Synergism of Hematopoietic Cytokines for Infarct Repair

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Over the past few years, the field of stem cell biology and its therapeutic application in cardiovascular diseases has expanded remarkably and moved to the forefront of cardiovascular science. Promising results from experimental studies with bone marrow (BM)-derived stem or progenitor cells \(^1\)–\(^3\) prompted initiation of clinical trials in ischemic heart diseases (IHD). Pilot clinical trials \(^4\), \(^5\) demonstrated that cell therapy using various BM-derived cells are safe and effective for treating IHD. The discovery that BM includes various stem cells spawned the strategy of directly mobilizing and homing BM cells into the heart to regenerate injured tissue. \(^6\) This concept is appealing because invasive procedures related to harvesting and delivering BM cells into the heart can be avoided.

The strategy of mobilizing stem cells from BM was initially contrived by hematologists to accelerate recovery after cancer chemotherapy. \(^7\) A number of hematopoietic cytokines, including granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony stimulating factor (GM-CSF), stem cell factor (SCF), flt3 ligand (FL), and erythropoietin have been tested for mobilization and repopulation of the hematopoietic system. G-CSF is the most widely investigated hematopoietic growth factor in animals and patients. In addition to mobilizing BM cells, G-CSF induces proliferation, differentiation, and survival of hematopoietic cells. Recently, G-CSF was reported to have a direct action on nonhematopoietic cells expressing G-CSF receptors such as cardiomyocytes, \(^8\) endothelial cells, \(^8\) and neuronal cells. SCF was cloned as a ligand for c-kit. \(^9\) SCF exerts its effects at the early stages of hematopoiesis in BM and acts synergistically with CSFs. Flt3 is a receptor expressed predominantly on HSCs and progenitors and has many overlapping activities with c-kit. FL belongs to a family of hematopoietic cytokines, including SCF and macrophage colony-stimulating factor (M-CSF), that are specific for class III tyrosine kinase receptors. \(^10\) FL plays a central role in the proliferation, survival, and differentiation of early hematopoietic precursor cells. FL is usually not efficient as a single cytokine, but with G-CSF it exerts synergistic effects on mobilization and engraftment of HSCs. \(^11\)

A series of experimental studies was performed to test the effects of hematopoietic cytokines on myocardial infarction (MI) with the hope that mobilized stem cells could regenerate injured heart. The first study was performed by Orlic et al., \(^6\) in which they injected G-CSF plus SCF to splenectomized MI mice before and after MI and showed improvement of cardiac function. Although this study demonstrated regenerating cardiomyocytes and vessels by BrdUrd and Ki67 immunostaining, it did not prove direct transdifferentiation of mobilized BM cells into myocardial cells. This role of G-CSF in inducing cardiac homing of BM cells to regenerate hearts was challenged by another study. \(^8\) They claimed that although G-CSF treatment is beneficial for infarct repair, the mechanism of action is different. They showed that G-CSF directly binds G-CSF receptors present on multiple myocardial cells and activated its downstream signals such as the Jak–Stat pathway, and thereby reduces myocardial apoptosis, increases angiogenesis, and favorably remodels infarcted myocardium. These promising results of experimental studies in conjunction with ease of administration led to clinical trials. Although initial phase clinical trials showed improvement of function in the groups treated with G-CSF, \(^4\), \(^5\) recent large-scale, double-blind, placebo-controlled trials \(^12\), \(^13\) demonstrated that G-CSF treatment in patients with acute MI after successful revascularization had no influence on infarct size and left ventricular function.

The timely study by Dawn et al. \(^14\) in this issue of Circulation Research addresses several important issues with regard to infarct repair by hematopoietic cytokines. The authors examined the therapeutic efficacy and underlying mechanisms of hematopoietic cytokines in repairing acute MI. They used a more clinically relevant experimental design, ie, reperfusion after coronary occlusion, administering cytokines after reperfusion, and selecting clinically appropriate doses of cytokines. They found that a combination of G-CSF plus FL regenerated injured heart to a greater extent histopathologically and functionally than G-CSF plus SCF or G-CSF alone. In this study, G-CSF alone yielded minimal benefits, in concordance with recent clinical trials. \(^12\), \(^13\) This study also addressed the remaining question of whether mobilized cells have the ability to generate new cardiac tissues by providing direct evidence of BM-derived cardiomyocytes and vessels using GFP-BM reconstituted mice. Another advance in this field is the discovery of the role of cytokines in modulation of adhesion molecules on the mobilized HSCs (lin\(^\neg\)/Sca-1\(^\neg\)/c-kit\(^\neg\) cells). It appears that the added benefits of cytokine combination is not restricted to the quantitative increase in mobilization but also to induce qualitative changes that favor the homing of HSCs into myocardium.

The underlying mechanisms are likely complex. The comprehensive study design and analysis uncovered intriguing clinicopathologic discrepancies, which may provide further mechanistic insight into the role of cytokines in infarct repair. Histologically, cardiac regeneration occurred mostly in the...
infarct area. However, the most significant functional improvement occurred in the nonischemic zone where there was negligible cardiac regeneration. It is also clear that there were no statistical differences in regional myocardial function in the infarct area regardless of cytokine treatment, but the regional function in the noninfarct region was enhanced in the cytokine treated groups. Therefore, enhanced wall motion from infarct tissue does not seem to account for the major differences in ejection fraction. Collectively, the data suggest that the enhanced global cardiac function should represent primarily the function in the noninfarct region, which is most likely attributable to nonregenerative effects of cytokines, rather than via cardiomyocyte regeneration. Several explanations are possible. One possibility is direct action of cytokines on the damaged but viable myocardial cells in noninfarct areas. This effect can prevent adverse remodeling of perinfarct areas and contribute to enhanced regional wall motion in noninfarct areas. It also needs to be investigated whether combined cytokines have additional benefit over G-CSF alone in preventing apoptosis and promoting angiogenesis. Another possibility is paracrine effects of recruited cells. Accumulating evidence suggests that paracrine action is a major mechanism to mediate therapeutic effects of BM-derived cells. Various biologically active molecules such as antiapoptotic and angiogenic factors derived from recruited BM cells in the myocardium could help endangered myocardium to survive, accelerate the recovery of ischemic and stunned myocardium, and amplify function in nonischemic zones. Whether the regeneration of new cardiomyocytes or vessels by cytokines in the infarct area is a prerequisite for producing paracrine effects in noninfarct zones needs to be explored further. In addition, many questions remain concerning mechanisms involved in cytokine therapy. Was there any dose-dependent relationship between the magnitude of mobilization and the extent of regeneration? The more cells you mobilize, the more repair you can achieve—or is there any ceiling to this effect? Which cell types of BM are responsible for generation of new cardiac tissues? Do we need to mobilize more secretory types of cells? What are the molecular events in BM-derived cells involved in the seemingly synergistic effect of cytokine combination? How can cytokines interact with specific adhesion molecules? What signals and downstream molecules are associated with induction of tissue regeneration? How do cytokines affect resident cardiac stem cells? How do cytokines influence matrix reorganization in cardiac remodeling? Many of the mechanisms will likely be difficult to dissect considering the wide spectrum and interconnected actions of hematopoietic cytokines.

We also would like to mention the safety of cytokine therapy. Prior hematological studies raised concerns on the safety of using G-CSF/GM-CSF in unstable patients. In fact, recent clinical trials using G-CSF/GM-CSF in patients with acute MI or angina revealed an apparent increase in acute coronary syndrome and in-stent restenosis. It is conceivable that the cytokines mobilize unwanted inflammatory cells and promote the secretion of inflammatory mediators such as MCP-1 and CRP, translating into the rupture of atherosclerotic plaques and aggravation of vascular inflammation. Therefore, more careful monitoring or screening of patients is warranted in potential clinical trials using cytokine combinations as these may pose a higher risk by mobilizing more unwanted cells.

Cytokine therapy for cardiac repair is an attractive approach for both patients and physicians. Although the outcomes of clinical trials with a single cytokine were disappointing, a revised protocol of using combinations of hematopoietic cytokines is expected to shed new light on this therapeutic modality. It also needs to be determined whether cytokine therapy may replace cell therapy or may complement cell therapy. Because candidate patients for cytokine therapy generally have multiple cardiovascular risk factors and it is now known that risk factors adversely affect function of BM cells, experimental studies have limitations to determining therapeutic efficacy. Clinical trials will ultimately determine the efficacy of this strategy and potentially reveal problems that are not currently apparent.

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