First Experience in the Use of Bone Marrow Mesenchymal Stem Cells for the Treatment of a Patient with Deep Skin Burns

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Female patient with extensive skin burn (I-II-IIIAB skin burn, total area 40%, area of IIIB degree 30%) was treated using transplantation of allogenic fibroblast-like bone marrow mesenchymal stem cells onto the surface of deep thermal burn. The study of wound healing dynamics after transplantation of allogenic fibroblast-like mesenchymal stem cells confirmed high tempo of wound regeneration in the presence of active neoangiogenesis. Due to this, autodermoplasty of burn wounds could be carried out with good results as early as on day 4 after transplantation of fibroblast-like mesenchymal stem cells; this led to more rapid healing of donor zones and accelerated rehabilitation of the patient.

Key Words: *bone marrow; mesenchymal stem cells; fibroblasts; burn*

Cell therapy of surface and deep burn wounds now became a part of combined treatment of patients with burns [1-3].

The following cells were used for transplantation onto burn surface: adult human cultured allofibroblasts [6,7,9], auto- and allogenic keratinocytes (alone or as components of bioengineering structures) [8,14,15]. Analysis of the results of transplantations of these cells showed that the use of keratinocytes and fibroblasts, though effective, is very expensive, which limits their wide practical use. Moreover, because of rapid transformation of fibroblasts into fibrocytes possessing less pronounced growth-stimulating and proliferative activity, cultured fetal fibroblasts showed be used.

Therapy with fetal cells in patients with burns promotes wound regeneration and reconvalescence of patients with burns of different severity; moreover, it reduces mortality [4,5].

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However, unsolved ethical, legal, and juridical problems and the absence of laws permitting collection and use of fetal cells necessitate the search for new effective means for the treatment of extensive deep skin burns. Studies of the use of bone marrow stem cells (BMSC) in rehabilitation treatment of damaged organs and tissues were recently carried out all over the world.

It is now proven that under certain conditions of culturing, BMSC can not only divide, but also predifferentiate into cells of other tissues, including fibroblast-like cells [13,16-19].

We previously showed the efficiency of allogenic and autologous fibroblast-like mesenchymal stem cells (FMSC) in the treatment of large skin burns of different severity in animals [10-12]. Based on these positive results, we used allogenic FMSC for the treatment of a female patient with severe skin burn.

In this paper we analyze the results of using allogenic FMSC for stimulation of neoangiogenesis in the wound and for acceleration of skin autotransplants take in a patient with extensive skin burn, 30% of which was IIIB degree burns.

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PREPARATION OF ALLOGENIC CELL MATERIAL FOR TRANSPLANTATION

Bone marrow cells for isolation of MSC and subsequent isolation FMSC were collected in adult donors under intravenous narcosis. The cells were collected from the ileac bone using a Kassirskii needle. Donors and their bone marrow were tested for hepatitis B and C, AIDS, syphilis, chlamydiasis, and other hazardous infections.

Bone marrow cells for isolation of MSC were cultured in Petri dishes at 37°C in a CO_2 incubator (5% CO_2) at 95% humidity in Iskov's medium (Gibco) with 10% fetal calf serum (HyClone). The medium was replaced every 3-4 days. MSC monolayer was formed over 14-17 days. Later MSC were cryopreserved as the initial material for the production of FMSC. For obtaining FMSC, suspension of MSC was defrosted and then cultured for 7 days.

Cultured cells were daily examined under a phase-contrast microscope (Nikon).

Suspension of FMSC was prepared from cells adhering to the plastic and pipetted onto burn wound surface (20-30×10^3 cells/cm^2), leaving free a 0.5-cm space from the wound edge. After cell transplantation the burn surface was dressed with a sterile gauze wetted in saline with gentamicin. The efficiency of cell therapy was evaluated visually during wound dressing on days 1, 3, 6, 8, 10, 14, and then as needed.

CASE HISTORY

Female patient S., 45 years, was hospitalized in traumatology ward of Central Regional Hospital in Okha town (Sakhalin) on May 9, 2003, with the diagnosis of thermal (flame) burn (I-II-IIIAB degree) of the neck, left half of the face, left upper limb, left half of the chest, lower third of the left thigh, and anterior surface of both shins, total area up to 40% body surface (30% IIIB degree). Burn shock of II-III degree, Frank index 70 U. According to the history of the injury, clothes inflamed during fire and stuck to the skin. In the hospital, the burn surface was cleansed and antishock therapy was started immediately. After shock was eliminated, combined treatment by traditional methods was started, including treatment of burn wounds for subsequent autodermoplasty (ADP).

Despite 20-day treatment (to 29.05.03), protein-free edemas appeared at different areas of the body; respiratory, cardiovascular, and hepatic insufficiency progressed. The therapy was supplemented by repeated infusions of blood, plasma, and erythrocyte mass, which led to stabilization of the general status of the patient.

Necrectomies of burn wounds were carried out 6 times in the operation room to 07.06.03, but necrosis repeatedly formed after necrectomy at some sites of the wounds because of poor blood supply and wound infection. Bacteriological analysis of smears from the wound surface showed *Pseudomonas aeruginosa* infection. Burn wounds were treated with detergents and antiseptics, levomecol ointment was applied. Despite these measures, granulation tissue in the wounds on the trunk remained loose, pale, with scanty vascular pattern, glossy at some sites, covered with fibrin and somewhere with purulent deposit; the wounds tended to deepen. By this moment hypergranulations developed on burn wounds on the anterior surfaces of both shins. Dressing was changed daily. Local therapy resulted in weak epithelial growth at some sites (at the wound edges), but the granulating wound surface remained unchanged. The last necrectomy was carried out on June 7, 2003, when a 9×8 cm fragment of necrotic tissue was removed from the left scapular area. Thorough cleansing of this area was carried out and a suspension of allogenic bone marrow FMSC was applied onto the burn wounds on the same day. After 3 days the greater part of granulating surface of burn wound was covered with granulations, patient's status improved; she easier contacted with other people, pain in the burn wounds was relieved. Visually, numerous small bright red vessels appeared; these new capillaries were plethoric and profusely bled even after careful dressing removal (Fig. 1). However, granulation tissue in the left scapular area remained pale and loose, as this area was the latest to be prepared to cell transplantation.

The first transplantation of skin grafts (SG) was carried out 4 days after the last necrectomy and FMSC transplantation (11.06.03). Skin grafts were resected from the anterior and outer surfaces of both thighs. After SG perforation they were used to cover the wound surfaces on the left upper limb, left arm joint, and upper portions of the chest. About 60% of wound surface was covered during the first operation (Fig. 2). Additional transplantations of allogenic FMSC on the donor wounds and on the spaces between SG were made directly after the operation. Due to formation of a protective film by transplanted FMSC covering the entire burn surface, plasmarrhea drastically decreased as early as during the first 30 min. Later the dressing did not stick to the donor zones and was just slightly wetted during the first 24 h. In order to prevent detachment of transplanted FMSC because of excessive wetting of the dressing, these sites were regularly dried with warm air. By the end of the first 24 h of cell therapy the patient noted pain relief in donor zones and in the burn wounds; hypersensitivity of burn wounds to atmospheric effects decreased. Visual examination in subsequent days showed that SG tightly adhered to the wound surface and were viable. Donor wounds

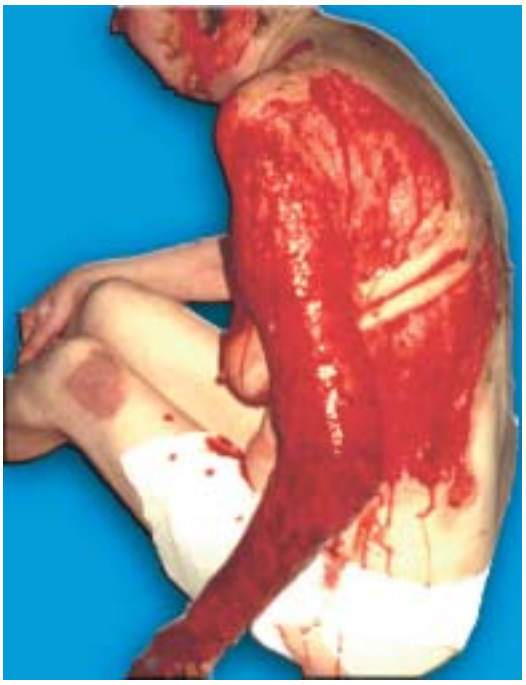

Fig. 1. Profuse bleeding from new capillaries during dressing 3 days after application of fibroblast-like mesenchymal stem cells (FMSC).

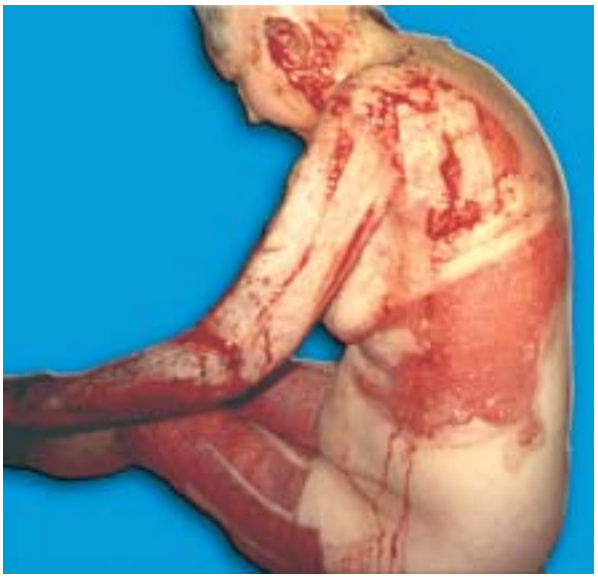

Fig. 2. Transplantation areas and degree of skin graft take after autodermoplasty (ADP) following application of FMSC.

remained clean due to formation of a protective film; no plasmarrhea was seen in these areas. Neoangiogenesis activated in donor areas and epithelialization was observed on days 7-8 due to high capacity of allogenic FMSC to stimulate epithelial and endothelial growth. This was paralleled by more active epithelialization of the burn wound edges, which led to shrinking of the burn area and active epithelial growth in zones with remaining epidermis stem zone (Fig. 3). On days 7-8 the biochemical values of the blood showed a trend to normalization. Patient's status improved significantly. Ten days after FMSC transplantation (21.06.03) we observed take of 99% transplanted SG. Granulation tissue on open sites of the burn wounds remained clean, no plasmarrhea was observed in these areas. The second operation (ADP) was carried out on June 24, 2003; SG were partially taken from the first donor sites. These SG were used to cover the remaining burn wounds, on which FMSC suspension was previously applied. Skin grafts took completely 10 days after the second ADP (04.07.03), including the burn wounds on both shins (hypergranulations before FMSC transplantations, Fig. 4). Skin grafts transplanted on the trunk looked like viable skin. On day 13 after the second ADP (07.07.03) the donor wounds were covered with crusts, some sites were epithelialized. By this time only small granulating wounds between SG and in areas of repeated resection of SG were seen, whose total area was no more than 8-10 cm^2 (Fig. 5). The patient was discharged from the hospital in a satisfactory condition for outpatient rehabilitation treatment on July 9, 2003 (28 days after the first ADP operation performed on day 4 after FMSC transplantation). The duration of hospitalization was 61 days; on August 1, 2003 the patient resumed her work.

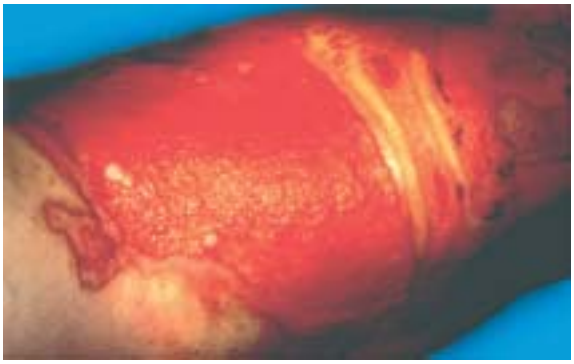

Fig. 3. Growth of marginal epithelium after application of FMSC.

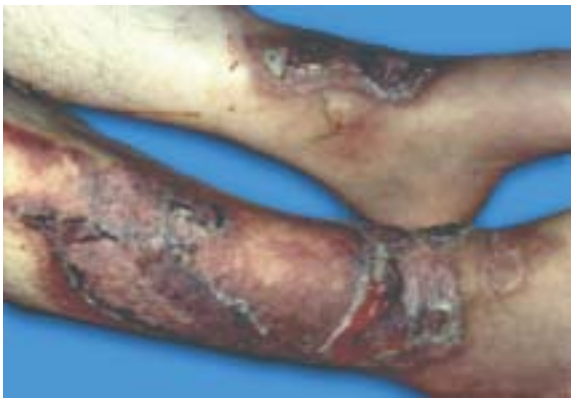

Fig. 4. Complete take of a skin graft on the lower third of the shin after application of FMSC.

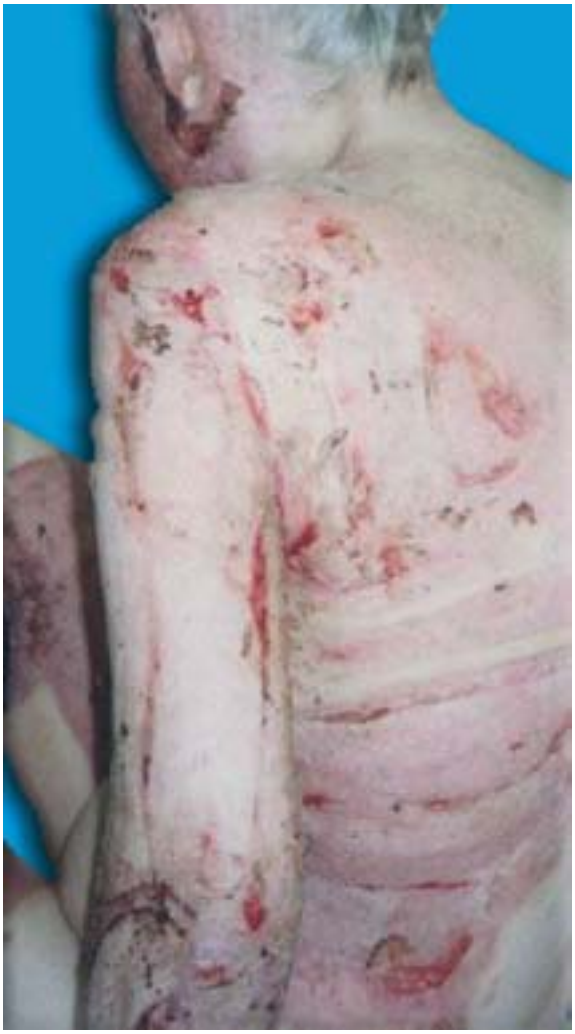

Fig. 5. Surface of burns on day 32 after application of FMSC and ADP.

Thus, transplantation of allogenic FMSC appreciably accelerated recovery of homeostasis and promoted healing of thermal burn, thus accelerating convalescence of a patient with burns.

Our previous study of the course of wound process in experimental animals with burns [10-12] and the results of the first clinical observation presented in this paper suggest that transplantation of allogenic FMSC on burn wound is a noninvasive and safe method for the treatment of severe (IIIAB degree) thermal injuries of the skin; the method of FMSC application leads to activation of new vessel formation, as a result of which marginal epithelialization of the wound after IIIB degree burns is stimulated and the risk of coarse cicatrices is reduced; due to transplantation of allogenic FMSC in IIIB degree skin injuries, ADP can be performed sooner with more rapid take of SG with good result.

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