Challenging the Surgical Rodent Hindlimb Ischemia Model with the Miniinterventional Technique

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ABSTRACT

Purpose: To develop an interventional hindlimb ischemic model and compare its angiogenic effect versus surgical ligation (SL) and excision of the femoral artery in rats treated with transplantation of bone marrow mononuclear cells (MNCs) as an angiogenic stimulator.

Materials and Methods: Forty-eight Lewis rats randomly received interventional embolization (IE) with hydrogel wire or SL and excision of the right femoral artery. Rodents were intraarterially transplanted with 1.5×10^7 MNCs in 500 μL medium from 24 isogenic donor rats. Functional and structural recovery was evaluated by laser Doppler imaging (LDI), cytokine/chemokine assay, and histologic staining.

Results: In vivo microscopic images showed significantly dilated vasa vasorum around the embolized segment of the right femoral artery at 3 days compared with disorganized tissue structure in the SL group. However, the LDI index was significantly higher in the SL group at 3 days compared with the IE group. LDI did not significantly differ between the two groups at 2 weeks after transplantation. Cytokine assay showed higher levels of interleukin (IL)–1α and IL-18 in the SL group; the IE group had higher levels of interferon-γ, IL-6, IL-13, and granulocyte colony-stimulating factor. Histologic examination demonstrated inflammatory infiltration near the incision within nerve fibers with dilated capillaries, showing nerve degeneration in the SL group. At 2 weeks, histologic analysis demonstrated massive scarring under the skin spreading into the musculature in the SL group.

Conclusions: A minimally invasive hindlimb ischemia model has been successfully developed that preserves tissue integrity and minimizes inflammation and confounding factors in the early stages of angiogenesis and arteriogenesis.

ABBREVIATIONS

GCSF = granulocyte colony-stimulating factor, IE = interventional embolization, IFN = interferon, IL = interleukin, LDI = laser Doppler imaging, MNC = mononuclear cell, SL = surgical ligation, VEGF = vascular endothelial growth factor

The prevalence of lower-extremity peripheral arterial disease is approximately 10% in people younger than 65 years and increases to 20% in people older than 75 years in the United States (1). Restoration of blood flow through angiogenesis and arteriogenesis may provide "biological revascularization" in these patients (2). In the past decade, intensive efforts have been undertaken to achieve neovascularization of ischemic limbs through the administration of growth factors that promote angiogenesis and/or arteriogenesis. Numerous animal studies (3–6) have shown the feasibility of enhancing vessel growth in ischemic tissues, and the clinical application of growth factors has shown encouraging results in several small studies (7).

Vascular endothelial growth factor (VEGF) plays an important role in the process of angiogenesis and promotes the proliferation and migration of endothelial cells (8); fibroblast growth factor is also a strong inducer of angiogenesis (9). In addition, it was recently demonstrated that VEGF and fibroblast growth factor had a synergistic effect on angiogenesis (10). However, two phase II randomized controlled clinical trials involving

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VEGF (11) and basic fibroblast growth factor (12) for intermittent claudication have not corroborated the results of these smaller studies.

Many issues may have contributed to the lack of success of clinical growth factor therapy (9,13). However, the problem may also result from inadequate animal models used in these animal studies. In particular, surgical ligation (SL) of the common femoral artery, frequently used as a model in such studies (4,5,14,15), may induce a number of artifacts as a result of extensive inflammatory injury that by itself can promote angiogenesis and alteration of the vascular bundle structure. Moreover, there is a persistent need for reliable noninvasive imaging techniques that allow monitoring of vessel development and function in response to therapy.

Animal models of human disease are a source of great insight, yet they also carry potentially crucial limitations that may confound experimental data. Useful models should incorporate the following essential features: (i) clinical relevance, (ii) low cost, (iii) small animal size, and (iv) provision of sufficient material for the study of cellular and molecular mechanisms. Rats meet these requirements, which ensure their popularity for models of vascular disease. However, the existing surgical hindlimb ischemic model is limited to surgical injury and wound healing in the targeted area. In addition, its clinical relevance to the anatomic and functional characteristics of intravascular atherosclerotic stenosis or occlusion is questionable. The present study addresses these issues through the development of an interventional hindlimb model, which compares the angiogenic effect versus SL and excision of the femoral artery in rats treated with bone marrow mononuclear cells (MNCs), an angiogenic stimulator (6).

We expect an interventional hindlimb model can not only preserve the tissue integrity, but also minimize inflammatory reaction from open surgery at the early stages and wound healing at the later stages, which will be conducive to the exploration of the cellular and molecular mechanisms in terms of functional and structural regeneration of blood vessels through pure angiogenesis and arteriogenesis.

MATERIALS AND METHODS

Animals

Seventy-two male inbred Lewis rats (weight, 250–275 g; Harlan, Indianapolis, Indiana), 10–12 weeks of age, were used for all experiments. By using an Internet-based source (http://randomizer.org/form.htm), 48 rats were randomly assigned to undergo interventional embolization (IE; n = 24) or SL (n = 24). The balance (n = 24) were saved as donors for MNCs. The study conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (publication no. 85-23, revised 1985) and was approved by the institutional animal care and use committee.

Hydrogel Coil in Vitro Study

The HydroCoil embolization system (MicroVention, Aliso Viejo, California) consists of a synthetic, polymeric hydrogel attached to a 0.014-inch platinum coil. Segments 10 mm long were cut from the 10-mm × 20-cm complex helical platinum coils. Images were obtained with the M2Bio three-dimensional microscopy system while the coil was immersed in the rat's serum at six different time points: pre-immersion; 10 seconds; and 5, 10, 15, and 20 minutes post-immersion. Axiovision 4.2 software (Zeiss) was used to assess the diameters of the coil.

Bone MNC Transplantation

Bone marrow was harvested from the femur and tibia of 24 isogenic Lewis donor rats and MNCs were isolated by Ficoll density gradient centrifugation (Lymphoprep; Nycomed, Zurich, Switzerland) as described previously (6). MNCs were used as a neovascular stimulus (6).

Interventional Hindlimb Model in Rats

Rats were anesthetized with intramuscular ketamine hydrochloride 80 mg/kg and xylazine 10 mg/kg. Heparin 100 IU was administered intraperitoneally. By using the sterile technique and with the assistance of a binocular loupe (magnification ×4; World Precision Instruments, Sarasota, Florida), a midline incision was made along the neck. The left common carotid artery was exposed and ligated distally with 4–0 silk suture, and a proximal suture was used to control bleeding during incision. A 2.4-F catheter over a 0.014-inch guide wire (Guidant, Santa Clara, California) was inserted into the incision and advanced to the proximal portion of the common femoral artery under C-arm fluoroscopic guidance (OEC 9900 Elite C-arm; GE Medical Systems, Milwaukee, Wisconsin). An angiogram was obtained with use of 0.5 mL Ultravist (Berlex, Wayne, New Jersey) to save as a roadmap. One 10-mm HydroCoil segment was loaded manually into a polyethylene tube (PE-50). This tube was opposite to the hub of the indwelling 2.4-F catheter. The coil segment was introduced into the artery with the same guide wire. The distal end of the coil segment was positioned approximately 2–4 mm proximal to the bifurcation of the popliteal and saphenous arteries. The catheter was then pulled back to the abdominal aorta. Complete occlusion of the artery was confirmed by injection of 0.5 mL Ultravist for angiography 20 minutes later. Then, 1.5×10^7 MNCs in 500 μL Dulbecco's modified Eagle medium was injected through the same catheter positioned proximal to the bifurcation of the internal and external iliac artery, followed by another 500 μL Dulbecco's modified Eagle medium for flush. The incision was sutured in layers with a 4–0 silk suture. Twenty-two of 24 interventionally successful rats were randomly assigned to one of three groups according to follow-up time: group 1 (n = 5; days