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Mesenchymal stem cells to augment therapeutic angiogenesis in hind-limb ischemia models: how important is their source?

Garry P. Duffy
Royal College of Surgeons in Ireland

Caroline C. Herron
Royal College of Surgeons in Ireland

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COMMENTARY

Mesenchymal stem cells to augment therapeutic angiogenesis in hind-limb ischemia models: how important is their source?

Garry P Duffy* and Caroline C Herron

See related research by da Cunha *et al.*, http://stemcellres.com/content/4/4/86

Abstract

Murine models of hind-limb ischemia are frequently used to assess interventions aimed at improving therapeutic angiogenesis in critical limb ischemia. Much of the current focus of angiogenesis lies with mesenchymal stem cells (MSCs). Important considerations when using these models include the strain of mouse, because some strains recover from ischemia more rapidly than others, and the MSC source. MSCs derived from certain strains generate increased levels of growth factors such as vascular endothelial growth factor. This may significantly affect the limb's ability to generate collateral vessels.

Atherosclerotic peripheral arterial disease is common, and critical limb ischemia (CLI) represents the end stage of this disease. Although the numbers of peripheral arterial disease patients progressing to CLI are low, there is a high morbidity and mortality associated with CLI and patients suffering from the condition have a poor quality of life. Therapeutic angiogenesis using stem cells and other biotherapeutics for the treatment of CLI is still under investigation. The authors of this study discuss the inherent variations in the ability of two different mouse strains, BALB/c and C57/BL6, to recover from ischemia [1].

C57/BL6 mice have a greater density of pre-existing collateral vessels, a higher rate of angiogenesis and increased expression of vascular endothelial growth factor and tumor necrosis factor alpha compared with other mouse strains [2]. These mice therefore have been shown to demonstrate a better recovery from ischemia [3]. This observation led the authors to question whether mesenchymal stem cells (MSCs) generated from different mouse strains would lead to different levels of recovery from ischemia. Choosing the appropriate mouse strain is therefore important not only when determining which strain to use in a hind-limb ischemia model but also when considering from which strain to source the MSCs. Use of MSCs has been at the forefront of investigations into therapeutic angiogenesis and they have shown promising *in vitro* [4] and *in vivo* [5] results.

Bone marrow cells were collected from the tibia and femur of 8-week-old BALB/c mice and C57/BL6 mice. BALB/c mice at 10 to 12 weeks old had ischemia induced surgically via removal of the femoral artery and closure of its branches. Five days postoperatively, the thigh muscles were exposed and 5×10^5 cells were injected into the quadriceps muscle. Aside from positive and negative controls, animals were divided into groups that received MSCs obtained from BALB/c mice or MSCs obtained from C57/BL6 mice (n = 6). The study ran for 35 days, during which period the degree of ischemia was assessed visually and scored accordingly. Isometric muscle force was compared in the gastrocnemius muscles prior to the conclusion of the study. Histological analysis assessed muscle regeneration and fibrosis, and immunohistochemistry assessed the presence of smooth muscle cells and endothelium. Due to the abovementioned factors, MSCs from C57/BL6 mice were expected to show greater improvements in angiogenesis and therefore greater benefit in the treatment of limb ischemia than MSCs derived from BALB/c mice. Indeed, during cell culture the MSCs derived from the C57/BL6 mice showed a higher growth rate and higher vascular endothelial growth factor expression.

The optimum time point for injection of proangiogenic factors has not been adequately defined, with most authors reporting immediate injection into the muscles

* Correspondence: garryduffy@rcsi.ie
Department of Anatomy, Royal College of Surgeons in Ireland, 123 St Stephens Green, Dublin 2, Ireland

upon ligation of the vessels [6]. In this study, however, the authors describe poor outcomes with this technique and therefore injection was delayed until day 5. Further investigation and analysis in this area is required. Comparison of the ischemic limbs visually illustrated that 16.7% of the animals in the treatment groups developed grade II necrosis. This contrasts with the untreated group, where approximately 70% reached grade IV necrosis. This difference suggests the efficacy of MSCs as a treatment option for limb ischemia. Histological analysis revealed no differences between the two treatment groups, but both showed increased muscle regeneration and vessel density when compared with the nontreatment groups.

Whilst there was no objective measurement of limb perfusion throughout the study and no differences were noted between treatment groups, this study nonetheless raises important questions both with regard to the timing of treatment post-creation of ischemia in murine models and with regard to the origin of MSCs. In view of the fact that there is a clear clinical need for novel treatment options in the subgroup of patients who are unsuitable for the traditional methods of revascularization in critical limb ischemia, and given the interest in MSCs as a potential treatment for ischemia, we feel further investigation into the optimum source for deriving MSCs for use in these models is warranted, and a concerted effort should be made to find agreement on the optimal model to use for future assessment of novel therapeutics.

Abbreviations

CLI: Critical limb ischemia; MSC: Mesenchymal stem cell.

Competing interests

Both authors declare that they have no competing interests.

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