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Natural and therapeutic myocardial regeneration

Naturalna i terapeutyczna regeneracja serca

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S u m m a r y

Despite intensive therapy post-infarction heart failure remains the leading cause of mortality worldwide. Heart failure is a progressive condition involving imbalance between cardiomyocytes death and cardiomyocytes renewal in the myocardium after ischemic damage. Stem cell-based therapy is particularly attractive because it could work additively with primary coronary angioplasty and can prevent development of heart failure. Recent therapeutic approaches involve bone marrow mononuclear cell (BM MNC), skeletal myoblasts, mesenchymal stem cells (MSC) and cardiac stem cells (CSC), embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC). The beneficial effect of stem cells is not restricted to their ability to differentiate, but is more likely to their ability to secrete a multitude of factors that modulate inflammation, apoptosis, angiogenesis, scar formation and endogenous cardiac stem cells activation. This review focuses on the natural heart regeneration. In addition, it summarizes the evidence related to use of sub-populations of stem cell as a treatment for ischemic injury, and discusses possible mechanisms of action.

S t r e s z c z e n i e

Pomimo intensywnej farmakoterapii pozawałowa niewydolność serca jest jedną z głównych przyczyn zgonów na świecie. U podłoża problemu leżą zaburzenia równowagi pomiędzy szybkością wymierania kardiomiocytów a ich regeneracją w niedokrwionym sercu. Wynik badań eksperymentalnych i pierwsze próby kliniczne sugerują, że terapia komórkami macierzystymi może być skutecznym uzupełnieniem pierwotnej angioplastyki wieńcowej i może zapobiegać rozwojowi niewydolności serca. W leczeniu chorób serca podjęto próby wykorzystania jednojądrzestych komórek szpikowych (BM MNC), mioblastów mięśni poprzecznie prążkowanych, mezenchymalnych komórek macierzystych (MSC), sercowych komórek macierzystych (CSC), embrionalnych komórek macierzystych (ESC) oraz indukowanych pluripotencjalnych komórek macierzystych (iPSC). Obecnie panuje opinia, że korzystne efekty terapii komórkowej mają związek z korzystnym działaniem różnych substancji czynnych produkowanych przez komórki macierzyste a nie z ich zdolnością do tworzenia nowych kardiomiocytów. Niniejsza praca przedstawia aktualną wiedzę na temat naturalnej regeneracji miokardium. Podsumowuje możliwości wykorzystania różnych subpopulacji komórek macierzystych w terapii regeneracyjnej niedokrwionego mięśnia sercowego oraz opisuje potencjalny mechanizm ich działania.

NATURAL MYOCARDIUM REGENERATION

Until recently it was thought that human heart (like other mammals' heart) is a terminally differentiated postmitotic organ. It was believed that shortly after birth cardiomyocytes lose their ability to proliferate and from this moment their amount is constant and further growth is by increasing the size of existing cells (hypertrophy). The following observations resulted in changing this dogma:

1. Between birth and early youth cardiomyocytes number increases (hyperplasia). In newborns both heart ventricles are built from ~1 x 10^9 cardiomyocytes while in 20-year-olds their amount is several times larger – in males there are ~5.8 x 10^9 cardiomyocytes in the right ventricle and 2.0 x 10^9 cardiomyocytes in the left ventricle, in women there are ~4.5 x 10^9 and 1.4 x 10^9 respectively (1).
2. Apart from apoptosis (2) and necrosis (3) in myocardium, the total number of cardiomyocytes in an adult heart is on a relatively constant level. It means that dying cardiomyocytes are replaced by new cells which are created from specific

cardiac stem cells (2). Estimated calculations based on the ^{14}C concentration measurements in myocardium of people exposed to increased ^{14}C concentration in the air in the youth (as a result of nuclear tests in the cold war period) suggest that cardiomyocytes are renewed with gradual decrease from ~1% turning over annually at age 20 to ~0.45% at the age 75. It means that during a norma lifespan about 50% cardiomyocytes would be exchanged (4). Newer analyses taking the frequency of apoptosis in myocardium into consideration show that the turnover of cardiomyocytes in human heart is much faster and this process even accelerates with age (5). According to these estimates in male heart cardiomyocytes replacement occurs at a rate 7, 12 and 32% per year at 20, 60 and 100 years of age. In women this process is even more intense and corresponding values are 10, 14 and 40% per year, respectively. It means that cardiomyocytes are renewed 11-15 times between 20 and 100 years of age. The rest of myocardial cells such as fibroblasts and endothelial cells are also dynamically restored (6).

3. The chimerism phenomenon. In males who had female heart transplanted after some time there are cardiomyocytes, endothelial cells and smooth muscle cells with male Y chromosome in myocardium (7). The similar phenomenon is observed in women being recipients of male bone marrow, with time in their myocardium there are cells with chromosome Y (8). These results suggest recruitment of peripheral stem cells to the heart (e.g. from the bone marrow) and creating myocardial cells *de novo*.
4. Cardiac stem cells (CSC) are the cells reside in the myocardium that can differentiate into cardiomyocytes, smooth muscle cells, endothelial cells and fibroblasts. At present there is not any good marker allowing to identify them and CSC include several subpopulations: (a) cell expressing receptor c-kit, (b) cell expressing stem cell antigen 1 (Sca 1), (c) cell expressing transcription factor Isl 1, (d) cells abilities to efflux the Hoechst 33342 dye so called side-population (SP) cells and (e) cardiosphere-derived cells (9-11). In the heart CSC are stored in a special structure called niches located in the apex and atria. CSC are sensitive to substances released from damaged cardiomyocytes, inter alia hepatocyte growth factor (HGF) and vessel endothelium growth factor (VEGF). These factors promote CSC proliferation and their migration from niches to damaged myocardial areas (12). According to the theory that in tissues there are heterogeneous populations of stem cells directed to different tissues and organs, CSC may also be found in bone marrow and peripheral organs.

Research of the past years show that cardiomyocytes lost in physiological apoptosis and/or necrosis are replacement by new cells formed by asymmetric mitotic divisions of cardiac stem cells.

In pathological conditions there may be disturbances in the natural processes of heart regeneration. This may be a result of increase in apoptosis/necrosis of myocardial cells or impairment of regeneration abilities of cardiac stem cells. As a result it may lead to net loss of constrictive cells with the following remodeling, hemodynamic decompensation and heart failure. For years there has been intensive research on the possibility of increasing regenerative potential of the heart and using it in treatment for acute myocardial infarction and post-ischemic heart failure.

THERAPEUTIC STRATEGIES IN HEART REGENERATION

At present there are two treatment strategies of increasing regenerative potential of the heart. **The first is to increase the regenerative ability of myocardium by mobilization of endogenous bone marrow derived and cardiac stem cells.** Experimental studies in this field showed that application of SCF (stem cell factor) and G-CSF (granulocyte colony stimulating factor) to mice with myocardial infarction leads to mobilization of bone marrow stem cells. After 27 days the infarcted area was occupied by 15 x 10^6 new cardiomyocytes, which resulted in 40% reduction of the infarct size. There were also new microvessels and hemodynamic parameters improved (13). However, the results of the clinical research on the influence of pharmacological stem cell mobilization from bone marrow by G-CSF in patients with acute myocardial infarction are unclear (14).

The second strategy is to increase the number of progenitor cells in the myocardium by application of different types of exogenous stem cells, which could potentially repair damaged myocardium. The direction of this research was determined by pioneer works of the Anversa's group (15), which showed that the subpopulation of mononuclear marrowy stem cells injected directly in the damaged mouse heart is able to regeneration it. After 9 days from cells application about 68% of the infracted area was occupied by newly formed cardiomyocytes, there were new and there was improvement in hemodynamic parameters of the left ventricle. Using exogenous stem cells, their different subpopulations, in regenerative heart therapy is at present the most dynamic field of research.

STEM CELLS AND THEIR THERAPEUTIC POTENTIAL

Stem cells are undifferentiated cells with the ability to (a) unlimited number of divisions (proliferation) which leads to their self-renewal and (b) differentiation into specialized types of cells (fig. 1). The direction of differentiation depends on the environmental conditions and so called differentiation potential of stem cells (fig. 2) (16). In heart diseases treatment, mainly in acute myocardial infarction treatment, there were attempts of using skeletal myoblasts, bone marrow mononuclear cells, mesenchymal stem cells, cardiac stem cells and embryonic stem cells (fig. 3).

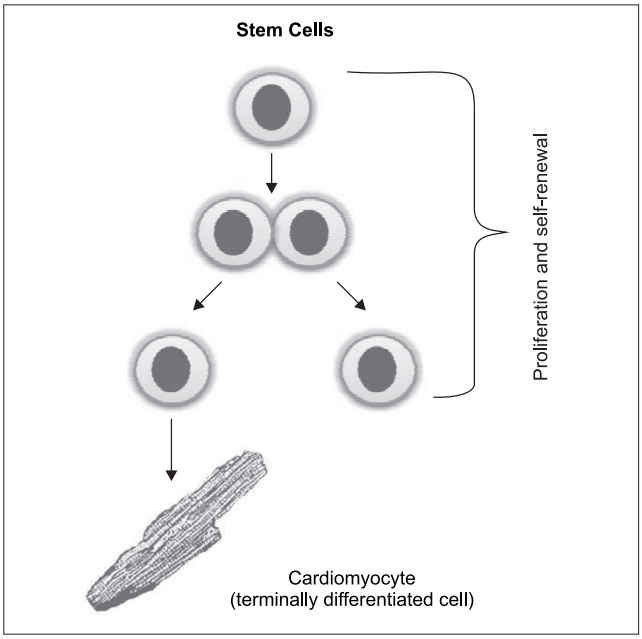

Fig. 1. Divisions and differentiation of stem cells.

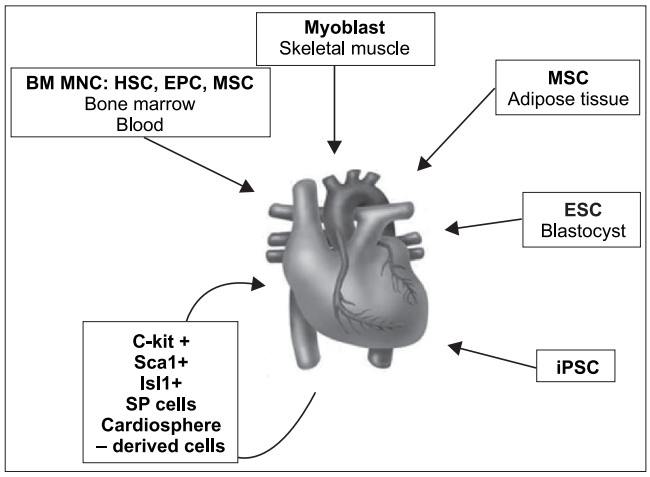

Fig. 3. Kinds of stem cells with potential therapeutic features and the sources of their extraction.
BM MNC – mononuclear cells derived bone marrow; HSC – hematopoietic stem cells; EPC – progenitor endothelial cells; MSC – mesenchymal stem cells; ESC – embryonic stem cells; iPSC – induced pluripotential stem

Skeletal myoblasts

Skeletal myoblasts (satellite cells) are stem cells taking part in physiological regeneration of skeletal muscle. Satellite cells were tested in regenerative therapy as first. Their advantage is a slight immunogenicity which makes them usable in autogenic as well as allogenic stem cell therapy. In addition they are much less sensitive to ischemia than cardiomyocytes. Experimental study showed that after application to post infarction scar myoblasts differentiate into constrictive muscle fibers. However, these newly produced myocytes do not create electrical connections with the surrounding cardiomyocytes, which is a consequence of the lack of expression of connexin 43 (gap junctions connections). Thus transplanted myoblasts are a barrier to spreading action potential in the heart muscle and may conduce ventricular arrhythmias which require additional antiarrhythmic treatment (17). Early observational clinical research without control group suggested beneficial effects of stem cell therapy with skeletal myoblasts. The only one randomized multicenter research with placebo usage was a failure and was prematurely ceased. In this research, during operation of coronary bypasses, a suspension of myoblasts was applied to the post infarction scar (ejection fraction < 35%). In six months observation there were no significant improvement in hemodynamic heart function in the treated patients (they had decreased left ventricular chamber dimension). However, the authors observed increased occurrence of arrhythmias (18). At present there are experimental works on such genetic modification of myoblasts that would enable to expression of connexin 43 (19).

Bone marrow derived mononuclear cells

Bone marrow derived mononuclear cells (BM MNC) are a mix of cells the most frequently used in the experimental as well as clinical research. Such mixture was used in the above mentioned pioneer research of

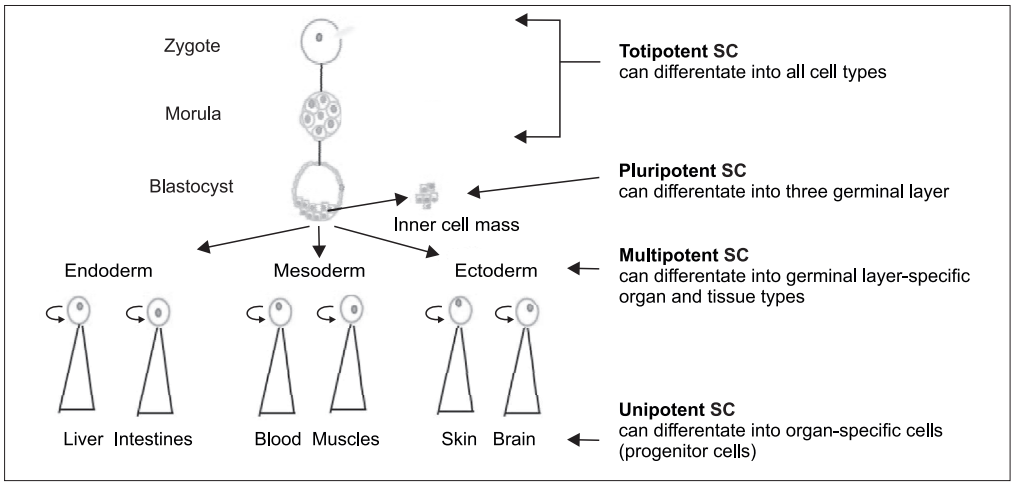

Fig. 2. The stem cells differentiation potential in the embryonic mammal development, based on Beręsewicz (16).
SC – stem cells

the Anversa's group (15). BM MNC is a heterogeneous cell population in which the majority is hematopoietic system cells on different stages of maturation. Stem cells are only 2-4% of population and they include: hematopoietic stem cells (HSC), endothelial progenitor cells (EPC) and mesenchymal stem cells (MSC). HSC are multipotential precursors of different hematopoietic cell lines and are the best known and characterized stem cells used in treatment of hematopoietic disease of a long time. HSC include also endothelial progenies (EPC). In physiological conditions EPC partly leave bone marrow and circulate in the blood stream taking part in constant replacement and regeneration of damaged endothelium (20). Populations of HSC, EPC and MSC may also be extracted from peripheral and umbilical blood.

BM MNC were applied mainly in treatment of acute myocardial infarction. The first attempts on little groups of patients reported beneficial effects of therapy; the results of newer randomized research with BM MNC are not so unambiguous and point out the moderate therapy benefits. Meta-analysis of 18 researches with 999 patients shows the increase in left ventricle ejection fraction (LVEF) by 3.6% and reduction of the infarct size by 5.5% (21, 22). While interpreting the results it should be remembered that the therapy involved patients after primary angioplasty with only a little impairment of contractility (LVEF about 50%), in whom it is difficult to expect a significant improvement of LVEF.

The results of randomized trials using BM MNC in the post-ischemic heart failure are also unclear. In the TOPCARE-CHD research in the third month after application of the cells statistically significant improvement in LVEF by 1.8% was observed (23). FOCUS-CCTRN research did not reveal any statistically significant differences between the group treated with BM MNC and a control group in a 6-month observation (24). Comparison of these researches results is, however, very difficult as their protocols were different in such significant elements as: the method of cells' isolation, the amount of cells administered, the way of administration, the moment of cells administration, analyzed end products or the length of observation.

Mesenchymal stem cells

Mesenchymal stem cells (MSC) are a little fraction (about 0.001-0.01%) of BM MNC. MSC are multipotential cells without expression of *CD34+* and *CD133* genes. They have the ability to differentiate into osteoblasts, chondrocytes and adipocytes and as some experimental research *in vivo* and *in vitro* also show, some populations of MSC may differentiate into cardiomyocytes (25, 26). From therapeutic point of view the MSC ability to produce a wide variety of cytokines, which have a potentially beneficial paracrine features is probably very important (see below). The advantage of MSC is their low immunogenicity. Beside bone marrow the easily available source of MSC is the fat tissue. MSC have successfully gone through the first phase of clinical trials serving the assessment of their safety and the technical abilities of their application (27).

Heart stem cells

Heart stem cells – the research on their application in the regenerative therapy are recent. The experimental research showed that human heart stem cells (hCSC) may rebuild the damaged by ischemia myocardium in mice and rats (animals with weakened immune response). hCSC after 5-21 days of application into myocardium differentiated into cardiomyocytes and, in a little degree, to cells building vessels and newly created cells integrated with the host cells (28). Thanks to genetic manipulations and analyzing the size of cardiomyocytes in the hearts, the authors excluded cell fusion of the applied hCSC and the cells of rodents. Cardiomyocytes differentiated from hCSC were small and measured from 100 to 2900 μm^3 if there was fusion new cardiomyocytes would be at least the size of of the rodent cardiomyocytes – 25 000 μm^3. The results of first clinical trials confirm the safety and effectiveness of CSC in treatment of post-ischemic heart failure. In SCIPIO research administration of the subpopulation of cardiac cells c-kit^{pos} to patients with post-ischemic heart failure resulted, in a year observation, in the increase in LVEF from 27.5 ± 1.6% to 41.2 ± 4.5% as well as the reduction of the infarct size by 30.2% (29). These results were confirmed in the CADUCEUS research, in which administration of cells subpopulation, creating *in vitro* cardiosphere, to the similar group of patients caused, in a year observation, reduction of the scarring area and the improvement of left ventricle contractility (30).

Embryonic stem cells (ESC)

Embryonic stem cells (ESC) are pluripotential cells collected from embryonic pole of blastocyst, which can differentiate into all types of cells building myocardium. The obstacle in using ESC is their ability to take over the pathological cell phenotype (the one we want to regenerate), transformation in teratoma, and their high immunogenicity (on their surface there are specific human leucocytes antigens – HLA) (31). The problem with using ESC is also of ethic nature.

Induced pluripotential stem cells

Induced pluripotential stem cells (iPSC) may be an alternative to ESC. Somatic cells, which as a result of genetic manipulations have morphological and functional features of stem cells, may be their source (32). In appropriate conditions iPSC may differentiate into cardiomyocytes, smooth muscles and endothelial cells. However, the effectiveness of the reprogramming process is still very low – from 0.001 to 0.1% – dependently on the kind of tissue (33). In 2012 research on iPSC were honored with Noble Prize in Medicine and Physiology. The assessment of iPSC usefulness in heart diseases treatment is not yet beyond experimental research.

THE MECHANISM OF BENEFICIAL STEM CELLS ACTION

Early experimental research suggested the ability of stem cells to differentiate into cardiomyocytes and regenerate infarcted myocardium (13, 15). Later works did not confirm these optimistic results. However, experimental as well as clinical research show that stem cells therapy results in the reduction of the infarct size (or infarct scar in patients with post-ischemic heart failure) and in improvement of hemodynamics. There is a wide spread opinion that beneficial effects of cell therapy are connected with beneficial activity of substances produced by stem cells and not with their ability to differentiation into cardiomyocytes. In reality, the media in which the cells were cultured have a similar beneficial effect as the cells themselves. Stem cells produce numerous biologically active substances which may: (a) increase the survival of cardiomyocytes during ischemia and lower their apoptosis; (b) stimulate angiogenesis, which prevents infract expansion and improves perfusion of the repaired area; (c) recruit peripheral CSC and EPC and activate resident CSC to regeneration of myocardium; (d) attenuate inflammatory responses; (e) modulate proteolytic activity and collagen synthesis in the extracellular matrix, which influences infarct scar formation and cardiac remodeling (fig. 4) (16).

Discovery of CSC makes to come back to the primary thesis of the regenerative therapy. Out of all cells used so far in the regenerative therapy CSC have the highest regenerative potential in the post-ischemic myocardium. The latest achievements in the research on stem cells show that regenerative medicine is a new promising field of cardiology. However, before we achieve full success in stem cell therapy usage there are further research required, which will allow to optimize the conditions of stem cells isolation, application and differentiation into myocardial cells.

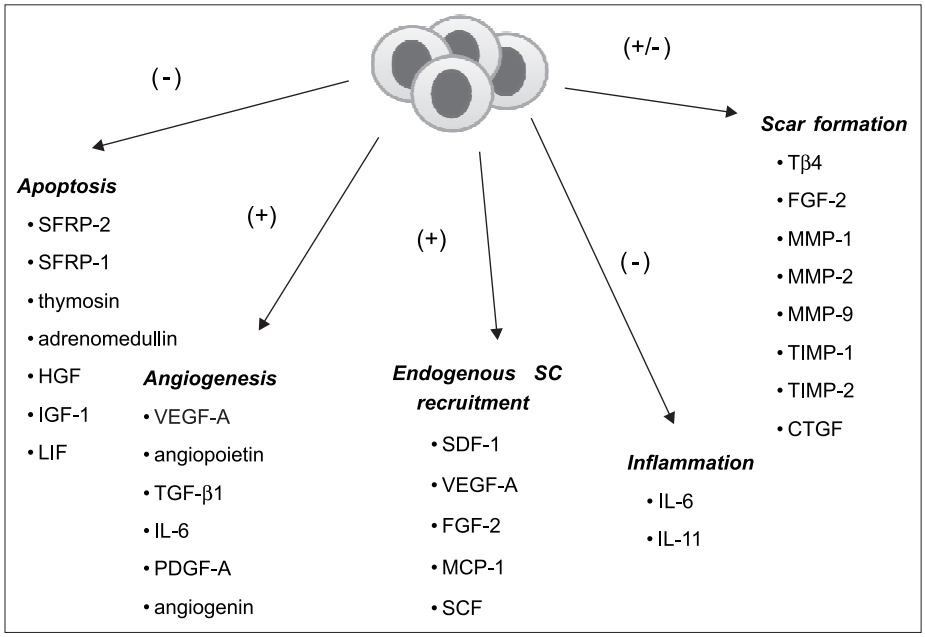

Fig. 4. Substances excreted by stem cells and their biological effect.
SFRP – secreted frizzled-related protein; HGF – hepatocyte growth factor; IGF – insulin-like grown factor; LIF – leukemia inhibitor factor; VEGF – vascular endothelial grown factor; IL – interleukin; TGF – transforming growth factor; PDGF – platelet-derived growth factor; SDF – stromal cell-derived factor 1; FGF – fibroblast growth factor; MCP – monocyte chemoattractant protein; SCF – stem cell factor; MMP – metalloproteinases; TIMP – tissue metalloproteinase inhibitors; CTGF – connective tissue growth factor

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