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Method to Improve Administered Cells Via Intramuscular Route to Augment Efficacy of Regenerative Cell Therapy in The Field of Peripheral Artery Disease

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ABSTRACT

In the field of peripheral artery disease (PAD), regenerative cell therapy has been expected to be a new alternative therapy especially for severely diseased patients. Although various preclinical studies showed promising results, those therapies failed to show sufficient efficacy for actual PAD patients and current guideline do not recommend regenerative therapy for PAD. The disappointing result may be caused by low retention rate of administered substance. Recently we published an article regarding novel method to augment the effect of cell therapy. In this review article, we would like to focus on the importance of improvement in cell survival and prerequisite factors to develop effective regenerative therapy for PAD patients.

INTRODUCTION

Peripheral artery disease (PAD) is a widespread problem due to the increased number of diabetes mellitus worldwide [1]. Chronic limb threatening ischemia (CLTI) is the most severe form of PAD and CLTI is basically treated with surgical or endovascular revascularization. However, approximately 25–40% of CLTI patients are untreatable due to poor general and anatomical condition [2]. Regenerative therapy, including cell therapy and gene therapy, has been expected to be an alternative therapy for such untreatable patients. However, current guidelines do not recommend regenerative therapy for PAD and CLTI due to limited efficacy despite promising results of various preclinical studies [3,4]. One of the potential causes of limited efficacy in real clinical settings would be attributed to the immediate loss of administered regenerative agents and inappropriate preclinical model to assess the therapeutic potential of target agents. Recently, we have published an article regarding regenerative cell therapy for PAD, which developed a new administration method to improve administered cell survival. Additionally, we assessed the efficacy of the new method using clinically relevant severe ischemia model [5]. In this concise review, we would like to focus on the newly developed method reported in the article and prerequisite factors to develop effective regenerative therapy for PAD and CLTI patients.

Cell administration via intramuscular pathway

For the regenerative cell therapy, there are mainly three administration pathways: intramuscular, intraarterial, and intravenous. Among the three pathways, intramuscular administration is the most favored one for PAD, because intramuscular injection enables direct administration of cells into ischemic area [6,7]. However, direct administration into ischemic muscles have a potential disadvantage of immediate loss of administered cells: Approximately 75% of administered cells would be lost within 4 hours in a preclinical model due to severe ischemic and inflammatory environment [8,9]. Early loss of administered cells would lead to insufficient reaction time to cause therapeutic effect. Therefore, to secure the regenerative effect of cell therapy via intramuscular administration, a way to improve administered cell retention and survival is mandatory.

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Clinically relevant preclinical model of PAD

Historically, rodent's acute hind limb ischemia model, including mice and rats, has been used as a preclinical model of PAD to confirm proof of concept of various regenerative therapy. The problem to use acute hind limb ischemia model would be high auto-regenerative ability of rodents and acute inflammatory status concomitant with induced acute limb ischemia [10]. High auto-regenerative ability would make the evaluation of the regenerative capacity of administered agents difficult: it would be difficult to discern whether the improvement after administration is attributed to the administered agents or self-recovery. Acute inflammatory status play a positive role for tissue regeneration, while actual PAD patients suffer from chronic inflammation, which is negative for regeneration [11]. Acute inflammation is essential part of repairment of damaged tissue by eliminating damaged tissue and induction of early phase angiogenesis [12,13]. To solve these two concerns, we excluded mice with high regenerative capacity by setting cut-off value of blood perfusion at 7 days after induction of limb ischemia [5]. By setting several days interval, at least three or four days, between induction of limb ischemia and administration of cells [9,14], rodents with high auto-regenerative ability would be excluded. Also, acute inflammation peaks at three to four days after induction of hind limb ischemia and then gradually falls in mice [5]. Therefore through the selection of appropriate animal models by setting cut-off value and several days interval after induction of limb ischemia, clinically relevant treatment refractory model may be established.

Required therapeutic mechanism for regenerative therapy in PAD

Historically, angiogenesis has been the main targeted mechanism in regenerative therapy. For PAD, muscle regeneration and regulation of inflammation are also important to induce therapeutic effect. PAD patients have deteriorated muscle function and are in potentially frail status due to impaired walking ability, which would be improved by muscle regeneration.[15,16] Regulation of inflammation is also important because PAD is caused by chronic inflammation. Chronic inflammation has various negative effect for tissue repair, including tissue scaring, inhibition of angiogenesis, and progression of atherosclerosis [17,18]. The major cause of chronic inflammation would be macrophage malfunction [12,19]. Macrophages have various phenotypes, and they are mainly classified into two phenotypes that are pro-inflammatory (M1) and anti-inflammatory (M2) macrophages [13]. Each phenotype is intrinsically labile and various signaling alters macrophage phenotypes, and macrophages change their phenotypes and roles depending on the phases of tissue regeneration. However, in certain diseased conditions, including obesity, infection, and diabetes, chronic inflammatory status persists with dominant and persistent pro-inflammatory M1 macrophages [19], which inhibits healthy angiogenesis and tissue regeneration in PAD. In chronic inflammatory status the ability to switch phenotype from pro-inflammatory M1 to anti-inflammatory M2 is impaired. Therefore, modulation of macrophage phenotype would be an important target of treatment in PAD. In short, to access the efficacy of newly developed regenerative therapy, angiogenesis, muscle regeneration, and modulation of inflammation should be evaluated to confirm proof of concept.

Novel method to augment administered cell survival

As described above, the low survival rate of administered cells is a cause of low efficacy of regenerative therapy. Previously, several methods to augment cell survival has been reported, including cell sheet, bioscaffold, and spheroid [20-23]. The key factor of those methods to improve cell survival is the existence of extracellular matrix (ECM). ECM has various roles, including maintenance of intracellular signaling that prevents cell death [24]. Therefore we focused on the importance of ECM and developed a new method, which we call clustered cell administration, to improve cell survival after intramuscular administration [5]. The clustered cells are lump of cells, which are created using cell sheet technology: Cell sheet is easily made by using a temperature-responsive culture dish and contains various kinds of ECM [20]. Clustered cells made of myoblast cells included fibronectin, laminin, and vitronectin. Those ECMs seemed to play different roles because the main location of each ECM differs: fibronectin and laminin located in the marginal area, while vitronectin located in the central area of the lump of cells. Fibronectin and laminin seemed to help the attachment of cells to the administered site, while vitronectin seemed to help conduct intracellular signaling [24,25]. Clustered cells composed of myoblast cells and ECMs showed significantly augmented cell survival after intramuscular administration. Owing to the improved cells survival, clustered myoblast cells showed marked therapeutic effect mainly via paracrine effect in the clinically relevant hind limb ischemia model regarding angiogenesis, muscle regeneration, and modulation of inflammatory status.

Future perspective

Although in the previous study regarding clustered cells technology, we applied autologous myoblast cells,[5] autologous cells would have disadvantages in real clinical settings.

Even stem cells are reported to be deteriorated in quality and function in the process of aging and disease progression [26]. In this sense, allogenic cells with high regenerative capacity may be more favorable cell source to develop highly effective regenerative therapy for PAD. The clustered cell technology would be applicable to various kinds of cells, and this new technology would help develop effective regenerative therapy to improve PAD patient's outcome.

CONCLUSION

To develop clinically effective regenerative cell therapy for PAD including CLTI, clinically relevant treatment refractory preclinical model should be used. Because of the potential loss of cells due to ischemic and inflammatory condition of PAD, a method to improve cell survival should be applied to maximize therapeutic effect of cell therapy. Regarding the evaluation of efficacy, angiogenesis, muscle regeneration, and modulation of inflammation should be performed.

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DECLARATION OF INTERESTS

None

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