduced intracellular HA pool of diabetic mice. Finally HA markedly improved skin mechanical properties. These promising results, if confirmed in a clinical setting, may improve the care and management of diabetic patients.

1. Introduction

Diabetic patients have impaired wound healing associated with increased morbidity and mortality [1,2]. The majority of non-healing wounds often leads to amputation, thus increasing the direct costs of their care, rehabilitation, and lost productivity [3]. Clinical and experimental evidence suggests that diabetic ulcers and other types of chronic wounds do not follow an orderly and reliable progression of [1].

Hyaluronic acid (HA) consists of a basic unit of two sugars, glucuronic acid and N-acetylglucosamine, polymerised into large macromolecules of over 30,000 repeating units. It is therefore one of the largest components of the extracellular matrix and its structure, highly conserved in evolution, appears identical in rodents and humans [4]. The molecule is readily soluble in water, producing a gel and has no allergenic properties, representing an excellent molecule for clinical applications. HA is formed at the cell surface of fibroblasts by extrusion into the extracellular matrix in close association with a dedicated receptor, CD44. Fibroblasts also elaborate hyaluronidase, the degradation enzyme, and are able to internalize both HA and, importantly, its breakdown products [5]. Enzymatic degradation cleaves the HA macromolecule into smaller polymers, each comprised of variable lengths of dimeric chains; many of which appear to modulate wound healing even if studies have indicated that most of the effects attributed to the molecule are applicable to only few products. Collagen deposition by fibroblasts is one of the key factors in reconstituting a supporting matrix at sites of scar formation and it is the nature of this deposition that largely determines scar quality. There is evidence that extracellular matrix (ECM) remodeling following HA application is enhanced and collagen deposition more

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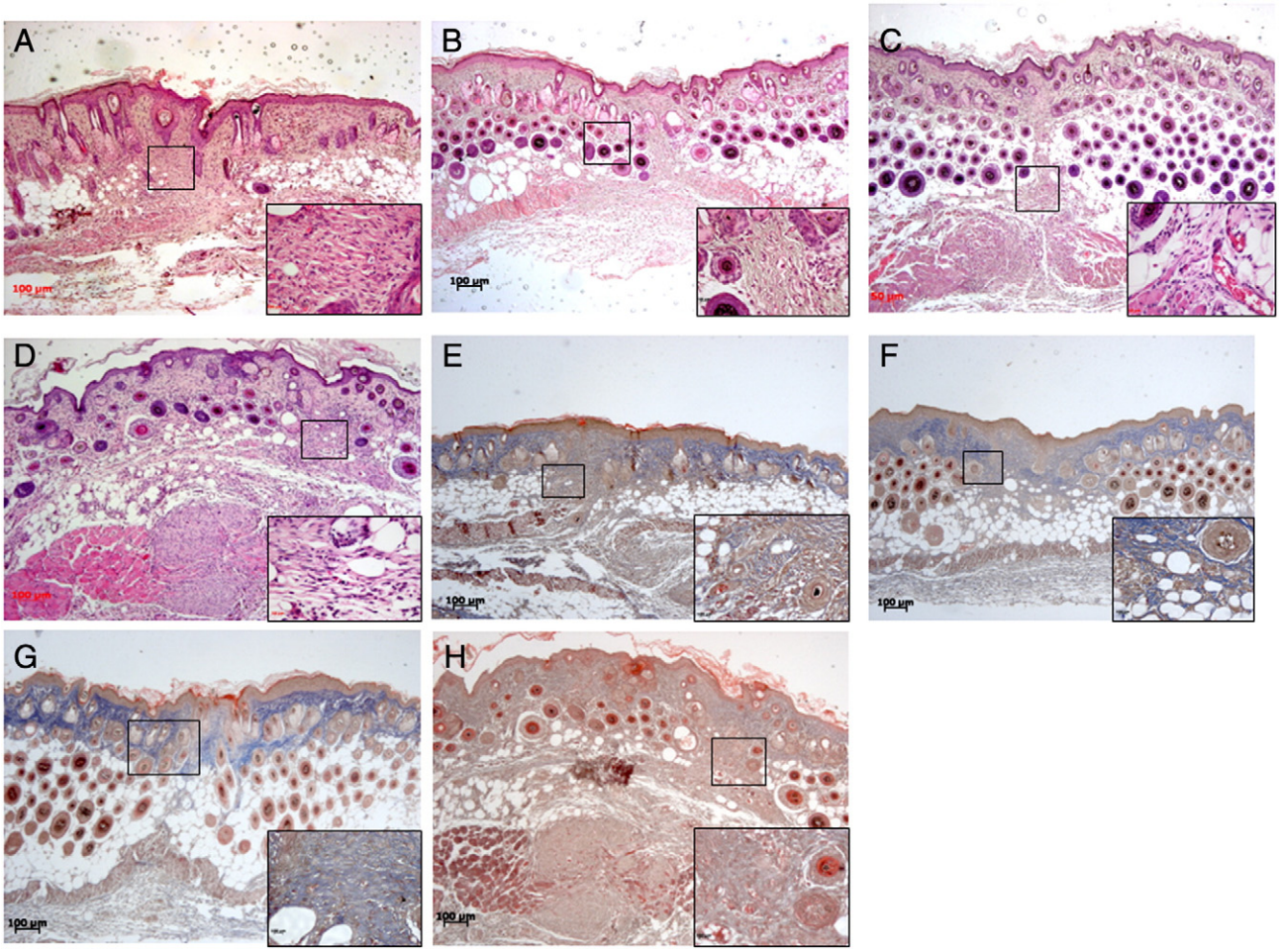

Fig. 1. A–D: Haematoxylin–eosin staining at day 12, original magnification ×10. Rectangle represents the area at higher magnification (×40) in the lower left corner. A: NDB + vehicle, shows almost complete re-epithelialization, presence of granulation tissue and inflammatory infiltrate (see rectangle in the corner). B: NDB + HA 7.5 mg/kg, shows advanced healing with initial retraction of the granulation tissue, less inflammatory infiltrate and presence of hair follicles (see rectangle in the corner). C: NDB + HA 15 mg/kg, shows complete healing process with retraction of the granulation tissue, more mature hair follicles and presence of vessels in the site of incision (see rectangle in the corner). D: NDB + HA 30 mg/kg, shows no further improvement in the healing process compared to NDB + HA 15 mg/kg. E–H: Masson's tricrhome staining at day 12, original magnification ×10. Rectangle represents the area at higher magnification (×40) in the lower left corner. Blue color identifies collagen tissue, red color identifies keratin and muscle fibers. E: NDB + vehicle, shows presence of few and poorly arranged collagen bundles at the incision site (see rectangle in the corner). F: NDB + HA 7.5 mg/kg, shows more and better arranged collagen fibers at the incision site (see rectangle in the corner). G: NDB + HA 15 mg/kg, shows completely restored and mature collagen tissue at the incision site (see rectangle in the corner). H: NDB + HA 30 mg/kg, shows no improvement in collagen tissue (see rectangle in the corner).

ordered [6–8]. Paradoxically, hyaluronidase (which it would be expected to increase tissue HA fragments) causes enhanced scarring [9], whilst persistently raised levels of HA decrease fibroblast contraction [10]. Furthermore, HA fibroblast production may be affected by a number of growth factors [11] and HA degradation products are pro-angiogenic, this effect being limited to fragments of between 4 and 25 disaccharides in length [12].

The mechanism underlying HA degradation is not completely understood, but according to the current model, it involves the concerted action of the somatic hyaluronidases (Hyal-2 and Hyal-1) with CD44 [13]. Very recently it was also shown that Hyal-1 and -2 mRNAs are up-regulated by exogenous lactate in normal fibroblasts [14]. After an injury, during the course of wound repair, the level of oxygen availability decreases whereas lactate production increases. This in turn leads to increased HA turnover and appearance of HA with smaller molecular weight; furthermore it helps to drive adequate wound healing. If the regulation of HA catabolism by lactate is impaired, as it has been shown in fibroblasts from diabetic patients with ulcers [15], tissue injury may promote accumulation of high molecular weight HA and intensification of reduced fibroblast proliferation. This effect may induce transformation of acute wounds into chronic ulcers. Thus, it is possible that both increased accumulation of high molecular weight HA and the elevated level of CD44 expression may be considered as additional factors enhancing susceptibility to chronic ulceration in diabetes.

In addition, HA binding to its cell surface receptor RHAMM is critical for the progression of cells through the cell cycle. The phases of cell cycle are controlled by different types of cyclins in a timely and orderly manner. Cyclins, cyclins-dependent kinases (cdks) and negative regulators such as cyclin-dependent kinase inhibitors work together to regulate cell cycle progression [16].

The genetically diabetic mouse represents an useful animal model for skin healing studies; in fact wound healing in these animals is markedly delayed when compared with non diabetic littermates. Healing impairment is characterized by delayed cellular infiltration, impaired granulation tissue formation, reduced angiogenesis, and decreased collagen synthesis and organization [17–19].

Our group already tested several approaches to improve wound healing by using an incisional wound model in genetically diabetic mice [20–24]. In the present study we investigated whether a systemic injection of HA might also exert beneficial effect on impaired cell cycle and tissue remodeling in diabetic wound healing.