Vascularized Bone Marrow Transplantation Model in Rats as an Alternative to Conventional Cellular Bone Marrow Transplantation: Preliminary Results

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ABSTRACT

The aim of the study was to follow the development of microchimerism after allogeneic vascularized bone marrow transplantation (VBMT) versus conventional bone marrow transplantation (BMT). In one group, a VBMT model consisted of donor Brown Norway rat hind limb heterotopic transplanted on recipient Lewis rats. An intravenous infusion of donor bone marrow cells in suspension equivalent to that grafted in the vascularized femur limb was administered intravenously to recipient rats in the second group. Cellular microchimerism was investigated in recipients of VBMT versus BMT. Donor-derived cells could be detected in VBMT recipients at 30 and 60 days but not in recipients of intravenous suspension of BMC. VBMT provides a theoretical alternative to conventional cellular bone marrow transplantation by addressing crucial clinical problems such as failure of engraftment or graft-versus-host disease.

CONVENTIONAL ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) represents an important therapeutic tool for the treatment of hematologic diseases, genetic disorders, and autoimmune diseases. A composite tissue allograft is a neurovascularized module of nonvital tissues, which include structural, functional, and aesthetic units, and is composed of a large spectrum of ectodermal tissues: epidermis and epidermal derivatives such as nails and hair, nerves and mesodermal tissues such as dermis, muscles, bones, articular cartilage, ligaments, tendons, paratenon and other supportive and connective tissues, adipose tissue, and vessels. In addition, there is a hematopoietic bone marrow component (bone marrow, bone, and stromal supportive microenvironment) that acts as a vascular bone marrow transplantation (VBMT) to the recipient on a vascular pedicle. VBMT has the advantage of providing a microenvironment and immediate engraftment of both mature and progenitor hematopoietic cells at the time of transplantation. VBMT works immediately as an instant bone marrow source of donor-derived immature hematopoietic stem cells in the recipients (no engraftment period) that differentiates to lymphocytes and granulocytes that contribute to mixed chimerism and facilitate the induction of immunologic tolerance. Later, the donor-derived hematopoietic stem cells engraft in recipient bone marrow and differentiate into mature hematopoietic cells.

The aim of this study is to study the applicability of VBMT as an alternative to conventional bone marrow cell suspension transplantation, comparing the development of microchimerism after allogeneic VBMT versus conventional BMT.

MATERIALS AND METHODS

Animals

The Animal Research Application for this study was approved by the Institution's Animal Ethics Committee (Carol Davila Medical University, Bucharest, Romania). All procedures utilizing experimental animals were carried out according to the National and European Health Medical Research Council's code of practice for the care and use of animals for scientific purposes. The experiments were conducted on Lewis and Brown Norway rats purchased from a local animal care facility. The rats were housed in a temperature-controlled environment with a 12-hour light/dark cycle and were maintained on a standard rodent diet and water ad libitum. The rats were divided into two groups: one group received VBMT and the other group received conventional BMT. VBMT was performed by heterotopically transplanting the hind limb of a donor rat onto the thigh of a recipient rat, while BMT was performed by intravenously transplanting a suspension of donor bone marrow cells into the recipient rats. Cellular microchimerism was assessed by flow cytometry at 30 and 60 days post-transplantation in VBMT recipients and at 30 days post-transplantation in BMT recipients.

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tual animals were male rats between 10 and 16 weeks (average 13) and weighing between 250 and 400 g (average 320 g). We selected two inbred strains (Brown Norway [BN] and Lewis [LEW]) due to their strong antigenic mismatch. BN (RT-1\(^n\)) rats were used as donors and LEW (RT-1\(^l\)) rats as recipients for heterotopic hind limb transplantation.

**Experimental Groups**

Twenty-five rats were included in this study. They were divided into three groups: group 1 (n = 10), LEW rats received a heterotopic BN hind limb (VBMT); group 2 (n = 10), from BN, 5 × 10\(^7\) bone marrow cells in suspension were given intravenously to LEW rats (BMT); and group 3 (control group; n = 5), untreated LEW rats.

**Transplantation Procedures**

In group 1, BN rats served as donors and LEW rats as recipients of heterotopic hind limb transplants (VBMT; Fig 1). The previously described surgical technique for heterotopic limb transplantation was used.\(^{10}\) In group 2, bone marrow cells were obtained by flushing the marrow cavities of donor BN femurs and tibias. Each host LEW rat received intravenously 5 × 10\(^7\) bone marrow cells, equivalent to that grafted in the VBMT model. In group 3, five untreated LEW rats were used as a control group.

**Immunosuppressive Drug Regimen**

All animals received standard immunosuppressive therapy, consisting of cyclosporine (CsA) in a dose of 17 mg/kg body weight for 60 days.

**Donor Chimerism Analysis**

Thirty and 60 days after transplantation, samples from the peripheral blood, recipient femur bone marrow, and donor femur were harvested and donor cell chimerism was evaluated. Donor-derived bone marrow cells were identified in recipient tissues by staining with the monoclonal antibody OX27 directed against MHC class I on BN cells and analyzed.

**Statistical Analyses**

Results were presented as mean percentage ± standard deviation. For statistical analysis comparing graft survival groups, Student t-test was used. Differences in the data were considered significant when \(P < .05\).

**RESULTS**

The cellular microchimerism was investigated in recipients of VBMT versus BMT, and also compared with a control group. Analysis of donor cell chimerism showed significant numbers of donor cells surviving after hind limb transplantation at 30 and 60 days, but not after injecting bone marrow cell suspensions. Evidently, the level of chimerism in the untreated (control) group was zero.

In group 1 (VBMT), the cellular microchimerism revealed the presence of BN cells in LEW recipient rats at 30 days: 5.3% ± 4.3% in peripheral blood, 0.6% ± 0.3% in bone marrow, and 28.5% ± 2.9% in donor femur. At 60 days, the donor BN cells were found in recipient rat peripheral blood in 3.3% ± 5.1%, bone marrow in 1.9% ± 0.7%, donor femur in 1.7% ± 3.3%.

In group 2 (BMT), the number of donor-derived cells detected in the recipient was very low at 30 and 60 days.

The difference between the two groups was highly significant \((P < .005)\), thus demonstrating better engraftment after VBMT compared with conventional BMT.

**DISCUSSION**

This study provided information that VBMT seeded from the bone marrow cavities of heterotopic transplanted limbs migrate to recipient tissues and can be detected there after 30 and 60 days of CsA therapy. A high number of donor bone marrow cells in recipient blood would point to their continuous release from the bone marrow of the grafted limb. The bone marrow cell were transplanted together with stromal cells in their spatial relationship. This natural environment could ensure proliferation of transplanted allogeneic bone marrow cells and their release to the circulation and homing to lymphoid organs. In the model of intravenous: suspension bone marrow cell transplantation, the grafted cells were deprived of their stromal cells, thus limiting their maturation and proliferation. The number detected in the recipient was very low at 30 and 60 days.

Further investigation is needed to determine the applicability of this concept in similar situations with clinical practice that requires cytoablation for the treatment of hematologic malignancies.

VBMT with its intact composite of marrow cells, stem cells, and supporting stromal environment function in a superior manner compared with conventional cellular marrow transplantation, with more robust bone marrow engraftment into nonmanipulated recipients, with prolonged level of microchimerism.

**REFERENCES**


![Fig 1. Heterotopic hind limb transplant (vascularized bone marrow transplantation).](image)


