Efficacy of intradermal allogeneic fibroblast injections in junctional epidermolysis bullosa

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Abstract: Objective — to assess the efficacy and safety of intradermal injections of allogeneic fibroblasts into non-healing wounds in a patient with junctional epidermolysis bullosa. Material and Methods — A 49-year-old patient with intermediate junctional epidermolysis bullosa was injected intradermally into the base of non-healing wounds with 1 mL suspension of allogeneic fibroblasts, which contained 5×10^6 cells/mL, 10×10^6 cells/mL, and 20×10^6 cells/mL. Immunofluorescence mapping exhibited reduced β3 chain of laminin 332 and collagen XVII expression in the basement membrane area. Paired erosions were injected with 2% albumin or saline solution. Results — At two weeks after treatment, wound areas reduced significantly, or 100% re-epithelialization occurred. Collagen XVII and β3 chain expression of laminin 332 increased at the dermal-epidermal junction. Conclusion — Our findings demonstrated that intradermal injections of allogeneic fibroblasts could be an effective therapeutic approach for treating small non-healing wounds in junctional epidermolysis bullosa.

Keywords: junctional epidermolysis bullosa, collagen XVII, laminin 332, allogeneic fibroblasts, non-healing wounds.

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Introduction

Junctional epidermolysis bullosa is a group of autosomal recessive disorders characterized by spontaneous or trauma-induced skin and/or mucosal blistering. The junctional epidermolysis bullosa is caused by a defect in the genes encoding structural proteins of lamina lucida of the basement membrane. These structural proteins serve to adhere keratinocytes to the basement membrane.

Mutations in LAMA3, LAMB3, LAMC2 and COL17A1 genes, encoding α3, β3 and γ2 chains of laminin 332 and collagen XVII, respectively, are found in majority of patients with junctional epidermolysis bullosa [1, 2].

Type XVII collagen is a transmembrane protein and a major component of the hemidesmosome. The hemidesmosome is a multiprotein complex that provides adhesion of basal epithelial cells to the basement membrane in stratified, pseudostratified and transitional epithelia [3]. Extracellular domain of type XVII collagen spreads to the lamina lucida, where it binds to laminin 332. Moreover, according to some studies, collagen XVII affects cellular motility [4].

Laminin 332 forms anchoring filaments, which provide mechanical stability of skin [5, 6]. Furthermore, by binding to α6β4 and α3β1, it plays a crucial role in keratinocyte migration and proliferation. Accordingly, its deficiency could lead to impaired wound healing [7].

Intermediate junctional epidermolysis bullosa is caused by mutations in LAMA3, LAMB3, and LAMC2 genes resulting in truncated and partially functional laminin 332 or aberrant protein. COL17A1 mutations lead to reduced, or non-existent, production of type XVII collagen [6, 8]. Intermediate junctional epidermolysis bullosa has a more favorable prognosis, compared with severe subtype. In adult patients, localized and predominantly traumatic blisters on the skin are detected, isolated in places of atrophic scars, along with cicatricial alopecia, mild damage to the mucous membranes and teeth, nail dystrophy, and hypo- and hyperpigmentation in the places of healed blisters [2, 6, 8]. In addition, stalled wounds could form within areas of chronic blistering.

Non-healing wounds significantly deteriorate quality of life in patients with intermediate junctional epidermolysis bullosa. Therefore, different treatment modalities should be explored to improve the healing process. Cell therapy is considered a perspective approach in the treatment of inherited epidermolysis bullosa. To date, there are scarce reports of successful treatment of junctional epidermolysis bullosa patients with autologous cultured reventant keratinocytes and punch grafting of reventant skin to isolated lesions [9, 10]. Implementation of these methods is complicated by the technical difficulties. Nevertheless, various cell types could be studied for cell-based approaches to the treatment — specifically, fibroblasts that are characterized by low immunogenicity and easy cultivation. Several publications reported the efficacy of allogeneic fibroblasts in the treatment of recessive dystrophic epidermolysis bullosa patients [10, 11].
In our study, we investigated the efficacy and safety of intradermal injections of allogeneic fibroblasts into non-healing wounds in a patient with junctional epidermolysis bullosa.

Material and Methods

The study was approved by the Ethics Committee at the State Research Center for Dermatovenerology and Cosmetology, Moscow, Russia. The prospective study subject has signed an informed consent to participate in the study. A 49-year-old female patient with intermediate junctional epidermolysis bullosa was admitted to our clinic with skin and mucous blistering, painful wounds on upper and lower limbs, hair loss and dental anomalies. The first clinical findings occurred at birth. Also, since birth, chronic (over a four-week period or longer) wounds were forming within healing blisters in friction areas. The patient was born to non-consanguineous parents. There was no familial history of epidermolysis bullosa. The patient had two healthy children.

Laboratory examination included clinical and biochemical blood tests, and clinical urinalysis. For immunofluorescence antigen mapping intended to detect collagen XVII and α3, β3 and γ2 chains of laminin 332, biopsy specimens were obtained at baseline and 14 days after intralesional injections of allogeneic fibroblasts. Normal skin specimens were employed as healthy controls. During a follow-up period, non-healing wounds repeatedly developed on different skin areas. The cell product used for treatment consisted of a suspension of allogeneic human fibroblasts in 2% human albumin solution or saline solution, containing 5×10^6, 10×10^6, and 20×10^6 cells/mL. The fibroblasts were prepared at N.K. Koltsov Institute of Developmental Biology. Eligible erosions were those, which failed to heal within a month and had no signs of infection. Local anaesthesia with lidocaine/prilocaine cream was provided an hour prior to injections of fibroblasts or of vehicle. The fibroblasts were injected intradermally into the base of the erosions at 1 cm intervals. The wound margin selection was determined by keratinocyte migration from wound margins to the wound bed which is a well-known event of a wound-healing mechanism. Suspension solution without fibroblasts was used for placebo injections. It contained 2% human albumin solution or saline solution.

At two weeks after the fibroblast injection or vehicle injection, clinical assessment was performed. Biopsy specimens for immunofluorescence antigen mapping were sampled after fibroblast injections. At 12 months after fibroblast injections, another clinical assessment was conducted.

Results

Physical examination demonstrated skin lesions within shoulder joint area, thighs and shins, and an isolated 3×4 cm blister on medial surface of the left thigh. Atrophic scarring and milia were present at shoulder joint, forearm, thigh, and shin skin areas. Scarring alopecia, anonychia, and caries were present as well. Anemia was diagnosed due to low levels of hemoglobin (10.9 g per dL vs. reference range of 12-16 g per dL). The results of biochemical blood analysis, including total protein, iron and transferrin, and of clinical urinalysis were within reference ranges. During the follow-up period, the patient had painful stalled non-infected wounds with no signs of exudation and an erosion area of up to 30 cm².

| 5×10^6 cells/mL | 10×10^6 cells/mL | 20×10^6 cells/mL |
|-----------------|-----------------|-----------------|
| Baseline        |                 |                 |
| Day 14 after fibroblast injections | | |

Figure 1. The results of 5×10^6 cells/mL, 10×10^6 cells/mL and 20×10^6 cells/mL allogeneic fibroblast injections into non-healing wounds in a patient with intermediate junctional epidermolysis bullosa.
Figure 2. The results of vehicle injections into non-healing wounds in a patient with intermediate junctional epidermolysis bullosa.

Figure 3. β3 chain and type XVII collagen immunolabeling at baseline (A, B) and two weeks after 5x10⁶ cells/mL (C, D), 10x10⁶ cells/mL (E, F), and 20x10⁶ cells/mL (G, H) fibroblast injections in a junctional epidermolysis bullosa patient (200x magnification).
**Table 1. Changes in erosion area after fibroblast and vehicle injections in a patient with junctional epidermolysis bullosa**

| Dose, million cells/mL | Vehicle       | Injection date | Wound location       | Erosion area before treatment, cm² | Day 14 erosion area, cm² | % of erosion area reduction |
|------------------------|---------------|----------------|-----------------------|------------------------------------|--------------------------|-----------------------------|
| 5                      | Albumin       | 11.19.2014     | Left shoulder         | 15                                 | 10.2                     | 56.6                        |
| 10                     | Albumin       | 04.08.2015     | Right shoulder        | 12.5                                | 6                        | 52                          |
| 10                     | Albumin       | 04.08.2015     | Lateral aspect of left thigh | 7.5                                 | 0                        | 100                         |
| 10                     | Saline solution | 07.20.2016   | Left elbow            | 2.21                                | 0                        | 100                         |
| 10                     | Saline solution | 07.20.2016   | Right knee            | 1.75                                | 0                        | 100                         |
| 10                     | Saline solution | 07.20.2016   | Right knee            | 0.8                                 | 0                        | 100                         |
| 10                     | Saline solution | 07.20.2016   | Right knee            | 0.84                                | 0                        | 100                         |
| 20                     | Saline solution | 03.23.2016  | Lower left buttock    | 2                                   | 0                        | 100                         |
| 20                     | Saline solution | 09.28.2017  | Left shoulder         | 30                                  | 1.2                      | 96                          |
| —                      | Albumin       | 11.19.2014     | Right shoulder        | 3                                   | 1.3                      | 56                          |
| —                      | Albumin       | 04.08.2015     | Left shoulder         | 1.5                                 | 0                        | 100                         |
| —                      | Saline solution | 03.23.2016  | Left elbow            | 6                                   | 0                        | 100                         |

Immunofluorescence antigen mapping yielded an abnormal staining of α3, β3 and γ2 chains of laminin 332 and collagen XVII, seen as a narrow line in the patient biopsy specimen, compared with bright linear staining in the dermal-epidermal junction observed in a healthy control skin. Immunostaining of other structural proteins was not altered. Clinical examination, family history, and immunofluorescence microscopy confirmed the diagnosis of the intermediate junctional epidermolysis bullosa.

Injections were administered into erosion margins with suspension of allogeneic fibroblasts in different concentrations. On days 3-6 after 5×10⁶ cells/mL allogeneic fibroblast injections, we detected an erosion area reduction, even though at two weeks, wounds did not heal completely (Figures 1 and 2).

Of seven wounds treated with 10×10⁶ cells/mL fibroblast suspension, six have successfully healed. The area of the seventh wound with an initial size of 12.5 cm², has reduced by 52%. Of two erosions injected with 20×10⁶ cells/mL of fibroblast suspension, one has epithelized. Reduction in initial area (12.5 cm²) of the second erosion constituted 96%. We detected complete closure of two erosions treated with vehicle solution (albumin and saline). Another erosion injected with albumin has diminished by 56% (Table 1). No adverse events were reported.

At two weeks after administration of both 10×10⁶ cells/mL and 20×10⁶ cells/mL, immunofluorescence microscopy demonstrated amplified β3 chain expression. The β3 chain expression was interpreted as a tendency to form a sustained line at the dermal-epidermal junction. We detected similar improved β3 chain expression after administering saline solution. At two weeks after 5×10⁶ cells/mL fibroblast and albumin injections, the β3 chain immunostaining remained unchanged, compared with the baseline level (Figure 3).

**Discussion**

Two weeks after 5×10⁶ cells/mL and 10×10⁶ cells/mL fibroblast injections, we observed an elevated collagen XVII expression, which exhibited an uninterrupted immunostaining line.

After administering 20×10⁶ cells/mL, the collagen XVII expression has also increased, compared with the baseline level. Granular fibrillar staining at the dermal-epidermal junction was detected albeit an uninterrupted immunostaining line was not formed. No significant differences in collagen XVII staining were observed after injections of saline and albumin solution.

The follow-up visit one year after the treatment has demonstrated no blistering within fibroblast injection sites. No adverse events were noted during the follow-up period.

Hence, immunofluorescence antigen mapping allowed detecting abnormal expression of type XVII collagen and β3 chain of laminin 332 in our patient biopsy specimen, thereby confirming the junctional type of epidermolysis bullosa. We discovered a reduced expression of type XVII collagen and β3 chain of laminin 332 implying digenic mutations that could be identified in our patient. Digenic inheritance is characterized by pathogenic mutations in two different genes [12]. Due to the correction of two genes, digenic mutations could become a challenge for gene therapy.

We administered allogeneic fibroblasts into the margins of non-healing wounds in a junctional epidermolysis bullosa patient, which resulted in improved healing. The limited number of observations did not allow evaluating whether the type of suspension solution impacted the efficacy of treatment. However, our data have suggested that the efficacy of treatment depended on the initial size of erosions. In two weeks, a complete closure of the erosions with the initial area under 12.5 cm² was achieved. Two weeks after fibroblast injections, the erosions with the initial area ≥12.5 cm² failed to heal entirely; nevertheless, their sizes reduced considerably (by 52%, 56.5%, and 96%). Increased β3 chain and collagen XVII immunostaining at the dermal-epidermal junction, observed at two weeks along with improved healing rates, suggested that allogeneic fibroblasts accelerated the production of aforementioned structural proteins. Although β3 chain of laminin 332 and collagen XVII are produced by keratinocytes, our data suggested that after fibroblast administration, developing subclinical inflammation was leading to autocrine upregulation of growth factors, which could enhance the wound healing process [13]. These observations could also explain re-epithelialization of non-healing wounds after vehicle injections.

**Conclusion**

Our findings demonstrated that intradermal injections of allogeneic fibroblasts could be an effective therapeutic approach for treating small non-healing wounds in junctional epidermolysis bullosa.
Conflict of interest
The authors declare that they have no conflicts of interest.

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Ethical approval
All procedures performed in studies involving human participants were in compliance with ethical standards of the institutional and/or national research committee and with 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards.

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