Successful treatment of chronic venous leg ulcers with lyophilized cultured epidermal allografts

V. Slonková, Z. Navrátilová, V. Semrádová and J. Adler

SUMMARY

The case of a 67-year-old woman with chronic venous leg ulcers and severe gonarthrosis is described. In spite of intensive therapy, the leg ulcers had persisted for 4 years and made the intended orthopaedic operation of the right knee impossible. The patient was treated with lyophilized cultured epidermal allografts and her leg ulcers healed within 40 days. Lyophilized cultured epidermal allografts represent a modern type of active wound dressing that leads to rapid healing of chronic venous leg ulcers and enables patients to undergo surgical intervention.

KEY WORDS

leg ulcers, cultured epidermal allografts, lyophilization, orthopaedic operation

Introduction

Standard treatments of leg ulcers comprise mechanical and enzymatic debridement, various types of dressing (alginates, hydrocolloids, hydrogels, dressing with active charcoal and silver etc.) and an appropriate compressive therapy.

Cultured epidermal allograft (CEA) is a method of active wound dressing and represents a modern therapy for leg ulcers. The best results are seen in venous leg ulcers while leg ulcers of other origin (ischaemic, diabetic, rheumatoid) heal more slowly (1-4). The CEA were prepared in the Tissue Bank under sterile conditions. Skin biopsies were obtained from patients undergoing plastic surgery. All donors were screened according to EATB general standards for HIV 1 and 2, hepatitis B antigen (HbsAg), hepatitis C virus (HCV) and syphilis. Skin samples were trypsinized which led to separation of keratinocytes. Isolated keratinocytes were resuspended in a culture medium and then inoculated to a 3T3 fibroblast feeder-layer. In the stage of subconfluency, repeated subculturing was made according to a slightly modified version of the Rheinwald-Green procedure.

The treatment can be recommended in the case of long-lasting, non-healing leg ulcers that are resistant to conventional therapy. In our patient, a rapid healing of the leg ulcers was necessary in order to prepare her for an orthopaedic operation.

Case report

A 67-year-old woman was admitted to the phlebology clinic of our department with ulcers on the right leg

which had persisted in spite of intensive therapy for four years. She was hospitalized in the Orthopaedic department where she was treated for gonarthrosis of her right lower limb, and an operation on the right knee (which displayed total endoprosthesis) was planned following the healing of the leg ulcers. The patient had diabetes mellitus that was being treated with oral antidiabetics, a history of hypertension, and had undergone hysterectomy and bilateral adnexectomy with subsequent radiotherapy because of a cancer 6 years previously. In August 2002 she was admitted to our department. At that time there were three ulcerations on the medial aspect of the right shin with a granulating, slightly covered ulcer base that encompassed an area of 22,7 cm^2 (Fig. 1). Phlebologic examination showed chronic venous insufficiency with incipient peripheral diabetic microangiopathy.

The patient was treated with mechanical debridement, nonadherent wound dressings and compression therapy. Systemic treatment included peroral ciprofloxacin (bacterial culture revealed Escherichia coli and Pseudomonas aeruginosa) administered for 10 days, and intravenous pentoxifylline. Lyophilized cultured epidermal allografts were applied on the clean, granulating wound bed under sterile conditions. The allografts were covered with a layer of nonadherent silicone dressing, several layers of dry gauze pads, a gauze bandage and a low-strength elastic bandage. The patient was advised to rest in bed with the limb elevated for 48 hours after grafting. The dressings were first removed after 5 days, and subsequent dressings were changed at 3-day intervals using a nonadherent dressing and low-strength bandages. The initial ulcer size (evaluated by planimetry) was 22,7 cm^2 before grafting (Fig. 1) and had reduced to 16,5 cm^2 on the 7^{th} day (Fig. 2), and to 2,4 cm^2 on the 28^{th} day after grafting (Fig. 3). The patient reported immediate pain relief following the application of CEA. The leg ulcers healed completely within 40 days of grafting (Fig. 4) and the patient was able to undergo the planned orthopaedic operation. To date there has been no recurrence of the leg ulcers. The patient has continued with an appropriate compressive treatment that constitutes the most important part of the conservative therapy of chronic venous insufficiency.

Discussion

Successful treatment of chronic venous leg ulcers with cultured epidermal allografts (CEA) was first reported in 1987 (5). Initially some authors (6, 7) hypothesised that the CEA were not rejected and that the absence of Langerhans´ cells in the allografts promoted a graft tolerance with respect to the major histocompatibility barriers. However it has been found that the allografts are rapidly colonized by Langerhans´ cells (8) and are rejected both in animals (9) and in humans (8). DNA fingerprint analysis has showed that allogeneic keratinocytes are rapidly replaced by the recipient's keratinocytes (10-12) and that the epidermal regeneration is due to the migration and proliferation of the recipient's residual keratinocytes (3). CEA release many cytokines, such as epidermal growth factor (EGF), transforming growth factors (TGF) alpha and beta, platelet-derived growth factor (PDGF), interleukin-1 and further mediators (2, 13, 14). These cytokines stimulate migration and proliferation of the recipient's keratinocytes and modulate fibroblast replication and collagen synthesis (2, 3, 15, 16).

CEA can be used fresh, cryopreserved or lyophilized. Fresh allografts are limited for common use because they must be applied immediately, and in a fresh state. Cryopreservation facilitates the availability and transport of CEA (1). Cryopreserved allografts can be produced on a large scale by the same laboratory and stored at -80 °C in a tissue bank where they remain ready for use at any time (1, 3). Their application is a simple and safe procedure that causes no discomfort to patients (1, 3, 17). Cryopreserved CEA give comparable results to fresh CEA in the healing of chronic leg ulcers (1, 3).

Although cryopreserved allografts are more convenient to use than fresh ones, there are a number of limitations to their common clinical use. They must be transported at the temperature of -80 °C, which is expensive and time-consuming in case of hospitals located at a major distance from the laboratory. Lyophilized CEA solve such problems. They can be stored and transported at room temperature and they are immediately available, and so reduce the cost of treatment considerably in comparison to cryopreserved allografts. During lyophilization, CEA are frozen at temperatures of up to -80 °C and then dried in high vacuum (10^{-5} Pa). This process leads to keratinocyte lysis, which means that the keratinocytes loose their integrity. However keratinocyte lysates contain the same cytokines as native keratinocytes and for this reason the effectiveness of lyophilized CEA is very similar to that of fresh or cryopreserved CEA (18).

There are only a few reports concerning the healing of wounds with lyophilized CEA in medical literature. Duinslaeger et al showed that lyophilized keratinocyte cell lysates contain multiple mitogenic activities and stimulate closure of meshed skin autograft-covered burn wounds with an efficiency similar to that of fresh allogeneic keratinocyte cultures (15). Somers et al reported stimulation of epithelial healing in chronic post-operative otorrhea using lyophilized cultured keratinocyte lysates (19). Recently we reported the results of a pilot study which compared cryopreserved and lyophilized cultured epidermal allografts in the treatment of leg ulcers (18). This study was performed at our department in the years 2000 – 2002, and included 50 pa-

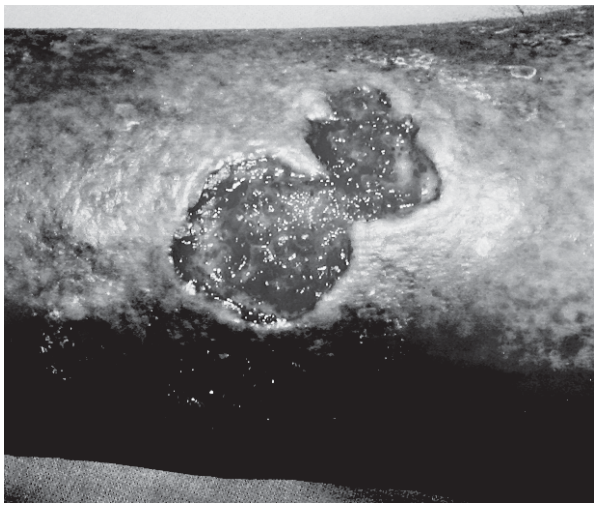

Figure 1. Leg ulcers before application of cultured epidermal allografts (CEA).

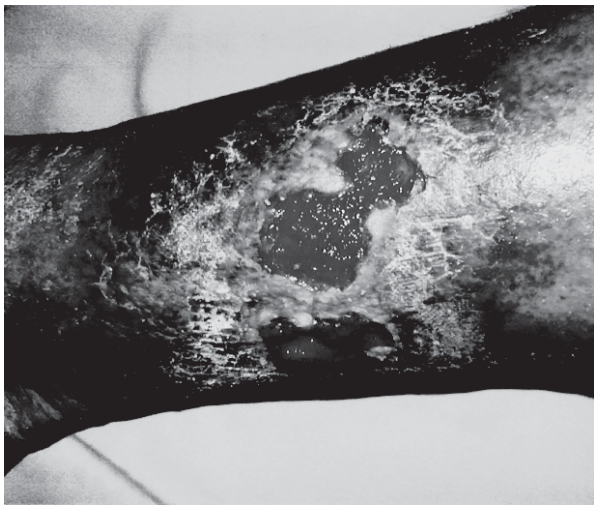

Figure 2. Seven days after application of cultured epidermal allografts (CEA), "edge effect".

tients with chronic leg ulcers of venous origin. The results of our study indicate that lyophilized CEA are comparable to cryopreserved ones in terms of the healing rate, the course of healing, relief from pain, and also in planimetric changes during the healing of venous leg ulcers (18).

In our patient, re-epithelization started at the wound edges, the so-called "edge effect" (Fig. 2), and later on islands of epithelium could be observed in the central part of the ulcer that represented keratinocyte outgrowth from the hair follicles (2-5, 20, 21).

Our patient reported immediate pain relief following the application of CEA in a way that is comparable with other studies dealing with cultured epidermal grafts (2, 13, 14, 22). Pain relief is singled out as a particular advantage of the method because it contributes to the improvement of quality of life even before healing of the leg ulcer.

In our patient, the rapid epithelization of leg ulcers was crucial to allow for the planned orthopaedic surgery. A pronounced improvement of the patient´s mobility led to the enhanced function of the musculo-venous pump which, in its turn, also improved the healing.

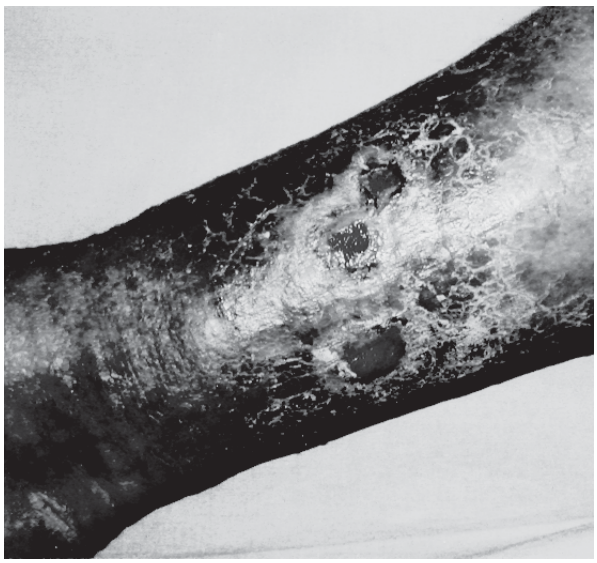

Figure 3. 28 days after application of cultured epidermal allografts (CEA).

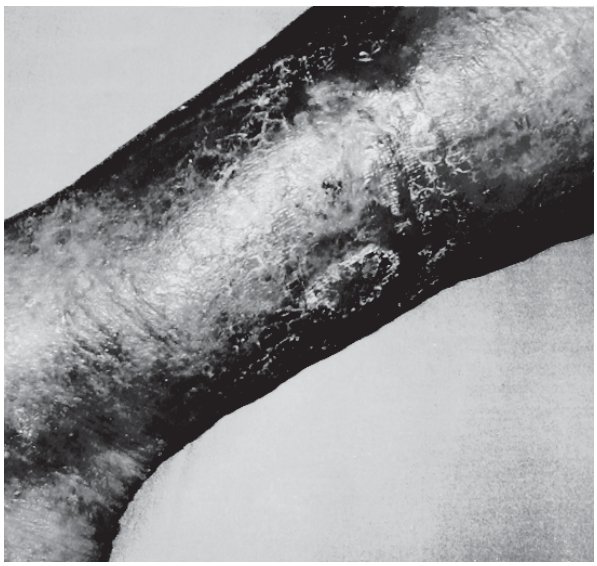

Figure 4. 40 days after application of cultured epidermal allografts (CEA), healed ulcers.

Conclusion

CEA treatment is relatively expensive but the relative cost-effectiveness depends on other circumstances. The healing time of leg ulcers is significantly reduced with CEA. For this reason the treatment is recommended in cases in which epithelisation has to be accelerated as well as a last resort in non-healing chronic leg ulcers that are resistant to conventional therapy.

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