Efficacy and mechanism of action of intravenous allogenic umbilical cord blood-derived mesenchymal stem cell therapy in patients with recessive dystrophic epidermolysis bullosa

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Directed by Professor Sang Eun Lee

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Seung-Ju Lee

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<ABSTRACT>

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(Directed by Professor Sang Eun Lee)

Recessive dystrophic epidermolysis bullosa (RDEB) is an incurable disease that causes severe mucocutaneous fragility due to mutations in *COL7A1* encoding type VII collagen (C7). In this phase 1/2a trial, we evaluated the safety and possible clinical efficacy of intravenous infusion of allogeneic human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) in patients with RDEB.

Four adult and two pediatric RDEB patients were treated with three intravenous injections of hUCB-MSCs (1-3 x 106 cells/kg) every two weeks and followed up for 8-24 months after treatment. The primary endpoint was safety. Secondary endpoints related to efficacy included clinical parameters such as disease severity score, wound assessment, itch and pain score, and quality of life. C7 expression levels and inflammatory infiltrates in the skin, as well as serum levels of inflammatory markers and neuropeptides were also assessed.

Intravenous hUCB-MSCs infusions were well tolerated without serious adverse events. Improvements in the Birmingham Epidermolysis Bullosa Severity Score, body surface area involvement, blister counts, pain, pruritus, and

quality of life were observed with maximal effects at 56 to 112 days post-treatment. hUCB-MSCs administrations induced M2 macrophage polarization and reduced mast cell infiltration in RDEB skin. Serum levels of substance P were decreased after therapy. Increased C7 expression was observed at the dermoepidermal junction in 1 in 6 patients at day 56.

To the best of our knowledge, this is the first clinical trial of systemic administration of allogeneic hUCB-MSCs in RDEB patients, demonstrating safety and transient clinical benefits.

Key Words : recessive dystrophic epidermolysis bullosa, mesenchymal stem cell, clinical trial

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I. INTRODUCTION

Epidermolysis bullosa (EB) is a group of genetic diseases characterized by mechanical fragility of skin and mucosa[1]. Recessive dystrophic EB (RDEB), caused by mutations in *COL7A1* coding for type VII collagen (C7), the main constituent of anchoring fibrils at the dermoepidermal junction (DEJ), is one of the most severe forms of EB, showing recurrent blistering, chronic wounds, and disabling scarring in the skin and mucosa and internal organ dysfunctions, leading to substantial morbidity and mortality[2,3]. Currently, there is no cure for this severe subtype of EB, however, novel therapeutic strategies have been developed in the fields of gene and cell therapies[4-15].

Mesenchymal stem cells (MSCs) have been identified as an attractive option for allogeneic cell therapy for RDEB based on their potential mechanisms of action including immunomodulation, migration to damaged tissue, stimulation of tissue

regeneration, and reduction of fibrosis, mainly through paracrine activities[4-6,8,12,13,15]. Locally injected allogeneic bone marrow (BM)-derived MSCs (BM-MSCs) have shown to accelerate wound healing with transient C7 restoration in RDEB patients and a mouse model of dystrophic EB[6]. Two early-phase clinical trials of systemic administration of allogeneic BM-MSCs in 23 pediatric patients with RDEB reported variable clinical benefits that lasted for several months, with satisfactory safety[5,8]. One additional, recently published, phase 1/2 trial of intravenous BM-MSCs injection in 10 adult patients with RDEB also showed transient, but clinically meaningful improvements in disease severity, skin inflammation, and pruritus with no serious adverse events (AEs)[4].

Until now, all clinical trials for RDEB have examined the potential of BM-MSCs[4,5,8]. However, umbilical cord blood (UCB) has become an attractive source of stem cells, since its non-invasive collection procedure and rapid availability from cord blood banking[16,17]. Human UCB-derived MSCs (hUCB-MSCs) exhibit higher proliferation capacity and lower immunogenicity compared to BM-MSCs[17-19]. A few data support that UCB-MSCs may have significantly greater immunosuppressive potential than other sources of MSCs[16-21]. In addition, hUCB-MSCs have been shown greater immunosuppressive and regenerative potential than BM- or peripheral blood-derived MSCs in murine wounding model[22]. A preclinical study has demonstrated that repeated systemic infusions of human UCB-derived unrestricted somatic stem cells, a subpopulation of non-hematopoietic stromal stem cells, significantly extended the life span and reduced blistering of RDEB mice model[15]. Given the promising results of the preclinical study, we conducted a first-in-human, phase I/2a clinical trial of intravenous administrations of allogeneic hUCB-MSCs in patients with RDEB to determine the safety, tolerability, and potential efficacy. We also analyzed changes in serum inflammatory markers and neuropeptides, and skin inflammatory infiltrates and

C7 expression following hUCB-MSCs treatment.

II. MATERIALS AND METHODS

1. Patients, study design, and procedures

This phase 1/2a, single center, non-randomized, open-label trial to evaluate the safety and efficacy of hUCB-MSCs for RDEB patients was conducted at Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea. The diagnosis of RDEB was made by immunofluorescence antigen mapping, transmission electron microscopy (TEM), and mutation analysis of the *COL7A1* gene. Patients who provided informed consent were screened within 4 weeks before the start of the cell therapy. The visit schedule consisted of a 4-month run-in period that included a screening visit and an enrollment visit, three times administration of hUCB-MSCs, and 8- to 24-month follow-up period. Patients received three separate intravenous injections of hUCB-MSCs every 2 weeks and then were assessed at days 56, 112, 168, and 8 to 24 weeks posttreatment. Following the first administration of hUCB-MSCs, the patients remained hospitalized for 24 hours for observation of possible adverse events. Peripheral blood samples were obtained at each visit for safety laboratory tests and biomarkers analysis. Skin biopsy samples obtained at visits 1 and 5 were examined for changes in C7 and anchoring fibrils expression by immunofluorescent staining and TEM, respectively and skin infiltration of immune cells after treatment.

2. Production of hUCB-MSCs

Production of hUCB-MSCs were manufactured and expanded according to Good Manufacturing Practice (GMP) regulations. hUCB-MSCs from umbilical cord blood of healthy donors were isolated, expanded in KSB-3 Complete Medium® Kit (Kangstem Biotech Co., Ltd) at the GMP facility of Kangstem Biotech Co., Ltd, South Korea. The manufactured cells were confirmed to meet the quality control criteria approved by the Ministry of Food and Drug Safety.

3. Outcome measures

The primary end points of the investigation were the safety and tolerability of three separate intravenous administrations of hUCB-MSCs. Safety was assessed through the monitoring of adverse events, laboratory assessments, vital signs, electrocardiograms, and abbreviated physical examinations at each visit during the 8 to 24-month posttreatment period. Secondary efficacy end points included (1) disease severity scores assessed by Birmingham Epidermolysis Bullosa Severity Score (BEBSS) and total body surface area (TBSA) affected by epidermolysis bullosa, (2) wound assessment by clinical photograph, blister count, and the ratio of blister area to body surface area (BSA) (3) visual analogue scale (VAS) for pain and pruritus, and (4) quality of life (QOL) questionnaire by QOL in EB questionnaire (QOLEB) during the 6-month posttreatment period compared with those in the screening period.

4. Immunofluorescence staining and TEM analysis

Frozen skin tissues from the patients were sectioned at 5 μm and stained with primary antibodies, including mouse monoclonal [LH7.2] antibodies to type VII collagen (ab6312; Abcam), mouse monoclonal antibodies to CD206 (#321102, Biolegend), mouse monoclonal antibodies to CD68 (ab955, Abcam), and rabbit

polyclonal antibodies to c-kit (A4502, Dako). Alexa Fluor 488-conjugated rabbit anti-mouse IgG and goat anti-rabbit IgG (all from Thermo Fisher Scientific) were used as secondary antibodies. Sections were stained with 4,6-diamidino-2-phenylindole (DAPI) (Thermo Fisher Scientific). Images were captured using an LSM 780 confocal microscope (Carl Zeiss, Oberkochen, Germany). Negative controls omitting the primary antibody were also performed (data not shown). The skin tissue sections were fixed in Karnovsky's fixative and examined under a transmission electron microscope (H-7600, Hitachi, Japan).

5. Mean fluorescence intensity (MFI)

MFI was calculated for each immunofluorescence stained images for C7 using Image J (National Institutes of Health, Bethesda, MD). Five measurements were taken at regular intervals using 8 × 8 pixels every 100 pixel along the dermal-epidermal junction. The values are presented as mean ± SEM.

6. Indirect immunofluorescence (IIF)

The detection of circulating anti-C7 autoantibodies was performed in all patients by an IIF study performed on salt-split normal human skin substrate. To evaluate the presence of anti-C7 antibodies, patient serum was obtained at baseline, day 56, day 112, and day 168 for evaluation of anti-C7 antibodies by salt-split IIF.

7. Serum biomarkers measurement

Levels of biomarkers were measured in pre-treatment and day 56 serum samples. IL-1β (Human IL-1β/IL-1F2 Quantikine ELISA Kit, catalogue number DLB50, R&D Systems), IL-6 (Quantikine ELISA Kit, catalogue number D6050, R&D Systems), substance P (Substance P Parameter Assay Kit, catalogue number KGE007, R&D Systems) and calcitonin gene-related peptide (CGRP) (human CGRP kit, catalogue number A05481.96, Bertin pharma) were quantified by

individual competitive enzyme-linked immune sorbent associated assays (ELISAs) according to the manufacturer's instructions. The normal reference range for the proinflammatory cytokines and neuropeptides were defined from a population of 10 healthy subjects ranging from 18 to 50 years of age.

8. Statistics

In this trial, six patients were enrolled and completed the follow up. For the secondary outcomes (clinical parameters), the mean differences from baseline were analyzed, together with a *P*-value and a 95% confidence interval (paired t-test). For the serum bloimarkers and the number of cell infiltrates in the skin, Wilcoxon signed-rank test was used in a statistical analysis to compare the paired samples of patients before and at different time points during treatment. Student t-test was used for comparing the secondary outcomes (clinical parameters) between age-subgroups. Statistical analysis were peformed using Prism 8 (GraphPad Prism). Statistical significance was defined as $P < .05$.

9. Study approval

All methods and procedures associated with this study was approved by institutional review board (IRB No. 3-2015-0285) of Yonsei University College of Medicine and was performed in compliance with the Declaration of Helsinki and Good Clinical Practice as defined under the Korea Food & Drug Administration (KFDA) regulations and the International Conference on Harmonisation guidelines. Prior to inclusion in this study, written informed consent was received from all participants or their guardians in case of pediatric patients.

III. RESULTS

1. Patient characteristics

Between Oct 2016 and May 2019, 6 patients with RDEB were assessed for eligibility. 3 adults and 2 pediatric patients were sequentially enrolled in the trial and received three repeated intravenous hUCB-MSCs injections. One additional adult patient was treated with the same investigational product under the treatment use approval from the KFDA due to being too late for the trial enrolment (Figure 1). All patients were moderate to severe or severe phenotypes with various extracutaneous symptoms. Negative or markedly decreased expression of C7 non-collagenous-1 domain was found in baseline skin biopsies. Analysis of circulating autoantibodies against C7 using IIF was negative for all patients (Table 1). All adult patients received 3 x 106 hUCB-MSCs/kg every 2 weeks, whereas two pediatric patients received 1-2 x 106 hUCB-MSCs/kg, with advancing the dose based on tolerance. Demographics and clinical characteristics of participants and trial flow are provided in the Figure 1 and Table 1. All patients completed at least 8-month (8-24 months) follow-up after the first infusion.

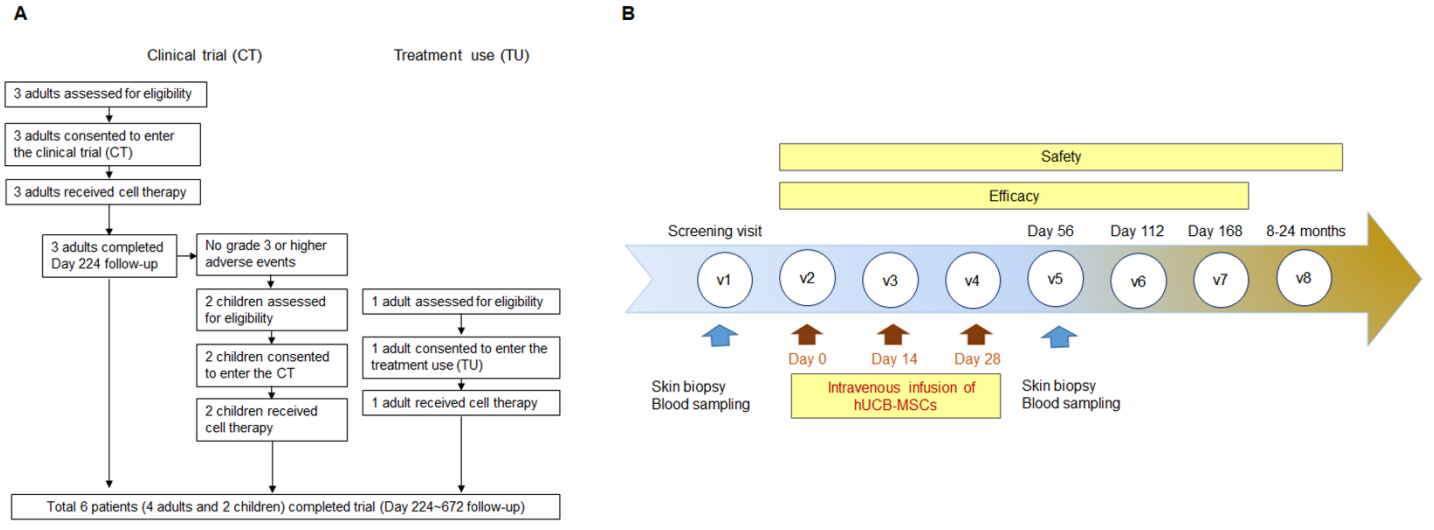

Figure 1. Study design. (A) Flow chart for clinical trial and treatment use (expanded access to investigational drugs for treatment use). (B) Study design for hUCB-MSC treatment and evaluation.

Table 1. Demographics and clinical characteristics of six RDEB patients

Subject	1	2	3	4	5	6
Sex/Age	F/60	F/25	M/21	F/13	F/8	F/28
***COL7A1* mutation**	c.3631C>T, p.Q1211X; c.8569G>T, p. E2857X	c.3139+12G>A; c.5188C>T, p.R1730X	c.2005C>T, p. R669X; c. 8569G>T, p. E2857X	c.3631C>T, p.Q1211X; c.3717_3721del5(TA CTC)	c.7371insA; c.2318_2319 del2 (C,T)	IVS22+T>G
Phenotype	Severe	Moderate-to-severe	Moderate-to-severe	Severe	Moderate-to-severe	Severe
C7 expression at DEJ (DIF)	Barely detectable	Barely detectable	Reduced	Undetectable	Undetectable	Undetectable
Circulating autoantibodies against C7 (IIF)	Negative	Negative	Negative	Negative	Negative	Negative
Major clinical features	Mitten deformity, cataract, corneal erosions	Mitten deformity (s/p hand surgery)	Esophageal stricture (s/p balloon dilation)	Mitten deformity (s/p hand surgery)	Mitten deformity (s/p hand surgery)	Mitten deformity (s/p hand surgery) Esophageal stricture (s/p balloon dilation)

C7, type VII collagen; DEJ, dermoepidermal junction; DIF, direct immunofluorescence test; IIF, Indirect immunofluorescence test; s/p, status post operation

2. Safety

AEs during the study period are summarized in Table 2 and 3. Overall, 50% of the patients treated reported ≥ 1 AE. The most frequent AE was wound infection in 4 of 13 AEs (30.8%), but all wound infections were thought to be due to the underlying RDEB. Only acute gastritis was considered as an AE determined to be possibly related to cell therapy. No severe AEs (defined by Common Terminology Criteria for Adverse Events) at grade 3 or higher were reported, suggesting that intravenous hUCB-MSC injections were generally well tolerated. There were no clinically significant changes in laboratory test values except increased basal levels of C-reactive protein (CRP) and fibrinogen, vital signs, or electrocardiogram results during the study period. There were no changes in tissue-bound immunoreactants using IIF following cell therapy.

Table 2. Summary of adverse events (AEs)

	Number (%)
Total number of patients	6 (100)
Number of patients who experienced AEs	3 (50)
Total number of AEs	13 (100)
Grade[A]	**Number of AEs (%)**
Grade 1	8 (61.5)
Grade 2	5 (38.5)
Grade 3~5	0 (0)
Relationship to the study drug	
Definitely	0 (0)
Possibly	1 (7.7)
Likely	0 (0)
Unlikely	1 (7.7)
Not related	11 (84.6)
Outcome	
Resolved	12 (92.3)
Continuing	1 (7.7)
Action taken	
None	3 (23.1)
Required concomitant medication	10 (76.9)

[A]Grading scales were measured according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Table 3. Adverse events (AEs) classified by systemic organ classes

		Relationship to the drug (n (%))				
System organ class	AEs	Definitely	Possibly	Likely	Unlikely	Not related
Generalized	General condition deterioration	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)
	Dizziness	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)
Gastrointestinal	Acute gastritis	0 (0)	0 (0)	1 (7.7)	0 (0)	0 (0)
	Nausea	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)
Infection	Fever	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)
	Herpes zoster	0 (0)	0 (0)	0 (0)	1 (7.7)	0 (0)
	Wound infection	0 (0)	0 (0)	0 (0)	0 (0)	4 (30.8)
	Upper respiratory tract infection	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)
Ocular	Lacrimal duct obstruction	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)
Ear, Nose, and Throat	Epistaxis	0 (0)	0 (0)	0 (0)	0 (0)	1 (7.7)

3. Clinical efficacy

hUCB-MSC treatment markedly reduced erythema and erosions in RDEB patients (Figure 2). At day 56, the mean clinical severity scores assessed by BEBSS and TBSA affected by RDEB significantly decreased by 5.6 (95% CI, -7.39 to -3.86) and 5.4 (95% CI, -8.14 to -2.61) points, respectively. Blister count and the ratio of blister area to BSA also decreased by 4 (95% CI, -6.74 to -1.26) and 2 (95% CI, -4.02 to -0.06) points, respectively at day 56 compared to baseline. After day 56, these clinical effects of hUCB-MSCs were either maintained or slightly attenuated over time until day 168 (Figure 3). Chronic non-healing wounds in RDEB are associated with decreased quality of life and increased risk of cutaneous squamous cell carcinoma (cSCC). We evaluated the effect of hUCB-MSCs on the healing of chronic open wounds that were unhealed for at least 12 weeks with wound size >100 cm^2. One pediatric (subject 4) and one adult (subject 6) subject each had two chronic open wounds. Of the four chronic wounds from two subjects, two wounds (one from subject 4 and one from subject 6) (50%) showed a 50% or greater reduction in wound size compared to baseline at day 56. Of these two wounds, only one remained at least 50% healed by day 112.

hUCB-MSC treatment resulted in a substantial mean reduction in pain (−3 points on VAS score, 95% CI, -4.76 to -1.24) and itch (−2 points on VAS score, 95% CI, -3.76 to 0.24) from baseline by day 56. Mean VAS scores for pruritus were maintained by day 168, while pain VAS scores showed a gradual increase over time (Figure 2). At day 56, QOL assessed by QOLEB was improved by 6.2 points (95% CI, -8.69 to -3.65). Age-subgroup analyses (children vs. adults) showed no significant between-group differences in the secondary outcomes, including BEBSS, TBSA, blister count and area, itch and pain scores, and QOLEB.

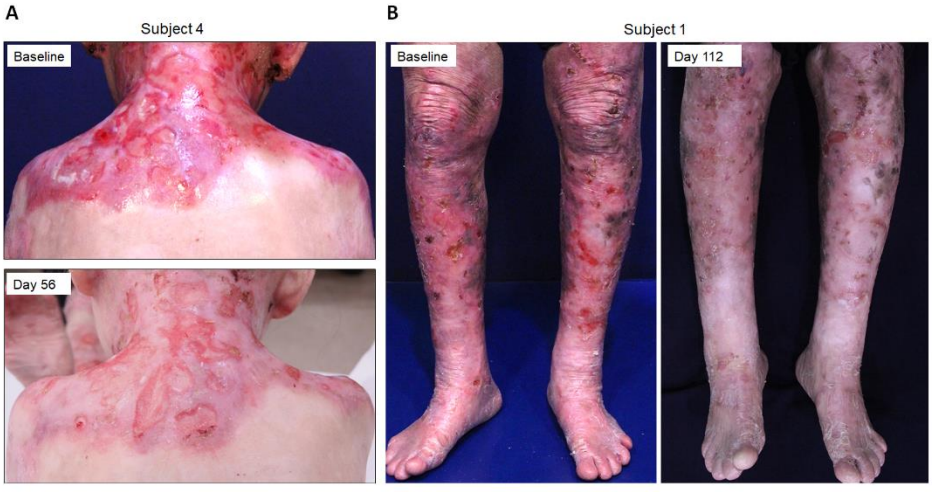

Figure 2. Baseline and post-hUCB-MSC treatment photographs of pediatric (A, subject 4) and adult RDEB patients (B, subject 1). Marked reduction in erythema and erosions is observed in patients after 3 repeated injections of hUCB-MSCs.

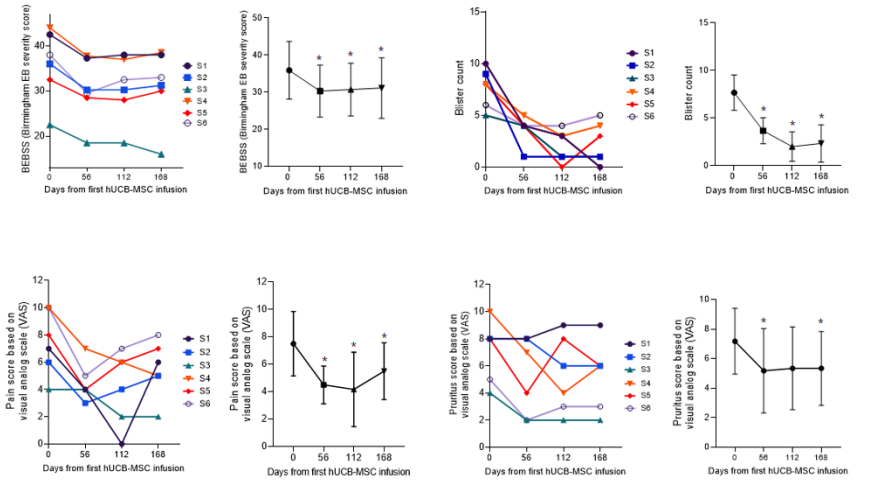

Figure 3. Systemic treatment with hUCB-MSCs improved clinical symptoms in RDEB patients. Time course of changes in disease severity (assessed by Birmingham Epidermolysis Bullosa Severity Score), blister count, visual analog scale (VAS) pain score, and VAS pruritus score was assessed throughout the trial. For each parameter, a graphical representation of mean score per visit with range per visit was added. Two-tailed Student′s t-test was performed for all the comparisons (n=6). ***$P < 0.001$. S, subject.

4. Molecular assays for C7 in skin

Then we evaluated whether the systemic infusions of hUCB-MSCs could restore C7 and anchoring fibrils in RDEB skin by immunofluorescence staining and TEM analysis of skin of patients before and after treatment. On day 56, one patient (subject 1) showed an increase in C7 expression level at DEJ as assessed by MFI compared to baseline, while others (subject 2~6) showed no significant changes in C7 expression in skin after MSC treatment (Figure 4). No obvious

differences in anchoring fibril structure or distribution were observed between baseline and day 56 in all 6 patients, as assessed by TEM (data not shown).

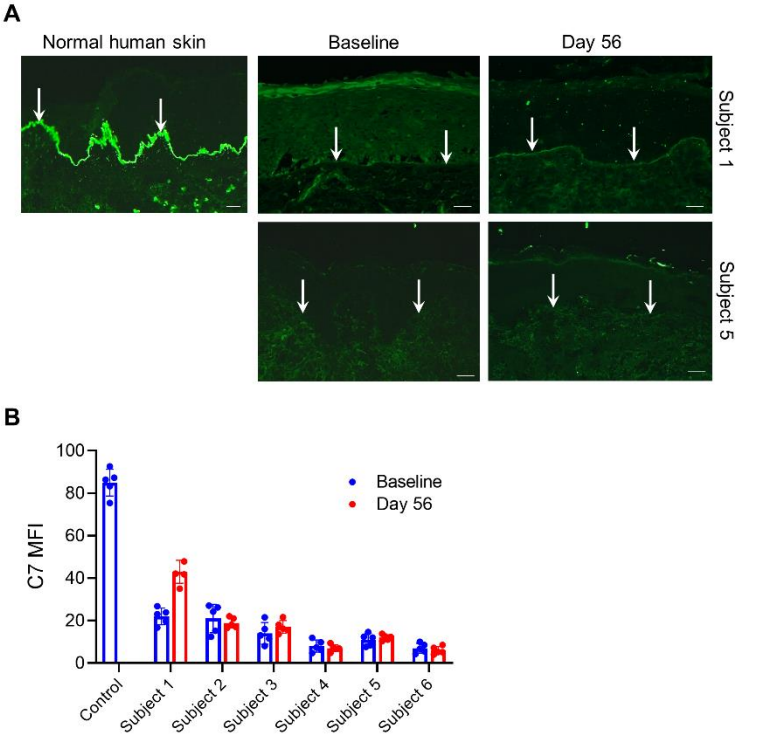

Figure 4. **Systemic treatment with hUCB-MSC does not significantly affect the expression levels of type VII collagen (C7) at dermoepidermal junction (DEJ) in most patients except one patient (subject 1) who showed an increase in C7 expression at day 56**. (A) Representative immunofluorescence staining for C7 using LH7.2, a monoclonal antibody that recognizes the NC1 domain of C7, on skin biopsy samples obtained before treatment (baseline) and at day 56 from RDEB patients (subject 1 and 5) receiving hUCB-MSC treatment. Scale bars= 20 μm. White arrows indicate C7

expression at DEJ (B) The intensity of the staining for C7 expression along the DEJ was morphometrically quantitated as MFI using Image J (National Institutes of Health, Bethesda, MD). Values are shown as mean ± SEM.

5. Changes in skin infiltration of macrophages and mast cells

We analyzed the phenotypes of macrophages in the skin of RDEB patients before and after hUCB-MSC treatment. The number of $CD68^+$ total macrophages was higher in the skin of RDEB patients at baseline than in healthy controls. Intravenous administration of hUCB-MSCs did not affect the density of $CD68^+$ total macrophages, but significantly increased macrophages expressing CD206, a marker of M2 macrophages, in RDEB skin at day 56 (Figure 5). Baseline skin biopsies of RDEB patients showed a significant increase of mast cell infiltration compared with normal human skin, but mast cell infiltration was significantly reduced 56 days after hUCB-MSC treatment (Figure 5).

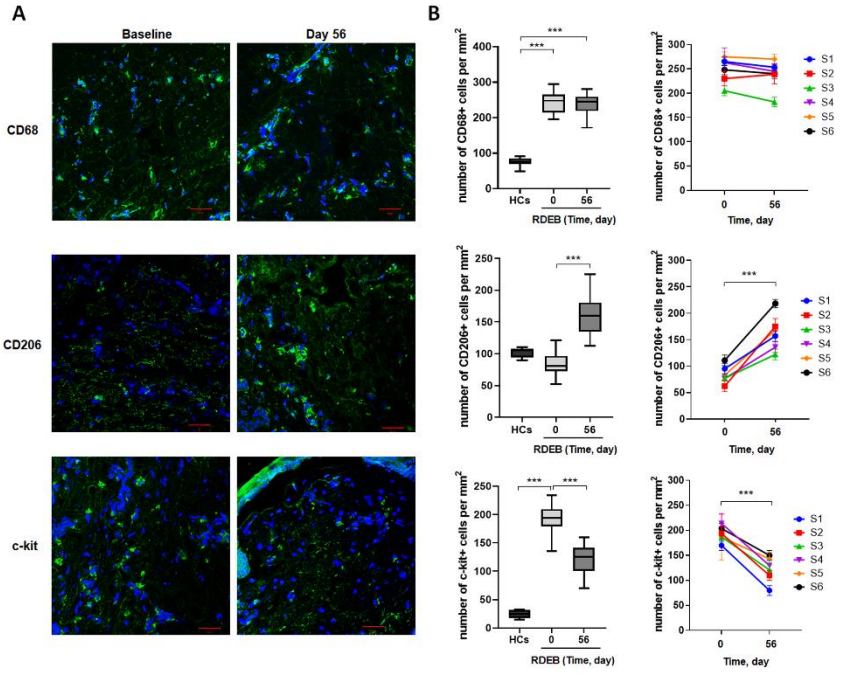

Figure 5. hUCB-MSC treatment modulates macrophage phenotype and mast cell infiltration in RDEB patient's skin. (A) Representative immunofluorescence staining for total macrophages (CD68), $CD206^{+}$ macrophages, and mast cells (c-kit) on skin biopsy samples before treatment (baseline) and at day 56 for 6 matched pairs of RDEB patients receiving hUCB-MSC treatment. Scale bars= 50 μm. (B) Mean total numbers of skin-infiltrating cells in biopsies from healthy controls (HCs) and RDEB skin by study day following hUCB-MSC treatment. By day 56, hUCB-MSC treatment markedly increased $CD206^{+}$ macrophage counts and reduced mast cell counts. Values are shown as mean ± SEM. The Wilcoxon signed rank test was performed for all the comparisons (n=6). ***$P < 0.001$. S, subject.

6. Changes in systemic inflammatory markers and neuropeptides

Chronic wounds in RDEB trigger systemic inflammation that may contribute to multiple organ damages[2,3,23-27]. Since MSCs have potent immunomodulatory capacities, we investigated the effect of hUCB-MSC infusion on the serum inflammatory markers in RDEB patients. Serum levels of CRP fluctuated in individual patients over time, but no significant change in the mean CRP values was observed 56 days after hUCB-MSC treatment (data not shown). Additionally, baseline serum levels of the pro-inflammatory cytokines, IL-1β and IL-6, were elevated in RDEB patients than in healthy controls, but these levels were not significantly altered by hUCB-MSC treatment on day 56 (Figure 6).

Given the remarkable efficacy of hUCB-MSC treatment in reducing pain and itch in patients with RDEB in this study, we also analyzed the changes in serum levels of neuropeptides. Baseline serum levels of substance P were significantly higher in RDEB patients compared to age-matched healthy control values, and notably, substance P levels were significantly reduced 56 days after hUCB-MSC treatment. Serum CGRP levels were also higher in RDEB patients than in healthy controls, and these levels were reduced from baseline after hUCB-MSC treatment ($P = 0.06$), but these changes were not statistically significant (Figure 6).

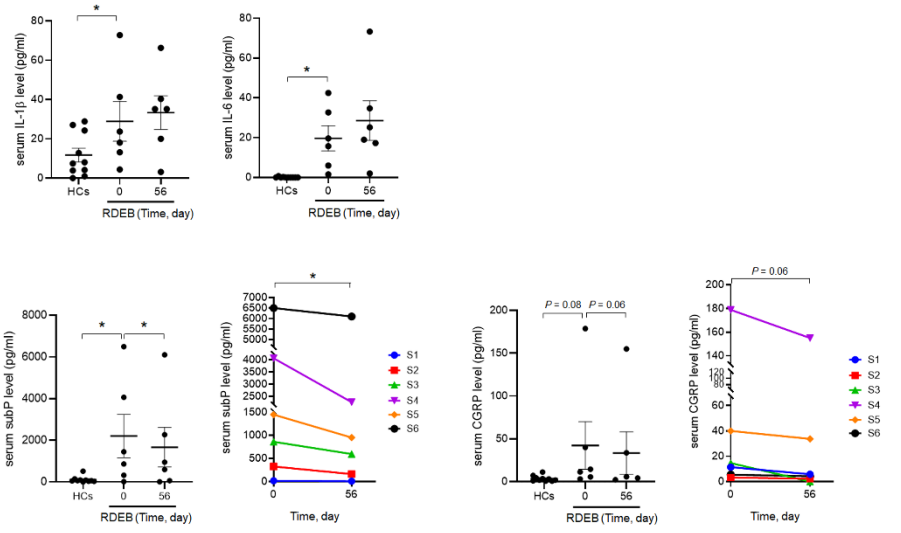

Figure 6. hUCB-MSC treatment reduces serum substance P levels in RDEB patients. Serum levels of inflammatory cytokines (IL-1β and IL-6) and neuropeptides (substance P and CGRP) were assessed in healthy controls (HCs) and RDEB patients (n=6) at baseline and at day 56 following hUCB-MSC treatment. Values are shown as mean ± SEM. The Wilcoxon signed-rank test was used to assess the statistical difference between the repeated measurements in the same patient. *$P < 0.05$. S, subject.

IV. DISCUSSION

This open-label, phase 1/2a clinical trial show that three repeated intravenous administrations of allogeneic hUCB-MSCs are well tolerated and potentially provide clinical benefits by reducing disease severity, disease-affected body area,

blister count, pain and itch and improving QOL in children and adults with moderate-to-severe or severe RDEB. This study is meaningful in that it is the first clinical trial to apply MSCs derived from UCB, systemically, to RDEB patients.

Three seperate intravenous infusions of hUCB-MSCs did not cause serious AEs. Previous clinical trial of BM-MSCs in 10 adult patients with RDEB reported development of cutaneous squamous cell carcinoma (cSCC) in two participants about six to seven months after the injections[4], suggesting careful monitoring of this potential complication, particularly in adult patients. In this trial, of the 4 adult patients, one patient (subject 6) was followed-up for 16 months and the remaining three patients (subjects 1, 2, 3) were followed-up for up to 24 months, and there was no development of cSCC during these follow-up periods. However, long-term follow-up data for more patients is needed to accurately evaluate the potential relationship between allogeneic hUCB-MSCs therapy and the risk of cSCC in RDEB.

Although the primary objective was to assess safety, our data provide evidence of the potential efficacy of hUCB-MSC therapy in various clinical aspects of RDEB. hUCB-MSCs infusions significantly reduced disease severity as assessed by using BEBSS, affected body surface area, blister count, and blister area with a maximal effect at day 56 in most patients. Over time, these clinical effects of hUCB-MSCs were progressively attenuated, but some patients showed sustained improvement up to day 168. With regard to wound healing, 50% (2 of 4) of large open wounds that were present for at least 12 weeks achieved 50% or greater healing by 56 days after treatment. With regard to wound healing, 50% (2 of 4) of large open wounds that were present for at least 12 weeks achieved 50% or greater healing by 56 days after treatment. Despite the small number of available chronic large wounds and the lack of control wounds, based on the previous report that the 50% of greater reduction in chronic RDEB wounds is clinically

meaningful in terms of improvement in patient-reported outcomes[14], our results indicate that hUCB-MSC therapy exerts beneficial effects on wound healing in RDEB. In addition to the improvement of cutaneous lesions, intravenous administration of hUCB-MSCs also relieved the symptom of dysphagia in one patient (subject 3), allowing the scheduled balloon dilation for esophageal stricture to be delayed.

Recalcitrant pain and pruritus are among the most bothersome symptoms of RDEB[28-30]. Pain in severe generalized RDEB is often very severe that it does not respond well to potent opioid analgesics and its intensity was shown to be greater than in post-herpetic neuralgia[31]. RDEB also causes severe pruritus that is thought to be associated with cutaneous inflammation secondary to barrier disruption, wound healing processes, and dysregulated activity of epidermal nerve fibers[28].

In this study, hUCB-MSCs markedly reduced pain and pruritus in RDEB patients by reducing average VAS scores by 3-cm and 2-cm on day 56, respectively. Given that the minimum important difference for clinical improvement of chronic pain or pruritus has shown to be 2 to 3 cm on the VAS score[32,33], our data suggest that hUCB-MSC treatment is effective in achieving a clinically relevant improvement in pain and pruritus in RDEB, that may lead to improved QOL.

When comparing the clinical efficacy of hUCB-MSCs with that of BM-MSCs in RDEB, the mean difference of BEBSS and QOLEB score at day 56 in our study were 5.6 points (95% CI, -7.39 to -3.86) and 6.2 points (95% CI, -8.69 to -3.65), which were comparable to those in previous studies using BM-MSCs in children (mean difference of BEBSS at day 60 was 5.2, QOLEB score was 4.4), and adults (mean difference of BEBSS at day 60 was 1.61, QOLEB score was 3.13)[4, 9]. These findings indicate that hUCB-MSCs provide comparable therapeutic effects to BM-MSCs in improving disease severity and quality of life in RDEB patients.

Regarding the itch and pain outcome, it is difficult to directly compare the effect of hUCB-MSCs with BM-MSCs because of the different measurement tools in each study and the lack of data on pain in the previous study using BM-MSCs in adult patients. The results of our trial show that hUCB-MSCs effectively ameliorate pain as well as pruritus in both children and adults with RDEB.

When comparing the clinical efficacy of allogeneic MSCs in pediatric and adult patients with RDEB, previous studies reported a better clinical efficacy of BM-MSCs in children (mean difference of BEBSS at day 60 was 5.2) than in adults (mean difference of BEBSS at day 60 was 1.61)[4, 9], which was speculated to be associated with more severe systemic inflammation and scarring in adults RDEB patients. In contrast, the therapeutic efficacy of hUCB-MSCs in this trial (mean difference of BEBSS at day 56 was 5.13 in children and 7.18 in adults) was similar in children and adults. Considering that the number of cells administered per kilogram of patient's body weight was less in children (three infusions, each dose 1–2×106 cells/kg) than in adults (three infusions, each dose 3×106 cells/kg) in this trial, additional clinical data are needed to accurately compare the effects of hUCB-MSCs in pediatric and adult RDEB patients.

Mechanistically, systemic treatment of hUCB-MSCs did not restore the expression of C7 and anchoring fibril at the basement membrane in the skin of most patients, except one who showed increased C7 expression on day 56. These findings are consistent with previous clinical trials of systemic administration of BM-MSCs[4,5,8] and suggest that the therapeutic benefits of hUCB-MSCs are not primarily caused by the recovery of C7 expression.

The mechanisms underlying hUCB-MSCs-mediated therapeutic effects on RDEB are still unknown. To understand their mechanisms of action, we further assessed the changes in blood biomarkers of inflammation and innate immune

cells infiltration in the skin following hUCB-MSCs treatment.

RDEB patients showed higher serum levels of CRP and pro-inflammatory cytokines, IL-1β and IL-6, compared to healthy controls, suggesting systemic inflammation in severe generalized RDEB. Despite the reductions in disease severity and cutaneous erythema, serum levels of CRP, IL-1β, and IL-6 showed no significant change after hUCB-MSC treatment compared to baseline, suggesting that these inflammatory molecules are not suitable biomarkers for monitoring therapeutic response to hUCB-MSCs. CRP and IL-6 are markers of acute phase response and fluctuated CRP values in individual patients might reflect the dynamic inflammatory status in RDEB patients. Our findings are consistent with a prior clinical trial of BM-MSCs in RDEB patients which reported that inflammatory molecules were generally unchanged, but high mobility group box-1 was significantly decreased after treatment[4,25].

In comparison between BM-MSC and hUCB-MSC therapy for RDEB, both of them did not show any adverse events related to the therapy[4,5]. With regard to efficacy, the mean difference of QOLEB score and BEBSS at day 56 in our study were 6.2 points (95% CI, -14.5 to 2.1), and 5.6 points (95% CI, -15.2 to 3.9), which were comparable to those in previous studies using BM-MSCs in children (mean difference of QOLEB score at day 56 was 4.4, BEBSS was 5.2)[5], and adults (mean difference of QOLEB score at day 56 was 3.13, BEBSS was 1.61)[4]. With regard to itch and pain, however, it is difficult to compare BM-MSC with hUCB-MSC because each study used different measurement tools to assess itch and pain.

Other than BM-MSCs or hUCB-MSCs, there is a clinical trial ongoing using allogenic $ABCB5^{+}$ MSCs in patients with RDEB, registered with ClinicalTrials.gov (NCT NCT03529877) since January, 2019. Recently, human

dermal ABCB5$^+$ MSCs are also a promising novel therapeutic candidate for the treatment in various uncurable diseases with their immunomodulatory effects and safety[34]. In addition, there are some growing evidence that MSC-derived extracellular vesicles augment therapeutic potential of MSCs in various pathways[35,36]. Especially, in RDEB, MSC-derived extracellular vesicles can function as assisting transport type VII collagen within extracellular space, and providing fibroblasts with mRNA which codes for type VII collagen[37]. Taken together, in addition to BM-MSCs and hUCB-MSCs, human dermal ABCB5$^+$ MSCs or MSC-derived extracellular vesicles also can be alternative therapeutic candidates in the field of cell therapy for RDEB.

To date, little is known about the pathophysiological mechanism of pain and pruritus in RDEB, but recent study found a decreased nerve fiber density and increased number of activated mast cells in skin of RDEB patients, indicating neuropathic pain and itch[38,39]. Sensory nerve-derived neuropeptides, substance P and CGRP, participate in neuro-immune crosstalk, thereby leading to neurogenic inflammation, neuropathic pain, and itch[40,41]. Moreover, the substance P-neurokinin 1 receptor antagonists have been reported to effectively reduce pruritus in patients with prurigo[42], cutaneous T cell lymphoma[43], as well as epidermolysis bullosa[44].

Interestingly, serum substance P levels were significantly higher and serum CGRP levels tended to be higher in RDEB patients compared to healthy controls. In addition, serum substance P and CGRP levels were reduced after hUCB-MSC treatment. Consistent with a previous study[38], increased numbers of mast cells were detected in the skin of RDEB patients compared to healthy skin at baseline. Of note, infiltration of mast cells was substantially reduced after hUCB-MSC treatment. These findings suggest the possible role of substance P and mast cells in the neuropathic pain and itch in RDEB patients. Furthermore, the effective

attenuation of pain and pruritus in hUCB-MSCs-treated RDEB patients could be due to the inhibition of substance P levels and mast cell activation.

Another interesting aspect of this work is the evaluation of changes in macrophage phenotype following hUCB-MSC treatment. Macrophages play an important role in immune modulation, tissue repair, and fibrosis[45,46]. In this study, we found that hUCB-MSC treatment did not alter the number of total macrophages, but markedly increased M2 macrophage infiltration in the skin of RDEB patietns. These findings are consistent with the observations from a preclinical study of human UCB-derived non-hematopoietic stromal stem cells in RDEB mouse model[15], supporting that hUCB-MSC therapy-induced M2 polarization of tissue macrophages also occurs in patients with RDEB. The increase in these pro-repair or alternatively activated M2 macrophages might contribute to the accelerated wound healing and the resolution of inflammation following hUCB-MSC treatment, however, further studies are necessary to elucidate the functional significance of macrophage M2 polarization.

Other than allogeneic MSCs from umbilical cord blood or bone marrow, there is a clinical trial ongoing using allogenic ABCB5-expressing MSCs (ABCB5+ MSCs) in patients with RDEB, registered with ClinicalTrials.gov (NCT NCT03529877) since January, 2019. Recently, human dermal ABCB5+ MSCs have emerged as a promising novel therapeutic candidate for the treatment of various uncurable diseases with their immunomodulatory effects and safety[47]. In addition, there are growing evidence that MSC-derived extracellular vesicles augment therapeutic potential of MSCs in various pathways[48, 49]. Especially, in RDEB, MSC-derived extracellular vesicles can support the transport of C7 within the extracellular space and provide fibroblasts with mRNA encoding C7[50]. Taken together, in addition to hUCB-MSCs and BM-MSCs, human dermal ABCB5+ MSCs or MSC-derived extracellular vesicles also can be alternative therapeutic

candidates in the field of cell therapy for RDEB.

The limitations of this open-label study were the small number of patients and the lack of a control placebo-treated arm.

V. CONCLUSION

In conclusion, allogeneic hUCB-MSCs were well tolerated when administered intravenously three times in both pediatric and adult patients with RDEB. hUCB-MSC therapy reduced disease severity, with significant improvements noted in erythema in the affected area, blister count, pain, pruritus and QOL. In addition, a transient clinical benefits of allogenic hUCB-MSCs were observed with a maximal efficacy at 56 to 112 days after treatment and a gradual attenuation of these clinical benefits through day 168. These findings suggest that additional treatments after 6 months might improve long-term efficacy. In the future, larger clinical trials are needed to investigate the optimal dosage, number of injections, differential efficacy of different tissue-derived MSCs, and the long-term safety of allogeneic MSC therapy for RDEB.

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ABSTRACT (IN KOREAN)

열성 이영양형 수포성 표피박리증 환자에서 동종 제대혈 유래 중간엽 줄기 세포 주입 치료의 유효성 및 작용 기전

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열성 이영양형 수포성 표피박리증은 제 7 형 콜라겐을 코딩하는 *COL7A1* 의 돌연변이로 생기는 피부 및 점막에 수포와 미란이 생기는 심각한 불치성 유전피부질환이다. 본 임상 1/2a 상 연구에서는, 열성 이영양형 수포성 표피박리증 환자들을 대상으로 동종 제대혈 유래 중간엽 줄기 세포의 정맥 주입 치료의 안전성, 유효성, 및 작용 기전을 평가했다.

열성 이영양형 수포성 표피박리증으로 진단 받은 4 명의 성인과 2 명의 소아 환자가 3 회의 동종 제대혈 유래 중간엽 줄기 세포 주입 치료 (1-3 x 10^6 cells/kg) 를 2 주 간격으로 받았으며, 치료 후 8 ~ 24 개월에 걸쳐 추적 관찰 하였다. 일차 평가 변수는 안전성이었으며, 이차 평가 변수는 질환 중증도 점수, 상처 평가, 가려움증 및 통증 점수, 삶의 질 평가를 포함한 임상적 유효성이었다. 피부에서의 제 7

형 콜라겐 발현과 염증 세포들의 침착과, 혈장에서의 염증 표지자 및 신경전달물질 또한 평가되었다.

동종 제대혈 유래 중간엽 줄기 세포 주입 치료는 모든 환자들에게서 심각한 이상 반응 없이 투여되었다. 수포성 표피박리증의 중증도 점수, 질환의 신체 표면적 침범 정도, 수포의 개수, 가려움증 및 통증 점수, 그리고 삶의 질 모두 치료 후 56 일에서 112 일까지 호전되는 효과를 보였다. 동종 제대혈 유래 중간엽 줄기 세포 주입 치료는 환자의 피부에서 M2 대식세포로의 분극화를 유발하였으며, 비만세포의 침착을 감소시켰다. 치료 후 환자 혈장에서의 신경전달물질 P 의 수치는 감소하였다. 치료 후 56 일차에서 1 명의 환자의 진피표피 경계부에서 제 7 형 콜라겐 발현의 증가가 관찰되었다.

본 연구는 현재까지 열성 이영양형 수포성 표피박리증 환자들을 대상으로 동종 제대혈 유래 중간엽 줄기 세포 주입 치료를 시행한 첫 번째 임상 실험으로, 안정성과 일시적인 임상적 효과성을 입증했다.

핵심되는 말: 열성 이영양형 수포성 표피박리증, 중간엽 줄기 세포, 임상 실험