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Safety and efficacy of cell-based therapy on critical limb ischemia: A meta-analysis

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Abstract

Background aims. Critical limb ischemia (CLI) is a major health problem worldwide, affecting approximately 500–1000 people per million per annum. Cell-based therapy has given new hope for the treatment of limb ischemia. This study assessed the safety and efficacy of cellular therapy CLI treatment. *Methods.* We searched the PubMed, Embase and Cochrane databases through October 20, 2015, and selected the controlled trials with cell-based therapy for CLI treatment compared with cell-free treatment. We assessed the results by meta-analysis using a variety of outcome measures, as well as the association of mononuclear cell dosage with treatment effect by dose-response meta-analysis. *Results.* Twenty-five trials were included. For the primary evaluation index, cell-based therapy significantly reduced the rate of major amputation (odds ratio [OR] 0.44, 95% confidence interval [CI] 0.32–0.60, $P = 0.000$) and significantly increased the rate of amputation-free survival (OR 2.80, 95% CI 1.70–4.61, $P = 0.000$). Trial sequence analysis indicated that optimal sample size (n = 3374) is needed to detect a plausible treatment effect in all-cause mortality. Cell-based therapy significantly improves ankle brachial index, increases the rate of ulcer healing, increases the transcutaneous pressure of oxygen, reduces limb pain and improves movement ability. Subgroup analysis indicated heterogeneity is caused by type of control, design bias and transplant route. In the dose-response analysis, there was no significant correlation between cell dosage and the therapeutic effect. *Conclusions.* Cell-based therapy has a significant therapeutic effect on CLI, but randomized double-blind placebo-controlled trials are needed to improve the credibility of this conclusion. Assessment of all-cause mortality also requires a larger sample size to arrive at a strong conclusion. In dose-response analysis, increasing the dosage of cell injections does not significantly improve the therapeutic effects of cell-based therapy.

Key Words: *Cell-based therapy, Clinical trial, Critical limb ischemia, Meta-analysis, Peripheral arterial disease*

Introduction

Critical limb ischemia (CLI) is an arterial disease of the extremities that causes pain at rest, ulceration and necrosis [1]. This disease affects approximately 500–1000 people per million per annum. Among adults over age 65, the morbidity rate is 10%–20% and increases with age. The disease is more prevalent in men than women [2,3].

CLI is associated with atherosclerosis, diabetes and thrombus occlusive vasculitis. The etiology of atherosclerosis is unclear; one epidemiological study found that 15%–20% of atherosclerosis patients who display symptoms will develop severe limb ischemia [4,5]. Diabetic limb ischemia often occurs with multiple arterial occlusions making revascularization difficult and complex [6]. Thrombus occlusive vasculitis (Buerger disease) is another segmental inflammatory disease that most commonly affects the small and medium arteries; almost everyone diagnosed with Buerger disease smokes or uses some other form of tobacco [7,8].

The pathological features of CLI are similar among patients: segmental arterial occlusion resulting in amputation or death. Arterial reconstruction and bypass surgery are common treatments for CLI patients; however, many patients cannot undergo surgical therapy, respond poorly to treatment or have a high recurrence rate [6,9]. The alternative, drug treatment with agents such as prostaglandin and iloprost, are also unsatisfactory in treating the disease [10].

Cell-based therapy has given new hope for limb ischemia treatment. In 2002, Tateishi-Yuyama was the first to transplant bone marrow mononuclear cells (BMMNCs) in patients with limb ischemia to perform therapeutic angiogenesis [11]. Since then, numbers of clinical studies have been executed for limb ischemia patients using cell-based therapy. However, the methods of cell-based limb ischemia treatment are

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relatively complex, and the number of patients in single clinical trials is low. Therefore, meta-analysis is necessary to assess the safety and efficacy of cellular therapy for treatment of limb ischemia.

Several meta-analyses have been carried out examining cell-based limb ischemia therapy, but the results are contradictory [12–17]. This study updates previous studies, expands the inclusion of cell types to include BMMNCs, bone marrow mesenchymal stromal cells (BMMSCs), peripheral blood mononuclear cells, VesCells (peripheral blood angiogenic precursors) and CD133 + cells and provides a comprehensive summary of outcome assessments. Lastly, we examine the relationship between the therapeutic effects of CLI treatment and the total dosage of mononuclear cells intramuscular injected using methods of dose-response meta-analysis.

Methods

Data sources and searches

We searched PubMed, Embase and Cochrane Central Register of Controlled Trials with keywords including "(Peripheral artery disease OR peripheral arterial disease OR limb ischemia OR limb ischaemia) AND (mononuclear OR mesenchymal OR stem cell OR cell transplantation) AND clinical trials." We did not apply any language restrictions and included all relevant articles up to October 20, 2015. We also searched the reference lists of identified trials.

Data selection

Two authors (FX and SZ) independently identified eligible reports. Discrepancies were resolved through discussion. Eligibility criteria included the following requirements: (i) the report described treatment of CLI, (ii) the study was a controlled clinical trial, (iii) the study included comparison groups in which one group received some type of mononuclear cells, mesenchymal cells, or other stem cells, and the control group received treatment without any cell-based therapy. Exclusion criteria were as follows: (i) the report described a retrospective or prospective observational cohort study, (ii) publication was a duplicate, (iii) the study included a control group that received cell-based treatment, and (iv) the study used granulocyte colony-stimulating factor treatments.

Data extraction

Two authors (JH and CPL) compiled data with a predefined information sheet. The following items were extracted from the included articles: author, year, country, number of patients, characterization of patients, type of control, intervention of experimental group, intervention of control group, cell transplant route, cell dosage and follow-up. Two authors (JH and CPL) independently conducted risk of bias assessments of the included studies using the Cochrane Collaboration tool [18].

Data analysis

The following outcomes were evaluated in this review: all-cause mortality, major amputation, amputation-free survival (AFS), number and area of healed ulcers, ankle brachial index (ABI), transcutaneous pressure of oxygen ($TCPO_2$), visual analogue scale (VAS), and walking distance. These outcome measures were ranked according to the Grading of Recommendations Assessment, Development and Evaluation (supplemental Table S1) [19].

The method described by Greenland and Longnecker [20] and Orsini et al. [21] was used to compute the trend from the correlated log odds ratios (ORs) across amounts of mononuclear cells, and the median or mean amount of cells was assigned to their corresponding ORs. When the median or mean was not provided in the article, we assigned the midpoint of the upper and lower boundaries as the average dose. We evaluated a potential nonlinear association between amount of cells and treatment effect (major amputation, AFS, all-cause mortality, ulcer healing). This was done by modeling the amount of cells with the use of restricted cubic splines with three knots at fixed percentiles (10%, 50% and 90%) of the distribution [22,23]. A P value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the second spline is equal to zero.

Statistical analysis

We used the inverse variance method to pool continuous data and the Mantel-Haenszel method for dichotomous data; the results were presented as standardized mean difference with 95% confidence intervals (CIs) and OR with 95% CIs. The I^2 statistic was calculated to evaluate the extent of variability attributable to statistical heterogeneity between trials. In the absence of statistical heterogeneity ($I^2 < 50\%$), we used a fixed-effect model; otherwise, we used a random-effects model. To investigate the sources of heterogeneity, predefined subgroup analyses were performed: cell types, follow-up time, control type, design bias, and transplant route. We assessed for publication bias by visually examining funnel plots and using the Begg-Mazumdar and Egger tests.

We also conducted trial sequential analysis to calculate the required sample size and the cumulative Z-curve's eventual breach of relevant trial sequential monitoring boundaries [24]. The required sample size of the trial sequential analysis was based on a type 1 error of 5% and a beta of 20% (power of 80%). A