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Research paper

Needle-free jet injection of hyaluronic acid improves skin remodeling in a mouse model

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ABSTRACT

Purpose: The purpose of this study was to improve methods of jet injection using a mouse model. We investigated the mechanism of action, efficacy, and safety of the pneumatic device using injection of hyaluronic acid (HA) solution into a mouse model.
Methods: We evaluated the efficacy and safety of an INNOJECTOR™ pneumatic device that pneumatically accelerates a jet of HA solution under high pressure into the dermis of mouse skin. We examined the treatment effects using skin hybrid model jet dispersion experiments, photographic images, microscopy, and histological analyses.
Results: Use of the INNOJECTOR™ successfully increased dermal thickness and collagen synthesis in our mouse model. Jet dispersion experiments were performed using agarose gels and a polyacrylamide gel model to understand the dependence of jet penetration on jet power. The mechanisms by which pneumatic injection using HA solution exerts its effects may involve increased dermal thickening, triggering of a wound healing process, and activation of vimentin and collagen synthesis.
Conclusions: Collagen synthesis and increased dermal thickening were successfully achieved in our mouse model using the INNOJECTOR™. Pneumatic injection of HA under high pressure provides a safe and effective method for improving the appearance of mouse skin. Our findings indicate that use of the INNOJECTOR™ may induce efficient collagen remodeling with subsequent marked dermal layer thickening by targeting vimentin.

1. Introduction

Skin aging is a complex biological process that is a consequence of both intrinsic and genetically programmed aging that occurs with time, and extrinsic aging caused by environmental factors [1]. The dramatic increase in the aging population and the psychosocial impact of skin aging have created a demand for effective interventions [2]. Currently, various nonablative skin resurfacing techniques are being used to rejuvenate facial skin including lasers and intense pulsed light (IPL) [3]. Several energy-based skin rejuvenation technologies such as lasers [4], radiofrequency [5] or ultrasound [6] aim to trigger collagen remodeling in response to controlled thermal damage of the dermal skin layers. All of these modalities induce controlled thermal damage to the dermal layers of the skin that can lead to limited long-term effects after several months of treatment but usually lack any immediate effect [7].

Jet injection uses a high-speed stream of fluid to puncture the skin and delivers drugs to the dermal or subdermal layer without the use of a needle [8,9]. Recently, jet injectors have also been applied in gene and vaccine delivery [10,11]; however, occasional pain and bruising limit their widespread use [12]. For skin rejuvenation, jet injection uses a precise technology to accelerate and laterally disperse skin-enhancing hyaluronic acid (HA) particles via microtrauma using a pneumatic needle-free mechanism. These particles penetrate the dermis to a controlled depth while leaving the surrounding tissue intact, and induce dermal microtrauma that mechanically stretches the fibroblasts, stimulating growth factors and inhibiting collagen breakdown [13]. Previous reports have demonstrated that Airgent™ triggers natural wound healing and augments collagen generation to enhance the effectiveness of the treatment and induce long-lasting changes to the dermis [13,14].

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However, the exact mechanism by which this process of collagen generation is mediated remains unclear.

Vimentin is a marker of fibroblasts and myofibroblasts and a component of the type III intermediate filaments [15]. Vimentin filaments in the mesenchymal cells are involved in motility, maintenance of cell shape, and endurance to mechanical stress [16]. In particular, vimentin-deficient mice exhibit delayed wound healing [17]. Therefore, vimentin filaments play a role in wound healing and the development of tissue fibrosis. The main aims of the current study were to evaluate the mechanisms of action, efficacy, and safety of the INNOJECTOR™ pneumatic device in a mouse model. Our findings indicate that the pneumatic injection of HA stabilized collagen synthesis by targeting vimentin.

2. Materials and methods

2.1. Jet production

The needle-free microjet injection device, INNOJECTOR™ (provided by Amore Pacific, Korea) is a novel technique for introducing various materials such as esthetic medicines, HA, botulinum toxin, and placental extracts into the skin without causing pain and bleeding. The accelerated jet penetrates the epidermis through a tiny entry point. This device produces a high-velocity jet (up to 180 m/s) with a nozzle diameter of 0.1 mm that penetrates the skin and delivers medicines intradermally using a liquid propelled by compressed gasses (nozzle diameter, 0.1 mm; max velocity, 180 m/s). In this study, a solution of bromocresol green dye (1% v/v) in deionized (DI) water was used as the jet fluid for the *in vitro* assessment of its penetration into gels. The liquid volume ejected by each jet was 0.15 mL, and the jets were ejected at a distance of 1 mm from 0.5%, 1%, 1.5% agarose or 20% polyacrylamide gels. For the mouse studies, the injection was performed by pneumatically accelerating a carrier fluid jet containing high-mass molecules of HA.

2.2. Animals and experimental design

Fifty female SKH-1 hairless mice (7-week-old) were purchased from Central Laboratory Animal Inc. (Seoul, Korea), and assigned to five groups of 10 mice in each. The animals were kept in a controlled environment at a constant temperature and humidity and a 12-h light/dark cycle and were fed a standard diet with water provided ad libitum. The mice were administered an anesthetic overdose of zoletil (25 mg/kg, intraperitoneally, i.p.) before their dorsal skin areas were treated with a conventional needle injection or an INNOJECTOR™ pneumatic device that delivered an HA dermal filler (Haviscoplus INJ 10 mg/mL, Pacific Pharma, Korea) diluted in saline, into the dermis via a pneumatic needleless action. A solution of the HA dermal filler (10% v/v) in saline was used as the jet fluid. The mice were monitored for 14 days post-treatment before being humanely euthanized. All the animal procedures were conducted in strict accordance with the guidelines of the Institutional Animal Care and Use Committee of Chung Ang University in Korea (Institutional Review Board, IRB Number: 13-0070).

2.3. Skin surface change

The morphological changes in the dorsal skin surface after treatment were examined by using a folliscope (LeedM, Seoul, Korea), which is a small handheld universal serial bus (USB) connection-based apparatus containing a high-definition microscopic camera that is operated by using a computer screen as an interface. We obtained photographs of the skin surface on days 0, 1, and 14.

2.4. Histological examination

The mouse skin samples of the areas treated by the pneumatic needleless injection or conventional needle injection were harvested immediately after measurement of the skin thickness. The skin tissues were fixed with 4% paraformaldehyde (PFA), embedded in paraffin, 5-μm thick sections were subsequently cut using a microtome, transferred to a probe-on-plus slides (Fisher Scientific, Pittsburg, PA, USA), and then they were stained with hematoxylin and eosin (H&E) to examine the epidermal and dermal changes. Ten sections were assessed for each experimental group. The dermal thickness was measured from the dermal–epidermal junction to the underlying subcutaneous tissue. The mean value was calculated and used as the final dermal thickness. The histopathological analysis of the images captured under a microscope (BX51®, Olympus) was performed using a computerized digital imaging micrometer software (Olympus Stream Modular Imaging Software®).

2.5. Picro-sirius red assay

The sections were stained with sirius red (Sigma, Steinheim, Germany) at room temperature for 1 h. After staining, the sections were hydrated with a series of graded concentrations of ethanol, cleared with xylene, and mounted with neutral resin. Collagen types I and III were differentiated by using polarization microscopy (PLM, Motic BA 300 POL, Richmond, BC, Canada) in which collagen types I and II appeared bright red and green, respectively.

2.6. Histological examination

The sections were stained by using mouse monoclonal antibodies against collagen I and vimentin (1:500 and 1:200, ab292 and ab92547, respectively, Abcam Cambridge, MA, USA). The immunohistochemical analyses were performed using a high-temperature antigen unmasking technique. In brief, the sections were heated in an unmasking solution (citrate buffer, pH 6.0), washed, and incubated with the primary monoclonal antibodies at room temperature for 1 h followed by incubation with secondary antibodies (Envision Detection kit K5007, DAKO, Glostrup, Denmark). The reaction products were developed using 3,3′-diaminobenzidine (DAB), and then the sections were rinsed and counterstained with hematoxylin to visualize the nuclei. The sections were dehydrated, covered with permount (Fisher Scientific, Fair Lawn, NJ, USA), and then coverslipped. The histological changes were assessed by using light microscopy. For the immunofluorescence, the sections were blocked at room temperature for 2 h in phosphate-buffered saline (PBS) containing 0.2% Triton X-100 and normal horse serum and then stained with mouse monoclonal antibodies against vimentin (1:500) by incubation at 4 °C overnight. Following incubation, the sections were washed thrice for 5 min each with 0.2% Triton X-100 in PBS, incubated at room temperature with fluorescein isothiocyanate (FITC)-conjugated anti-goat anti-rabbit (1:200, sc-2012, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 30 min, and then counterstained for 5 min with 4′,6-diamidino-2-phenylindole (DAPI).

2.7. Statistical analyses

The statistical comparisons of the treated and untreated groups were performed by using a one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for direct comparison. The results are expressed as mean ± standard deviation (SD) of at least three independent experiments, and P-values <0.05, <0.01, and <0.001 were considered statistically significant.