The Longevity of a Bilayered Skin Substitute After Application to Venous Ulcers

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Background: A bilayered skin substitute composed of allogeneic keratinocytes and fibroblasts in a collagen gel has been approved by the US Food and Drug Administration for the treatment of venous and diabetic ulcers. Its mechanism of action has not been fully determined.

Objective: To determine the longevity of allogeneic fibroblasts and keratinocytes in a bilayered skin substitute in patients with venous leg ulcers.

Methods: Ten patients with venous leg ulcers were treated with a bilayered skin substitute on day 0, days 3 to 5, and weeks 1 through 3. Biopsy specimens of the grafted wound were taken. We used polymerase chain reaction analysis to determine whether allogeneic DNA was present in the biopsy specimens.

Results: We detected allogeneic DNA in 2 of 8 specimens at 1 month after initial grafting. Neither of the 2 patients showed persistence of allogeneic DNA at 2 months after initial grafting.

Conclusions: Allogeneic cells from a bilayered skin substitute do not appear to survive permanently after grafting for treatment of venous leg ulcers. Other mechanisms of action might include cytokine release, structural support, or provision of a moist wound environment.

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PATIENTS AND METHODS

Patients with venous ulcers of greater than 1 month’s duration (confirmed by means of duplex ultrasonographic findings) and an ankle-brachial pressure index of greater than 0.65 were enrolled into the study. Patients enrolled in other investigational studies within the past 3 months, patients previously treated with the BSS, and patients with HLA class I antigens common to the patient and the BSS were excluded from participating.

All patients received up to 5 applications of the BSS during the 3-week treatment. At the screening visit (days 7 to 5), blood was collected as a DNA control sample. The BSS was applied to a clean, debrided wound after thorough irrigation with isotonic sodium chloride solution. Oozing or bleeding caused by debridement was stopped by means of gentle pressure. The graft was covered with a non-adherent dressing, premeasured gauze, and a self-adherent elastic wrap from the metatarsals to the tibial plateau. At 4, 8, and 12 weeks after the first BSS application, 3-mm punch biopsy specimens were taken from the area judged by the investigator (T.J.P. or V.F.) to be most likely to contain BSS. If the BSS was not visible on the ulcer, specimens were taken from the center of the wound. Biopsy sites were rotated so that the specimen was not taken from the same location at each visit. When DNA of the BSS could not be detected in the specimen, no further samples were taken.

To test for the persistence of the BSS on patients, we used the expression of specific HLA genes by BSS cells. The HLA phenotypes of the BSS have been determined previously (data not shown). We used this information to create sequence-specific primers directed toward the DNA of the BSS. In the present study, HLA-DQB1*0201-specific primers were used (Figure 1). Keratinocytes and fibroblasts from the BSS carry this gene. Primers were amplified using polymerase chain reaction (PCR) analysis.6

not be detected in biopsy specimens from 6 patients (Figure 2). Results of PCR testing showed that 2 of these wounds demonstrated the BSS DNA at 4 weeks (Figure 3).

No correlation between the clinical appearance of the wound and the BSS persistence was found. Neither wound showed persistence of DNA from the BSS at 8 weeks. One of these ulcers healed; the other did not.

The BSS has been approved by the US Food and Drug Administration for the treatment of venous and diabetic ulcers. The healing process has not been entirely elucidated.

Some authors postulated that the presence of a dermal-like substitute in the BSS may create conditions sufficiently different from cultured epidermal allografts to allow long-term engraftment. In patients with acute wounds due to epidermolysis bullosa, extensive erosions were treated with the BSS, and the treated areas remained blister free, with clinical evidence of graft take and no signs or symp-

COMMENT

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toms of acute graft rejection. Molecular genetic testing using a specific marker for the tissue-engineered cells confirmed persistence of the skin substitute in 4 (33%) of 12 patients with these acute wounds at 4 weeks. Delayed rejection of allograft may occur in these patients because of immune tolerance to allogeneic tissue in early life or because of abnormalities in immune function in patients with epidermolysis bullosa. Alternatively, the mechanism of action of cultured allogeneic tissue may be different in acute wounds compared with chronic wounds. In other acute wounds (eg, split-thickness skin-graft donor sites), persistence of allogeneic DNA from the BSS occurred in 3 (27%) of 11 patients at 4 weeks. Additional data are needed to elucidate whether the BSS persists for longer than 4 weeks in acute wounds.

In chronic wounds, clinical investigators have observed clinical graft take and temporary persistence of the
BSS in approximately 41% of patients in whom the wounds healed. Clinical remodeling of the graft and probable replacement with the patient’s own skin appeared to occur in at least 63% of these patients. The authors commented that the BSS could benefit wounds as a temporary skin replacement or as a stimulus for wound healing.

In the small group of patients with chronic venous ulcers undergoing testing in this study, allogeneic DNA from a BSS could not be detected at 2 months after grafting. A weakness in this study is that during the period of observation, only 2 of the patients experienced complete healing. Persistence is probably related to successful initial graft take and wound closure, and it is difficult to determine persistence in an unhealed wound. If a wound heals completely and rapidly after application of the BSS, persistence of allogeneic cells would be more likely, as occurred in patient PM-02-05. However, allogeneic DNA was also detected in patient PM-02-06, who did not experience complete wound healing. Our data did not support survival of allogeneic BSS cells at 4 weeks. We did not experience complete wound healing. Lanes A and B show DNA from the BSS in specimens from both patients collected 4 weeks after the initial treatment.

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REFERENCES


