Re-growth of the adult heart by stem cells?

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CELL REPLACEMENT IN HEART TISSUE—OPEN QUESTIONS

The potential of the human heart to regenerate cells in tissue in adult life by local replacement of cells either by cell division or by differentiation of cardiovascular or mesenchymal progenitor cells (here referred to as stem cells) has been a subject of major controversial discussion in the last 10 years [1]. The debate and studies to understand physiological and pathological regulation of cell turnover in different parts of the heart organ is currently ongoing. Basic science is gradually evolving data on cell turnover and replacement in cardiac tissue with respect to considerable differences between the various cell types of the heart as well as to the generation and turnover of cardiac extracellular matrix components. Different kinetics of cell turnover have been discussed for endothelial cells and myofibroblasts to be at least in part derived from bone marrow stem cells and cardiomyocytes to be potentially derived from resident cardiac stem cells. First reliable human data on cell turnover and cardiomyocyte replacement has been generated by Bergmann et al. [2], observing a slow physiological replacement of cardiomyocytes throughout adult life. The source of supply of new cells either by cell division of cardiac stem cells or by cardiomyocytes is currently under debate in recently published papers. The three possible modes of replacement are (i) cell division of mononuclear cardiomyocytes [3] vs (ii) replacement by resident cardiac (progenitor) stem cells [4, 5] vs (iii) dedifferentiation and proliferation of cardiomyocytes [6]. All three proposed mechanisms remain speculative to be involved in human hearts disease. This is reflected by the divergence of the calculated rate of annual replacement of cardiomyocytes in adult humans ranging from 1 [2] to 40% [4].

The potential to stimulate cardiomyocyte growth by pericellular-based integrin stimulation discovered by Kühl et al. [7] fostered research on pathways for therapeutic use. Another interesting advance is coming from cell programming technology, raising prospects for clinical translation. Qian et al. [8] and Song et al. [9] have demonstrated the direct intracardiac programming of fibroblasts to cardiomyocytes by the use of a combination of transcription factors. Actually, this has been complemented by a paper by Eulalio et al. [10], demonstrating the induction by cardiomyocyte proliferation by specific microRNA. The generation of autologous cardiomyocytes from induced pluripotent stem cells (iPS) [11] for myocardial transplantation has been demonstrated to be another promising option for myocardial transplantation of cells or myocardial sheets and to induce cardiac recovery [12]. Thus, a number of tools for cell manipulation and site-directed application in cardiac disease have been discovered and can be developed for preclinical evaluation. Taken together, however, basic science understanding of the mechanism and control of cardiac growth, especially with respect to cardiomyocytes, is still quite limited to open the road for rapid clinical translation in the near future. The use of extracardiac or cardiac stem cells for therapeutic approaches to induce angiogenesis and cardiac growth, however, has gained some early advance in clinical translation, reaching clinical trial level since 2001 [13–15].

STEM CELLS FOR CARDIAC REPAIR

The unique feature of asymmetric cell division of stem cells causes a lifelong undifferentiated cell reservoir to allow tissue regeneration. Stem cells resident in the adult body outside of the germline system are restricted to tissue-specific differentiation (multipotency). By this, they are ideal candidates for the repair of damaged cardiovascular tissue and the replacement of increased cell loss. Experimental studies have demonstrated the positive effect of bone-marrow-derived hematopoietic, mesenchymal stem cells and cardiac stem cells on postinfarction myocardial recovery [1, 13, 14]. The mechanisms of cardiac regeneration by stem cells are not yet understood. However, most effects seem to be related not only to cell replacement but also to a major extent to paracrine effects [13]. A major mechanism of action of hematopoietic stem cells seems to be the induction of microvascular angiogenesis. Derived from this are clinical studies in cardiac surgery and interventional cardiology, ongoing employing stem cells derived from bone-marrow, fat tissue or cardiac tissue specimens [14]. However, complex cell technology, application techniques for cell retention and survival, as well as variable clinical outcomes in early clinical trials unravel a typical scenario of a complex and difficult clinical introduction [13].
CELL PRODUCTION

Good manufacturing practice (GMP)-standard is required in the European legislation for the isolation and purification of stem cell products from autologous or allogenic tissue sources to be used for specific therapeutic applications. Pharmaceutical and device-related legislation is controlled by the food and drugs administration (USA) and the European medicines agency (European union). Table 1 lists the cell types that have been employed for cardiac clinical trials so far. Non-selected mononuclear cell preparations have mainly been used for intravascular application. This represents simple ‘buffy-coat’ centrifugation from bone marrow or peripheral blood [16, 17] or cell centrifugation of adipose fat tissue aspirates [18]; both contain limited absolute numbers of stem cells. In the case of intravascular application, the recruitment of circulating stem cells to the heart is dependent on the expression of stem cell-specific endothelial homing factors like stromal cell-derived factor 1, expressed by cardiac endothelia.

Cell selection and enrichment technologies using monoclonal antibodies to stem cell-specific membrane proteins have been employed for the isolation of stem cells. On this basis, clinical isolation of cluster of differentiation (international classification of cell surface epitopes) CD34+ or CD133+ or c-kit+ stem cells has been applied to bone-marrow and peripheral blood apheresis after G-CSF induced stem cell mobilization, leading to purified cell preparations usable for direct tissue application [19]. Cultivated cell products are using cell culture expansion and modifying factors for cell selection. Clinical applications of cultivated progenitor or stem cell preparations have been satellite cells from striated muscle biopsies [20], mesenchymal stem cells from bone-marrow [21], as well as expanded cells from atrial [22] or right ventricular [23] heart tissue.

The production and purification of defined (stem) cell products is a major research and development area in regenerative medicine [24]. The award of the Nobel prize for Medicine 2012 shared by the British cell cloning researcher John B. Gurdon and the Japanese stem cell researcher Shinya Yamanaka for the development of technology for induction of cell cloning and generation of pluripotent stem cells (iPS) from differentiated cells rewards the fast progress of this field of cell programming technology [11]. The generation of specifically engineered autologous cells by this technology for therapies is a major prospect for future clinical cell therapies. The evolving technology for safe cell production may need several years before reaching clinical trials in cardiovascular indications. In contrast, the clinical application of differentiated cells derived from embryonic stem cells is still an ethical conflict. Moreover, problems with allogenic rejection and teratogenicity are strongly limiting the clinical potential of ES-cell-derived cell products [1, 13].

APPLICATION TECHNOLOGY

In the last decade, a number of interventional and surgical application techniques for cardiac cell transplantation have been developed and applied in clinical studies. A survey on clinically used intracoronary and intramyocardial application procedures, including ventricular patch, is depicted in Fig. 1 [14]. These techniques are in clinical studies and need further methodological standardization and validation [1]. Especially, techniques improving cell seeding and retention or specific targeting for tissue engineering need to be improved [25].

CLINICAL RESULTS

The majority of clinical studies using stem cells have been applied to myocardial infarction and ischaemiac heart disease. The clinical results were summarized in 2012 in a meta-analysis [15]. In this analysis, 50 clinical studies and 2662 patients were studied. Compared with control patients, bone-marrow stem cell treated patients had an improvement in left ventricular ejection fraction (LVEF) (3.96%; 95% confidence interval, 2.90–5.02; P < 0.00001), reduced infarct size (−4.03%, 95% confidence interval, −5.47 to −2.59; P < 0.00001), reduced left ventricular end-systolic volume (−8.91 ml; 95% confidence interval, −11.57 to −6.25; P < 0.00001) and reduced left ventricular end-diastolic volume (−5.23 ml; 95% confidence interval, −7.60 to −2.86; P < 0.0001) [15]. These improvements were independent of study design and type of ischaemic heart disease and showed long persistence. All-cause mortality, cardiac mortality and incidence of reinfarction were significantly reduced in the bone-marrow stem cell-treated patients when compared with control patients [15].

We started the first Phase I study in cardiac surgery in 2001, applying purified hematopoietic CD133+ stem cells to the infarction border-zone during coronary artery bypass graft (CABG)-surgery [19]. Since then, Phase II and Phase III (ongoing) studies have been installed to study safety and efficacy of the treatment. The improvement of LVEF at 6 months vs controls was 6.3% [19, 26]. With patient selection dedicated to the detection of hibernation
myocardium (by positron emission tomography - computed tomography, single-photon emission computed tomography – computer tomography or adenosin-Cardiac magnetic resonance imaging), the responder rate to stem cell therapy could be improved. A long-term follow-up [27] and a meta-analysis [28] of intramyocardial bone-marrow stem cell therapy in CABG-patients confirm the positive clinical effects and safety of this procedure in cardiac surgery.

Recent studies have used cardiac-derived stem cells, c-kit+ cells in the cardiac stem cells in patients with ischaemic cardiomyopathy (phase I clinical trial) trial [22] and CD105+ cells in the intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (phase I clinical trial) [23], observing signs of cardiac growth. Higher stem cell numbers were used in both trials as well as in recent trials using CD34+ bone marrow stem cells for cardiac delivery, showing significant improvement in non-ischaemic cardiomyopathy [29]. The impact of dose–response according to the extent of myocardial tissue treated and retention rate, may be a major issue in future developments using high-dose purified stem cell preparation.

**IMPACT FOR CARDIAC SURGERY AND INTERVENTION**

Since the first cardiac stem cell therapies in 2001 in cardiac surgery (Rostock, intramyocardial purified CD133+ bone marrow stem cells) and in cardiac intervention (Duesseldorf, intra coronary bone marrow mononuclear cells), a vast number of clinical studies have been performed worldwide. However, in these studies, a wide variety of cell preparations have been used and the number of randomized trials and trials under good clinical practice (GCP)-standard is very low. Thus, standardization and quality improvement of trial conditions is mandatory for a successful progress. So far, clinical development has reached Phase III-level in very few trials as listed in clinicaltrials.gov. Interestingly, at present, three of six currently recruiting clinical Phase III trials are studying cardiac stem cell therapy in the context of cardiac surgery (left ventricular assist device or CABG) like the first initiated Phase III trial PERFECT [30] lead by the Rostock Center (NCT00950274). Intramyocardial injection of purified stem cells (CD34, CD133, mesenchymal stem cell) is the preferred application technique in five of the six currently recruiting Phase III trials. Interestingly, intravascular stem cell therapies may shift to medical treatment with GCSF stem cell mobilization and drugs like sitagliptin to enhance cardiac homing postinfarction as currently studied in the SITAGRAMI-trial (NCT00650143). Interventional approaches at present are focusing on endocardial injection of stem cell preparations accompanied by electrophysiological mapping. Expecting the further exploitation of c-kit+ cardiac stem cells in clinical studies designed in the context of cardiac surgery, the field of stem cell-based therapies will most likely continue and enhance trial activity in specialized cardiac surgery units. The specialization of these centres requires standardization in GMP-cell preparation, employment of special myocardial tissue diagnostics, GCP-standard and specific patient management for long-term vigilance after stem cell therapy.

The development and application of therapies may be restricted to specialized centres like the national institutes of health (USA)-funded CCTRN cardiovascular cell therapy research network (USA) in the USA or the BMBF German ministry of research and education -funded Reference and Translation Center for Cardiac Stem Cell Therapy (RTC) in Germany. For further development, a close interaction of preclinical research, clinical research and multicentric clinical trials is mandatory to enable safe clinical instalment of these new (stem) cell therapies. This especially holds true for the combination of drug or gene therapies and nanotechnological cell modification technology. In Rostock, we are aiming to reach this integration in the RTC together with national and international partner institutions.

**Figure 1:** Intracoronary (A) and intramyocardial (B) cell transplantation methods in heart disease. Adopted from Strauer and Steinhoff [14].
REFERENCES