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REVIEW ARTICLE

Review and Clinical Report Comparing Herbal Remedies and Stem-Cell Application to Chronic Leg Ulcers

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ABSTRACT

Conventional treatments of leg ulcers includes repeated debridements, herbal dressings and interventions like split skin graftings. With adverse predisposing factors such as diabetes, aging and foot deformities, many ulcers fail to heal.

For chronic ulcers of over 2 years duration, treatment was done by applying Mesenchymal Stem Cells derived from adipose tissues (MSC) directly into the ulcers: 4 cases of Chronic Non-Healing Ulcers (CNHUs) were managed with herbal treatments, and 4 other CNHUs cases were treated with MSC. This report compares management success of those leg ulcers treated with herbal treatment alone or with using MSC after herbal treatment fails. MSC therapy seems to enhance re-epithelialization and angiogenesis in ulcer healing.

Introduction

Chronic non-healing ulcers have been characterized as those that do not heal over a span of more than 8 weeks in spite of receiving conventional wound care and treatment [1]. Such ulcers are expected to be related to vascular insufficiency, loss of skin sensation, deformities of the extremity and/or other related medical conditions like diabetes, peripheral vascular diseases and chronic infections like leprosy under rare occasions [2].

Conventional treatment of leg ulcers consists of repeated debridements, dressings and interventions like split skin graftings. With adverse backgrounds like diabetes; aging and foot deformities, the ulcers fail to heal [3].

A previous report indicated favorable outcomesusing herbal treaments for chronic leg ulcers among patients suffering from type 2 diabetes [4]. With limited minor surgical procedures like wound debridements, skin grafting and toe amputations, over 80% of patients escaped the need for more extensive amputations [5].

In spite of all these efforts, some chronic ulcers did not heal.

Since 2 years ago, for those chronic non-healing ulcers with 7 months to 11 years duration, we started a novel attempt of applying Mesenchymal Stem Cells (MSC) directly onto the ulcers as the last resort of getting them healed.

This report records treatments of Chronic Non-Healing Ulcers (CNHUs) of more than two years' duration, using herbal treatment alone, or using MSC after herbal treatment fails.

Herbal Treatment

Herbal treatment consisted of two herbs: viz. Radix Rehmanniae and Radix Astragali. The herbs in assigned proportions, were boiled together in water in standard procedures, hydrolyzed into standard granules of 5 gm per sachet, to be consumed daily as a drink. Details have been reported in past publications [4]. The pharmacological effects of the herbal formula have also been described [6-8]

MSC Treatment

MSC's are multipotent adult stem cells present in different types of tissues such as bone marrow and adipose tissue. MSCs are capable of self-renewal and differentiation into mature cells of various lineages [9]. MSCs from adipose tissues are chosen to be our media of cell therapy for chronic non-healing skin ulcers because of the ease of isolation and efficient expansions under laboratory conditions.

Under local anesthesia, about one gram of subcutaneous adipose tissue was surgically removed from below the umbilical level of the abdominal wall. The procedures of MSC preparation consisted of the following: -

i) Washing extensively with phosphate buffered saline and digesting with 0.1% collagenase (type I: Sigma-Aldrich) for 30 minutes at 37°C.

ii) Filtration through 100-µm mesh filter, washing filtrate with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum.

iii) Maintaining cell cultures in a humidified atmosphere of 5% CO2. MSCs at cell passage 3-5 were suitable for transplantation.

The MSCs had the potential to differentiate into adipocytes, chondroblasts and osteoblasts under specific in vitro conditions. Flow cytometry showed expressions from CD13, CD29, CD44, CD90 and CD105, but not CD34, CD31 and CD45.

On transplantation, around 2 x 107 MSCs suspended in 500ul PBS were topically applied to the ulcer surface together with superficial injection around the ulcer edges. As a result, cells and supernatant fluid i.e. the paracrine, were applied together.

Results of treated Patients using adipose tissue stem cells.

In the past 2 years, we started to apply adipose stem cells to heal chronic unhealing foot ulcers. Initially, we selected those patients who failed to respond well straight-away to herbal treatment later, we attempted to use adipose stem cells application. There were 4 cases in either category and the summaries are given in Table 1 and Table 2. (Figure 1 & Figure 2)

Figure 1a: Foot ulcer in Leper for 11 years.
ST-P001(Leprosy)
- Lt sole ulcer
- Ulcer since 2008 or earlier
- 1st visit in ICM 2010

Figure 1b: After 8 MSC graftings in 2 months
8th graft : 27 Jul 15

Figure 1c:
Ulcerhealed after 14 MSC graftings in 13 months
Cast removed: 17Dec15

Figure 2a: Chronic ulcer 9 months
ST-P004B
1st graft : 12 Oct 2016

Figure 2b: Ulcer healed after 4 graftings
Ulcer healed in 5 months

Discussion

Treatment of MCHU's in patients with complex pathologies demands special care. Quick successful outcomes are not expected and previous use of synthetics, growth factors and circulating stem cells have been disappointing [10-12].

The simple two herbs formula has been shown to greatly facilitate fibroblast proliferations in the laboratory [6,7]. Clinical results of using the formula for chronic ulcer have indicated not only slow but reliable healing processes which are well supported by apparent molecular pathways of repair [13]. However, should failure or delay of healing be encountered, MSC therapy is indicated, as it may accelerate the healing. In the case of chronic infection of leprosy, the multiple adversities of sensory and motor loss, together with foot deformities, are all indications for novel measures.

Wound healing depends on angiogenesis and active granulation tissue formation both of which depend on a well-orchestrated interactions among the infiltrating and resident cells, mediators, growth factors and cytokines.

Concluding Remarks

Growing evidence showed that differentiation of transplanted MSCs into phenotype of epithelium is not considered as the major recovery mechanism to accelerate cutaneous epithelialization [9,14].

Instead, the release of a variety of cytokines from MSCs is thought to play a significant role in tissue repair through suppression of inflammation and apoptosis, as well as stimulating angiogenesis and re-epithelization. In fact, the beneficial effects of MSCs on wound healing have been demonstrated in a variety of preclinical and clinical studies [15-17].

Our MSCs application could have enhanced re-epithelialization, angiogenesis and granulation tissue formation. MSCs have been administrated by

Table 1: Patients with Chronic Non-Healing Ulcers (CNHUs) treated with stem cell application after herbal treatment failed

Patient M/F/Age	Pathology	Ulcer duration before treatment	Treatment received before MSC	No. of tissues of MSC treatment	Total duration	Final Result
M/68	Leprosy renal insufficiency (Figure 1)	11 years	- Herbal formula treatment failed - Special topical agent failed	14 times	13 months	healed
F/44	Fibromatosis multiple excisions	7 years	- Herbal formula treatment failed - Special topical agent failed	19 times	24 months	healed
M/58	Diabetes Heart disease	3.5 years	Herbal formula failed	2 times	6 months	healed
M/73	Diabetes	7 months	Herbal formula failed	2 times	4 months	healed

Table 2: Patients with chronic unhealingfoot ulcer treated with stem cell application

Patient M/F/Age	Pathology	Ulcer duration before treatment	No. of tissues of MSC application	Total duration of MSC application	Results
M/59	Diabetes amputation stump	2-5 years	3	9 months	healed
M/60	Diabetes Renal failure amputation stump	3-5 years	2	6 months	healed
M/82	Peripheral arterial disease Necrotizing fasciitis	10 months	4	10 months	healed
M/56	Diabetes Amputated toe stumps	7months	3	9 months	healed

intramuscular/subcutaneous injections or topical application onto the recipient wounds of ulcers. In this series of case reports, the MSCs application could have enhanced re-epithelialization, angiogenesis and granulation tissue formation. In order to sustain the continuous effects of MSCs, a film of fibrin was sprayed on top to prevent the escape of MSCs under the dressings. Injecting the MSCs together with the paracrine would be further assurance to the sustenance of the MSC effects.

Early experiments done by our research group in the laboratory on a rat model with an artificially created ulcer subsequently treated with topical MSC had shown that the MSC could successfully attach to the ulcer for 5-6 days, then they were not seen any more while the ulcer healing continued [18]. It was therefore postulated that the healing enhancement provided by the MSC was probably working via paracrine influences, rather than involving a direct transformation of the MSC to fibrocytes or epithelial cells.

This report indicates a proof-of-principle: Chronic Non-Healing Ulcers (CNHUs) which fail to respond to herbal therapy may respond well with Mesenchymal Stem Cell (MSC) therapy.

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