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What would convince us – From bench to bedside and back

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Abstract

The relatively recent identification of stem cells within the adult heart and the possibility of regenerating or repairing the injured myocardium through autologous stem cell administration have started a revolution, opening the doors to "regenerative medicine" for cardiac diseases. However, the great expectations generated from the results of the first animal studies and early phase clinical trials have turned now into a kind of spreading scepticism when inconsistencies were found to be the rule of the first randomised, placebo-controlled trials. At this point, it seems unavoidable to be self critical, analysing every point of view on what has been done and where we are going. In this commentary, we have tried to show the clinicians' point-of-view on what information we need from basic researchers, and what we should offer them for the success of this emerging field. Today, more than ever, coordination between basic and clinical researchers within multidisciplinary teams is needed. Along with basic studies designed to unravel the mechanisms of action, parallel intermediate sized randomised, placebo-controlled clinical trials are needed to confirm basic findings or redirect bench research according to their results, test safety issues in a real scenario, and reject or modulate future large sized studies.

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Introduction

Heart failure, considered as the end-stage of several cardiac diseases, remains as a major health problem in most developed countries despite a trend over time to a better survival owing to preventive strategies[1]. Even more recent advances in the treatment of heart failure patients have failed to reduce mortality, and are thus not fully satisfactory[2]. The unacceptable death rate, along with the social and economic burden derived from the increase in its prevalence, make primary prevention and the search for new effective therapies a compelling endeavour[3]. Yet, the old concept of the adult heart as a post-mitotic and terminally differentiated organ implied until recently that therapeutic efforts should only be focused on palliating the adverse clinical course of this severe condition.

In the past few years, however, subpopulations of cardiac cells were discovered which re-entered the cell cycle under certain pathological and experimental conditions in both animals and humans[4-7], and especially the first evidence came out of the existence of true cardiac stem cells by Beltrami et al[8], which definitively called the previous paradigm into question, and opened the doors of "regenerative medicine" for cardiac diseases, thus starting, at least in theory, a new era of curative therapies. Almost simultaneously, bench works and early phase clinical trials to assess the safety and usefulness of autologous adult stem cell transplantation/mobilisation for the prevention or reversal of adverse ventricular remodelling were conducted. Subsequently, on the basis of their positive preliminary short-term results about safety and promising signs about cardiac functional recovery, the first "efficacy-assessing" trials were carried out[9]. Nevertheless, the initial great expectations derived from basic findings of studies using bone marrow derived progenitors[10,11], still have not found correlation in randomised clinical studies, particularly those assessing either intracoronary injection or bone marrow cell mobilisation, whose results have been less outstanding in some cases[12-14], neutral in others[15-19], and in general terms not supported by a deep knowledge of the mechanistic basis. As a result, a certain feeling of scepticism about the potential usefulness of cell therapy is spreading within the medical research community. Moreover, a heated controversy over the existence of true cardiac regeneration through cell transdifferentiation in animal experiments has to a certain extent focused the attention of basic researchers.

At this point, it seems unavoidable for both basic and clinical investigators to look back and think whether we have actually worked together as a team, carrying out parallel and coordinated studies to ask the many questions still waiting to be answered, or as disconnected parts of the same engine, trying separately to achieve satisfactory results as soon as possible in response to the always impatient requirements of society. In this commentary, we have tried to show the clinicians' point of view on what information we need from basic researchers, and what we should offer them for the success of this emerging field.

What do clinicians need from bench researchers?

Although there is a considerable amount of experimental data supporting the physiological benefit derived from transplanting stem cells into the healing heart, a great number of uncertainties regarding basic aspects of cell therapy for cardiovascular repair are left to be resolved. Success in clinical trials relies on the ability of preclinical scientists to find the right answers to such questions. To begin with, the identification of the stem or progenitor cell capable of rendering a significant benefit in the various clinical settings we are facing, is far from being accomplished. Several types of cells were observed to improve cardiac function when transplanted into the infarcted heart in bench research[20]. However, most experts agree that the generation of new cardiomyocytes, if it occurs, is not quantitatively sufficient so as to produce a significant increase in pump function due to a greater amount of contractile myocardium. So, what does such a wide range of cell types have in common to produce a similar benefit? Nowadays, a deep understanding of the paracrine effect of stem cells is one of the most challenging tasks which basic researchers confront. The identification of several populations of cardiac resident stem cells[21] and the mechanisms by which these latter could be stimulated remain unresolved. The use of chemokines, pro-survival genes or growth factors along with extra-cardiac stem cell administration will play an important role in future experiments. Indeed, some initial studies have documented that administration of anoxia-preconditioned bone marrow stem cells[22] or bone marrow derived mesenchymal stem cells overexpressing Akt, a protein kinase with anti-apoptotic activity, contributed to the repair of the infarcted rat myocardium as early as 3 days after treatment, modifying the secretion of cytokines and growth factors[23,24]. With this background in mind, the existence of true cardiac regeneration through transdifferentiation of extracardiac stem cells should not divert scientist's attention any more from the fact that the heart does have, though limited and probably designed to maintain cardiac cell turnover throughout life, a self regenerative potential.

Regardless of the main mechanism by which transplanted stem cells exert their benefit, there is also lack of information about factors that mediate their homing, engraftment and survival. In addition, no study has addressed the long-term fate of any cell type as yet. Improvements in current imaging modalities (magnetic resonance, SPECT, PET) and techniques of direct and indirect labelling of cells (cell tracking and reporter genes) make us feel optimistic about the future[25]. They would allow us to assess the influence of every possible variable (cell phenotype, timing of administration, delivery method, etc.) on the aforementioned factors. Apart from analysing the pharmacokinetics and pharmacodynamics of cell labelling molecules, their potential impact on stem cell differentiation, proliferation and survival should be carefully observed.

With respect to current methods of isolation, expansion and administration of stem cells, they need to be improved. Some authors have pointed out the enormous difference between infarct mass in current animal experiments and human studies, being in the latter 7,000 fold greater[26]. In addition, they also call for experiments performed on animals with similar characteristics to humans. Once a significant number of autologous cells is available, dose-escalating trials will determine how many cells would be needed to obtain comparable results in human experiments.

Regarding the current models of ischaemia/infarction in bench research, they actually do not resemble accurately the pathophysiology of either acute myocardial infarction or chronic ischaemic heart disease in humans. Apart from the size of the animals used,

the methodology and biology of such experiments is clearly different from that of humans. For instance, abrupt occlusion of a coronary artery by means of vessel ligation in a previously young and healthy animal has hardly anything to do with an acute coronary syndrome in an aged human with comorbidities (dyslipidaemia, diabetes, etc.) that led to atherosclerosis, conditions where not surprisingly, senescence, functional impairment and a decrease in number of vascular progenitor cells take part in the pathophysiology[27,28]. Such differences may be responsible for the discrepancy between results from bench and bedside studies, as they are for the lack of efficacy of numerous cardioprotective therapies to prevent reperfusion injury in humans[29].

Another aspect not fully addressed by basic research thus far, is the optimal cell delivery route for each clinical scenario. A crucial goal for experimental studies should be to determine the method which best guarantees the administration of the adequate number of cells within the target area, and their longest engraftment without other additional risks than those inherent to the delivery technique. Although some authors have analysed this facet, solid conclusions for every cell type and cardiac condition cannot still be drawn. Hoffman et al elegantly demonstrated that the percentage of unselected bone marrow cells retained within the heart through different intravascular methods is trivial, and found that cell phenotype (CD34+ enrichment) had a strong influence on cell homing[30]. Besides, Hou et al observed in a swine model of acute myocardial infarction, that the majority of the peripheral progenitors they implanted reached other organs than the primarily targeted, irrespective of what delivery method was used (intramyocardial, intracoronary or interstitial retrograde coronary venous injection)[31]. As a result, concerns about long-term effects of multi-organ seeding have arisen. Besides, differences in short term engraftment among such means of administration were not highly significant and, in the case of the most efficient (intramyocardial), it was inconsistent. Some other specific situations should be studied in basic stem cell research, particularly microvascular obstruction following reperfusion, and diastolic heart failure. Microvascular obstruction, a well-characterised complication of reperfusion therapies, is known to have severe prognostic implications[32]. We and others have observed in the human setting that microvascular obstruction following infarct related artery revascularisation is still a limitation which preclude patients from any benefit derived from intracoronary bone marrow stem cell transplantation with the current approach and methodology[15,33]. Therefore, animals suffering from experimental "non-reflow phenomenon", despite restoration of epicardial coronary patency, should be a target for new investigations. With regard to diastolic dysfunction, it accounts for almost half the symptomatic heart failure cases in the clinical setting, and mortality and rates of hospitalisation are as high as those among patients with systolic heart failure[34]. Still, no basic study has addressed so far the utility of stem cells to improve cardiac mechanical properties in animals with preserved systolic function and experimental diastolic impairment.

Above all, safety issues should remain a priority in stem cell preclinical research. For instance, it could be argued that further preclinical experience with myoblasts for cardiac repair before starting the first clinical trial may have warned clinicians about possible pro-arrhythmic effects, as some authors found later[35]. Thus, a thorough evaluation of every single safety aspect of future experimental studies should be undertaken, particularly when new sources of stem cells are used, to avoid as many unexpected clinical adverse events as possible.

What should clinicians offer bench researchers?

From the bedside of stem cell research, clinicians have tried for the last six years to emulate the results of basic experiments in animals. Apart from the evident divergence between outcomes, probably owing to differences between animal models and human heart disease (heart size, atherosclerotic background, exclusive biological properties of certain species, etc.), there is a lack of consistence between the results reported from the first randomised trials in the human setting[12-19]. Although the inhomogeneity in almost every variable from clinical trials may account for it (different cell products administered, timing of cell transplantation, disease severity, surrogate endpoints, imaging techniques, etc.), it seems compelling to be self-critical and analyse every point of view on what has been done and where we are going.

In this regard, although the majority of clinicians consider it appropriate to continue to carry out human clinical trials testing diverse approaches to stem cell therapy for cardiac repair, not everyone agrees on how large such studies should be or what clinical endpoints should be established (surrogate or hard clinical endpoints) at the present time. More surprising, is the wide variety of opinions among basic scientists, ranging from claims for a moratorium on clinical stem cell testing for cardiac repair until further preclinical data answer essential questions relative to stem cell biology[26], to suggesting the beginning of early-phase clinical trials using embryonic stem cells in patients who awaits heart transplantation, as they should be less affected by some expected adverse events[20], even though the tumorigenicity and immunogenicity of such cells with current techniques may put patients at serious risk.

The position of the European Society of Cardiology with this regard has been recently stated through a consensus document[36]. The task force encourages future clinical trials using autologous adult stem cells since the amount of data provided by animal experiments is thought to be sufficient. We subscribe to this opinion, stressing the importance of the guidance of bench scientists whenever starting new protocols. As some authors have suggested, clinical and basic research should go forward in a parallel manner[37]. In this way, modest advances through bench work supported by confirmatory results from carefully designed clinical trials – both being desirably conducted by multidisciplinary teams – will allow us to deepen the knowledge of cardiac regeneration and optimising current approaches. However, we feel that it is not yet the time to carry out large size studies as the lack of consistency among published phase II-III clinical trials, which assessed the efficacy of intracoronary bone marrow stem cell transplantation or cytokine-induced stem cell mobilisation, strongly suggests that the outcomes will not satisfy unreasonably idealistic expectations. Instead, intermediate-sized, randomised, controlled trials (double-blinded whenever possible) to establish the effects of stem cell therapy on surrogate markers should be conducted.

Nevertheless, it is essential when designing ideal future clinical trials, to take into account some lessons learned from the past. To start with, the selection of patients should be based on the severity of the disease entity. As the REPAIR-AMI trial investigators found, patients with lower ejection fraction were the most benefited from cell therapy[13]. Likewise, Janssens et al detected an increase in metabolic activity in the infarcted area after cell transplantation only in larger infarctions, as well as an improvement in wall motion index in transmural scars, even though no effect on global ejection fraction was found[15]. Thus, if the goal of the study is to induce a recovery in contractile function, patients with mild or even mild-to-moderate systolic dysfunction could not be the target any more. Similarly, patients at risk for ventricular remodelling after acute myocardial infarction, such as those with microvascular obstruction despite reperfusion therapies should be selected. Several imaging techniques have been shown to help detecting such patients[38]. However, no randomised trial in this setting, with only one exception[15], has as yet evaluated the impact of microvascular dysfunction on the results of intracoronary bone marrow transplantation. According to Janssens et al[15], this complication precluded systolic function recovery, was associated with adverse remodelling, and was not affected by the treatment assignment. Consequently, the selection of patients for future trials should rely on imaging modalities to optimise the risk-benefit ratio of any therapeutic intervention. Finally, and also regarding imaging modalities as well, the selection of proper surrogate endpoints and noninvasive imaging techniques with high reproducibility should be encouraged, as they can reduce dramatically both the number of patients required as well as the economic cost of a given clinical trial. For example, the large sample size calculated and the use of two-dimensional echocardiography in some ongoing trials are directly related[39]. Other techniques, such as magnetic resonance imaging, in addition to offering in detail morphologic and functional information, would have reduced substantially the number of patients to enter into the trial[40].

Conclusions

Stem cell therapy investigations are one of the most characteristic examples of translational research, as the purpose of their discoveries, generated from bench works, is the direct translation, into clinical applications, for the treatment of highly prevalent and severe conditions. Today, more than ever, coordination between basic and clinical researchers within multidisciplinary teams is needed. Along with basic studies designed to unravel the mechanisms of action and complex biology of stem cells when used as “living-drugs”, parallel intermediate-sized, randomised, placebo-controlled clinical trials are needed to confirm basic findings or redirect bench research according to the results, test safety issues in a real scenario, and reject or modulate future large-sized studies.

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