Brief Report

Topical Gentamicin 0.1% Promotes Collagen 7 Expression in Recessive Dystrophic Epidermolysis Bullosa

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Abstract

Background: Currently, there is no cure for epidermolysis bullosa (EB) but few studies have explored the role of aminoglycosides in promoting collagen 7 expression in recessive dystrophic EB (RDEB). **Materials and Methods:** Consecutive patients aged >1 year with a confirmed diagnosis of dystrophic EB (DEB) were advised to apply 0.1% w/w gentamicin cream in a collagen base (Derbriment G™) twice daily on a representative area on right lower limb (RLL) and paraffin gauze dressings on the corresponding opposite side on the left lower limb (LLL). Skin lesions were evaluated clinically during the 12-week treatment period at the end of which a repeat skin biopsy was sent for immunofluorescence antigen mapping (IFM). **Results:** Twelve patients with DEB were recruited but only eight completed the study and were analyzed. The mean fluorescence intensity (MFI) of the study cohort increased from 2765 ± 1732.07 (263–4845) at baseline to 5412.75 ± 3937.64 (2100–13536) at 12 weeks; a 95.75% (range 5.34%–775.14%) increase in the MFI of collagen 7 from baseline (P = 0.06). Among patients with a known termination codon mutation (n = 3), the percentage increase in MFI was greater among patients with known premature termination codon (PTC) mutations compared to those with unknown mutations. The clinical severity did not change significantly in terms of the mean number of blisters, erosions, and scarring during the study period. None of the parents reported any adverse effect. **Conclusions:** Topical gentamicin 0.1% w/w is a safe and effective way to promote the expression of COL7A1 in DEB patients, especially those carrying PTC mutations.

Keywords: *Epidermolysis bullosa, gentamicin, premature termination codon*

Introduction

Epidermolysis bullosa (EB) represents a wide spectrum of phenotypes, ranging from mild blistering to severe scarring of the skin, extracutaneous manifestations, and early mortality due to secondary malignancies.[1] Aminoglycosides are a class of antibacterial agents which reduce discrimination between cognate and near-cognate tRNA, permitting an amino acid to be inserted at the stop codon. This mechanism has been used in the treatment of cystic fibrosis,[2] Duchenne muscular dystrophy.[3] In few recent studies, it has been seen to have similar effect in recessive dystrophic EB (RDEB), and junctional EB.[4-8] In this pilot study, we planned to evaluate the efficacy of topical gentamicin in promoting collagen 7 expression in the EB skin, promoting wound healing and prevention of new blister formation in patients of EB.

Materials and Methods

This was a 3-month open label study undertaken by the Department of Dermatology and Histopathology, PGIMER, Chandigarh initiated after obtaining approval from the Institute's ethics committee. Patients aged >1 year with diagnosis of dystrophic EB (DEB, confirmed by immunofluorescence antigen mapping [IFM] and/or molecular diagnosis). Patients who had received any topical/systemic antibiotics and so on in the last 6 weeks or were a part of any other clinical trial in the past 6 months, patients with a frank secondary bacterial infection or sepsis were excluded.

All EB patients presenting in the study period were screened and those satisfying the inclusion/exclusion criteria were enrolled over a period of 1.5 years after obtaining written informed consent/assent from the patients' parents. All patients

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were advised to apply 0.1% w/w gentamicin cream in a collagen base twice daily on a representative area on right lower limb (RLL) and paraffin gauze dressings on the corresponding opposite side of the body. The amount of cream to be applied was explained to parents using finger tip unit (FTU) rule (one FTU for one leg from knee to ankle). Skin lesions were evaluated clinically once in every 4 weeks during the 12-week treatment period for the development of any secondary bacterial infection when a repeat skin biopsy was taken from the same initial site, and sent for IFM. General care was the same in all patients. Risk of nephrotoxicity is low with topical administration. However, serum urea and creatinine were monitored at baseline and weekly for 4 weeks and thereafter monthly for 3 months. Mean fluorescence intensity (MFI) of collagen 7 expression was measured by ImageJ software. For immunofluorescence, statistical analyses were performed using GraphPad Prism (v5) software. The statistical analyses were performed with the help of Statistical Package for the Social Sciences (SPSS) statistical software, version 20.0 (SPSS, Inc., Chicago Il, USA), $P < 0.05$ as significant.

Results

During the study period, 12 patients with DEB were recruited – 6 boys and 6 girls. The mean age of the study cohort was 5.44 ± 5.2 years (range 12 months to 14 years). The mean disease severity as measured by instrument for scoring clinical outcomes of research for epidermolysis bullosa (iSCOREB) was 42.82 ± 71.90 [Table 1]. At the time of recruitment, severe mucosal scarring in the form of ankyloglossia was present in 8 out of 12 patients, and dysphagia due to esophageal strictures in 3 out of 12 patients. Severe cutaneous scarring in the form of contractures was present in three out of 12 patients. Of the 12 patients, only 8 adhered to the treatment protocol and were analyzed at the end of 12 weeks of study period. The remaining four patients expressed inability to go for sequential biopsy citing lack of clinical improvement.

After 12 weeks of treatment, the MFI of the study cohort increased from 2765 ± 1732.07 (263–4845) to 5412.75 ± 3937.64 (2100–13536). This represented a 95.75% (5.34%–775.14%) increase in the MFI of collagen 7 from baseline. However, this was not found to be statistically significant ($P = 0.0635$; paired student *t*-test) [Figures 1a–h and 2]. Among patients with a known termination codon mutation ($n = 3$), the percentage increase in MFI was 173.69% (increase in a MFI of 2777.3 ± 2046.96 to 7590.33 ± 5731.81) compared to only 48.77% increase in MFI (2760 ± 1776.24 to 4106.2 ± 2240.1) among the remaining five patients.

The clinical severity did not change significantly during the study period. At baseline, the mean number of blisters, erosions, and scarring in the treatment limb was 2.0, 5.0, and 5.8, respectively, compared to 2.375, 6.0, and 5.625,

Table 1: Baseline disease characteristics of the study patients

Patient S.No.	PTC Mutation	Age (years)	Sex	iSCOREB	Blisters				Erosions				Scarring				MFI COL7A1 only of active treatment arm			
					RLL	12 weeks	LLL	12	RLL	12 weeks	LLL	12	RLL	12 weeks	LLL	12 weeks	Pre-treatment IFM (mean)	Pre-treatment (SD)	Post-treatment (mean)	Post-treatment (SD)
Patient 1	COL7A1 Exon 54 c. 5000G>A COL7A1 Exon 112 c. 8329C>T	8	M	78.6	2	2	3	3	4	3	5	5	7	7	8	8	4845	1239	7135	3407
Patient 2	COL7A1 Exon 5 c. 553C>T p.Arg185Ter	1	F	16	1	2	1	2	3	3	4	4	5	6	6	7	752	241	2100	1012
Patient 3	COL7A1 Exon 5 c. 553C>T p.Arg185Ter	1	F	42	2	2	1	1	8	7	5	6	6	6	5	5	2723	1322	13536	4800
Patient 4	Report not available	1.5	M	50	2	2	2	1	6	5	9	10	7	7	6	6	2487	1126	2620	1261
Patient 5	Report not available	7	M	72	3	2	4	3	4	4	9	10	7	7	4	4	2104	620	2522	987
Patient 6	Report not available	1	F	54	3	3	3	3	6	7	6	4	7	7	5	5	4549	2544	6855	3100
Patient 7	Report not available	1	M	16	1	1	2	2	3	3	4	2	5	6	7	7	263	140	2299	1046
Patient 8	Report not available	14	F	54	2	2	3	3	6	6	6	7	3	3	4	6	4397	2542	6235	2932
Patient 9	Report not available	8	M	42	1	1	1	1	12	10	7	5	3	3	5	5		Did not complete study protocol		
Patient 10	Report not available	9	M	18	2	1	3	3	15	15	3	4	6	6	7	8		Did not complete study protocol		
Patient 11	Report not available	14	F	44	1	1	1	2	3	4	5	6	1	2	2	2		Did not complete study protocol		
Patient 12	Report not available	2	F	26	1	2	1	1	3	4	5	5	5	5	6	6		Did not complete study protocol		

LLL – control; RLL – active treatment

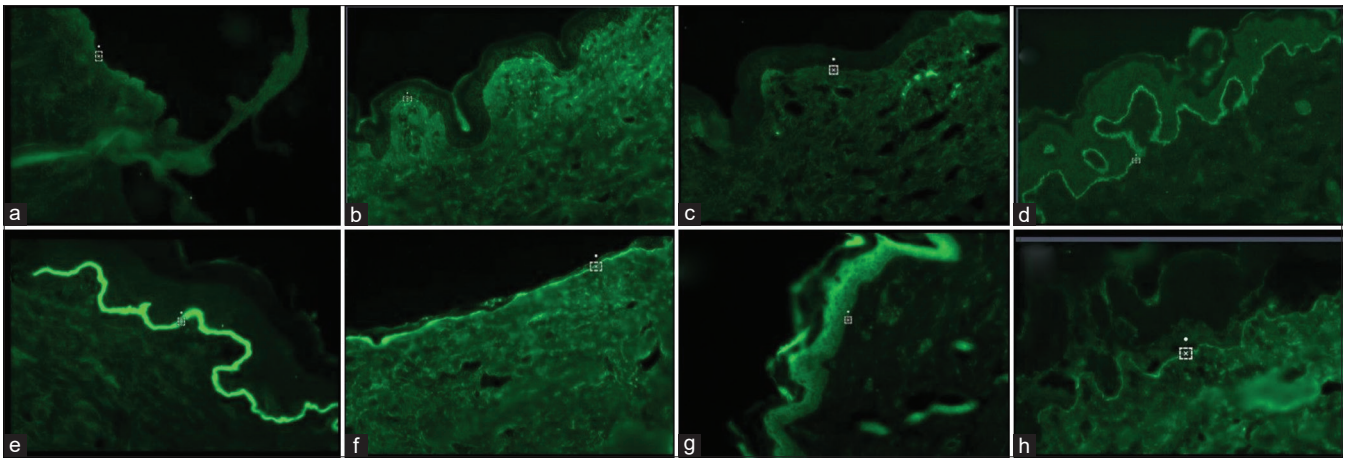

Figure 1: (a-d) Pre-treatment IFM images of patient 3, 1, 5, and 8. (e-h) Post-treatment IFM images of patient 3, 1, 5, and 8

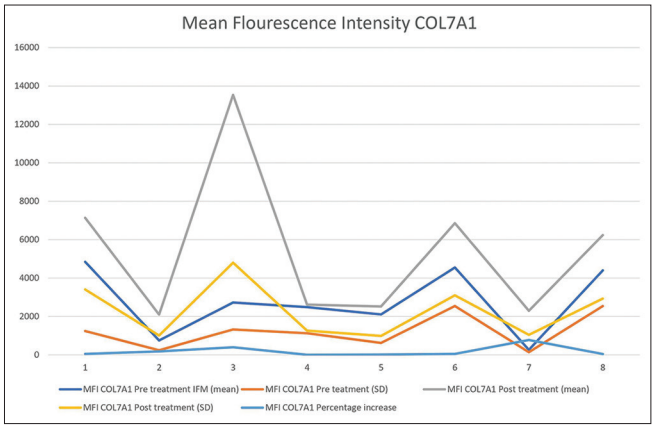

Figure 2: Pre- and post-treatment mean immunofluorescence intensity of the active treatment arm of the study patients

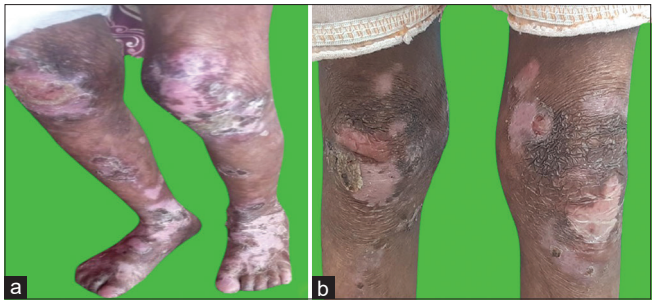

Figure 3: (a) Pre-treatment image of patient 1. (b) Post-treatment image of patient 1; note the mild improvement in wound healing on the side of the intervention (RLL)

respectively, in the control limb. At the end of 12 weeks, mean number of blisters, erosions, and scarring in the treatment limb was 2.0, 4.75, and 6.125, respectively, compared to 2.25, 6.0, and 6.0, respectively, in the control limb [Figure 3a and b]. None of the patients/parents reported any adverse effect.

Discussion

The management of EB, in general, and DEB, in particular, is difficult and aimed at improving the patient's quality of life by providing symptomatic relief. With an increase in the understanding of the disease etiopathogenesis, there is an increased emphasis on exploring the role of gene- and cell-based therapies.[9]

Although antiseptics and antibacterial creams are frequently prescribed in EB wound care as they can assist in the healing process and help prevent secondary infection, the role of aminoglycoside group of antibiotics is particularly interesting. In the study by Cogan *et al*.[4] incubation of RDEB keratinocyte cell lines and primary RDEB fibroblasts cells with aminoglycosides led to the synthesis of a full-length collagen 7 in a dose-dependent and sustained manner along with reversal of abnormal RDEB cell phenotype. In a double-blind, placebo-controlled pilot trial by the same group, Woodley *et al*.[5] observed that use of topical 0.1% gentamicin ointment applied three times daily for 2 weeks or intradermal gentamicin injection (8 mg) for 2 days induced type VII collagen (varying from 20% to 165%) and anchoring fibrils at the dermal–epidermal junction of treatment sites. They observed that the newly induced collagen persisted for 3 months but did not generate anti-type VII collagen autoantibodies. These results are consistent with our observations where we observed a mean increase in collagen 7 expression of 95.75%. The increase in expression was particularly remarkable for patients with a known premature termination codon (PTC) mutation. In fact, on further dissection, the child with the compound heterozygous mutation (one PTC and the other missense mutation) had a lower percentage increase compared to those with homozygous PTC mutation. However, we did not observe a corresponding improvement in the clinical condition.

Similar results have been seen in junctional EB too with increase in Laminin 332 expression after use of topical gentamicin.[6-8] There were several limitations of the present study. One was the small sample size, and a short follow-up period of 3 months. Because the active treatment had additional collagen in addition to topical gentamicin,

it could have influenced any clinical outcome in terms of wound healing although we did not see an obvious clinical benefit in the active treatment arm. The follow-up biopsies were not compared with biopsies from the control site, as parents did not want the children to undergo repeated skin biopsies. Finally, not all patients were genetically characterized. In the absence of a confirmed mutational diagnosis, it can only be speculated that patient 7 who showed a 774% increase in collagen 7 expression too had a PTC mutation.

Concluding, topical gentamicin 0.1% w/w is a safe and effective way to promote the expression of COL7A1 in DEB patients, especially those carrying PTC mutations although the clinical improvement may take longer than 3 months. Further large and multicentric studies are needed to study the strength and dose of the cream to be administered for long-lasting clinical effectiveness.

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Conflicts of interest

There are no conflicts of interest.

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