Benefits of Stem Cell Therapy for the Heart

Summary

A heart attack occurs when the blood supply to part of the heart is suddenly interrupted, either because of a blood clot or from a piece of ruptured atherosclerotic plaque. The resulting deprivation in oxygen can lead to the death of heart muscle tissue. Heart attack is generally considered an irreversible injury. Heart diseases are the leading causes of mortality in developed countries and the World Health Organization estimated in 2002 that roughly 12.6 % of deaths worldwide were from ischemic heart disease.

Although the heart has been traditionally seen as having, like the brain, little regenerative capacity after birth, recent studies have clearly shown that the heart can regenerate substantially, largely based on BMSC migrating to the heart and becoming heart cells. In individuals at risk for cardiovascular diseases, studies have shown that people with more stem cells circulating in their blood have a lower incidence of cardiovascular problems. In other words, higher stem cell circulation prevents cardiovascular problems or helps maintain cardiovascular health. Increasing the number of circulating, stem cells by supporting the release of stem cells from the bone marrow can, improve recovery from heart attack.

Acute myocardial infarction (AMI), more commonly known as heart attack, occurs when the blood supply to part of the heart is suddenly interrupted. Most commonly, this is caused by a blood clot or by a piece of ruptured atherosclerotic plaque - a cluster of lipids (like cholesterol) and white blood cells (especially macrophages) in the wall of an artery - which lodges itself at the entrance of a network of blood vessels. The resulting ischemia (restriction in blood supply and consequent oxygen deprivation) can then lead to the death (infarction) of heart muscle tissue (myocardium).

Myocardial infarction is generally considered an irreversible injury. The cardiac muscle has high metabolic needs and is thus very vulnerable to lack of blood flow, which deprives the heart of both oxygen and nutrients. Irreversible injury to the cardiac muscle begins after 15 minutes without blood supply and the extent of an infarction is related to the duration and severity of the blood flow reduction. Following the death of a portion of the cardiac muscle, scarring and remodeling of the cardiac tissue can further reduce cardiac function in the weeks

to months after the initial event. So far, no medication or clinic procedure has proven effective in repairing myocardial scar tissue and restoring contractility of the heart.

Heart diseases are the leading causes of mortality in developed countries. In the United States, for example, diseases of the heart are responsible for more deaths than cancer. Although much has been done to prevent heart disease (campaigns promoting stress reduction and management, smoking cessation, weight loss, increased consumption of vegetables and reduced meat consumption, physical exercise, etc.), nearly 1.2 million heart attacks occur every year, and little treatment is offered to the 65% of people who survive. Survivors of heart attacks are often left with a reduced quality of life and little prospect for improvement.

Many recent studies have challenged the view that nothing can be done to improve cardiac health post-AMI. Evidence clearly suggests that there is a low level of constant regeneration of the cardiac cells, most likely coming from the migration of circulating stem cells into the cardiac tissue.[1-3] For example, analysis of cells undergoing cellular division in the human heart revealed that proliferating heart cells can account for about 14 cells per million in the normal heart, for a total of an estimated 80,000 cells undergoing cellular division at any given time. On the other hand, there is an estimated progressive loss of approximately 7 million heart cells per year. So the normal process of ongoing regeneration appears sufficient to maintain normal function. However, while the number of dividing cells can increase by up to 10-fold in chronic heart disease, this level of proliferation still seems insufficient to rescue the cardiac muscle after a heart attack.[4]

Recent advances in stem cell research offer, for the first time, a tangible hope for people recovering from heart attack and even for the prevention of heart disease. It is now well established: that BMSC have the ability to migrate to the infarcted area of the heart and differentiate into functional heart cells.

For example, in one study GFP-positive BMSC from male] mice were injected into the ventricular wall of female mice, adjacent to the infarcted area, 3-5 hours after triggering AMI. Nine days later, microscopic analysis revealed the presence bands of GFP- and Y chromosome-positive donor-derived heart!; cells within the damaged cardiac muscle. The new band or heart muscle occupied 68% of the infarcted area and extended across the entire region of infarcted tissue.[5-7]

These new heart, cells showed many characteristics of functional cardiac muscle' cells, such as the presence of connexin 43, a protein commonly found in heart cells. Using bromo-d-uridine (BrdU), a marker of cellular proliferation, it was observed that the new developing cardiac cells were actively dividing. Furthermore, many endothelial cells and smooth muscle cells forming new blood vessels in the regenerated cardiac muscle were positive for GFP and the Y chromosome, indicating that they originated from the injected stem cells. Therefore, injected BMSC strongly contributed to the regeneration and development of both cardiac muscle anal new blood vessels.

In the past, it was believed that new blood vessels in the heart were formed following the migration and proliferation of neighboring mature endothelial cells. However, as show in many recent studies, the formation of new blood vessel involves the migration of circulating endothelial progenitor cells (EPC) - a type of stem cell - that home to the injured site and participate in its repair.[8]

These results prompted a number of studies investigating whether injections of BMSC into the human infarcted heart could bring about significant regeneration of the cardiac muscle. In one study, after standard treatment for AMI, 10 patients were transplanted with BMSC via a balloon catheter placed into the artery supplying blood flow to the infarcted area. These patients were matched and compared with 10 patients who received only standard treatment.[4] After 3 months, the size of the infarcted region in the group treated with BMSC had decreased dramatically and was significantly smaller than the non-treated group. In the group treated with BMSC, the force of contraction of the infarcted cardiac muscle and the velocity of the ventricular wall had increased markedly. Further investigation showed a significant improvement in stroke volume, left ventricular end-systolic volume, and, as in the mouse study by Orlic et al.,[6] an increased blood supply to the infarcted region.[9] So far, injection of BMSC directly into the vicinity of the infarcted region seems to improve a patient's condition only when injections take place soon after a heart attack, not when attempting to treat an old myocardial infarction.[10]

But as with other organs and cell types, BMSC have been shown in humans to naturally migrate from the bone marrow to the heart and become functional heart cells. Using a sex-mismatched paradigm in which a male patient received a heart transplant from a female donor, it was observed that up to 10% of the cardiomyocytes and blood vessels were positive for the Y-chromosome within days

of the transplant, indicating that, they originated from the man's own bone marrow.[11]

As with conditions of the central nervous system and' diabetes, it appears that the migration of BMSC to the heart is a natural process that contributes to the regeneration of the cardiac muscle after a heart attack. Therefore, it is possible that simply supporting the release of BMSC could constitute; an effective way of tapping into the regenerative power of stem cells and improve the outcome of AMI.

When tested in mice, this strategy proved extremely promising. It is important to mention that despite the significant improvement in cardiac functions obtained in animal studies after injection of stem cells directly into the cardiac muscles, closer analysis revealed that the newly formed cardiac cells and blood vessels had often failed to fully integrate structurally with the remaining viable portion of the cardiac tissue. In other words, the regenerated tissue formed a secondary structure which, though part of the heart, was not functioning in complete harmony with the rest of the heart. Therefore, although positive, the! results of injecting stem cells directly into the heart remained unsatisfactory. However, in the course of these investigations, two elements emerged as essential for the regeneration of the cardiac tissue with stem cells: the presence of tissue damage and a high level of circulating stem cells. On this basis, research was undertaken to test whether BMSC mobilized by stem ce1t factor (SCF) and G-CSF could home to the infarcted region on' their own, proliferate, differentiate, and ultimately promote cardiac repair after a heart attack.[5,6]

As described before, injection of the cytokines SCF and G-CSF during 8 days after inducing a heart attack dramatically increased the number of circulating stem cells, which led to the migration of BMSC into the cardiac tissue. Twenty-seven days after the heart attack, a band of newly formed cardiac tissue occupied more than 75% of the infarcted region of the ventricle. By comparison, the ventricular wall in control animals was filled with scar tissue covering the entire area of the infarct.

Aside from the actual regeneration of the cardiac tissue, increasing the number of circulating stem cells also led to the development of new blood vessels supplying the infarcted region of the ventricle. The blood vessels were surrounded by smooth muscles and microscopic observations revealed the presence of red blood cells, indicating that the newly formed arterioles had integrated structurally with the remaining functional vasculature. No new blood vessels were seen in the

untreated group.

In all, while only 17% (9 out of 52) of the untreated animals survived the heart attack, showing severe signs of cardiomyopathy and compromised blood circulation, 73% (11 out of 15) of the animals treated with G-CSF survived with quasi-normalized cardiac function and restored blood circulation. After 27 days, ejection fraction was 114% greater in the treated group than the untreated group. Likewise, end-diastolic pressure, systolic pressure, and other parameters of cardiac health were all improved in treated versus non-treated mice. The simple support of BMSC mobilization therefore appears to be an effective, non-invasive and safe approach in the treatment of AMI.

When applied to humans, however, this approach did not, lead to the same success. While some groups did report very; promising results,[12,13] others reported no effect at all.[14-16] Al comprehensive review of the various studies reveals that each study used slightly different protocols with regard to the time) of treatment after AMI (from hours to 3 months), as well at the intensity and duration of the treatment, suggesting that the treatment can indeed be successful once the most effective treatment protocol has been developed[17]

For example, Wojakowski et al. reported in a study with 43 , cardiac patients that if the patients were treated with G-CSP1 early after AMI (<12 hours), the number of circulating stem cells following the BMSC mobilization correlated with the extent of cardiac repair.[18] In other words, the greatest improvements in ejection fraction were seen in patients having the largest number of circulating stem cells, if mobilization took place soon after the heart attack.

In another study, BMSC mobilization was shown to not only improve ejection fraction but also to prevent left ventricular remodeling. Left ventricular remodeling is a term used to describe the structural changes caused by the formation of scar tissue in the ventricular wall as a consequence of AMI. Scars formed in the cardiac muscle can limit mobility, similar to scars on the; skin. Left ventricular remodeling reduces the heart's ability, to contract optimally, leading to significantly reduced cardiac functions. It is believed that the extent of scarring in a tissue is inversely linked to the number of stem cells available for healing. In the presence of abundant stem cells, a tissue would heal faster and more adequately, limiting the formation of scar tissue. But when stem cells are deficient, the tissue would heal through the formation of scar tissue. In one study, within 5 days after a severe heart attack, Leone et al. injected G-CSF in 41 patients at high risk of

unfavorable left ventricular remodeling. Five months after G-CSF treatment, ejection fraction had improved 12.5% compared to no improvement in the control group.[19] The improvements in cardiac function were linked to the prevention of left ventricular remodeling.

A meta-analysis reviewing the effectiveness of BMSC mobilization for the treatment of AMI included 7 studies and a total of 364 patients. The analysis concluded that treatment with G-CSF can improve the left ventricular ejection fraction, if the treatment is administered early after the heart attack.[20] However, in spite of the improvements in ejection fraction, other general parameters of cardiovascular health such as ventricular arrhythmia, re-hospitalization for heart failure, and the composite of other cardiovascular events (i.e., death from heart attack, recurrent heart attack, and stroke), were not significantly different in the G-CSF treatment groups compared with the control groups. Similar results were reported by another meta-analysis that included eight studies and 385 patients.[17]

Thus, the extent with which the simple mobilization of BMSC can constitute an effective treatment for AMI remains unclear. It would be improper scientific protocol to deny the positive results obtained in some studies on the basis of the' negative results obtained in others. Reconciliation of all the' data and the development of an effective treatment will most likely come through the determination of the optimal treatment parameters: 1) intensity of stem cell release, 2) duration of the. treatment, 3) time after AMI, 4) number of treatments received over time, and 5) other yet to identified parameters. Compounds other than G-CSF might also be discovered that could provide more consistent results or could be safely used for more than 5-6 days, providing results comparable to those obtained by Orlic's group in mice.

Already other compounds have been discovered that trigger BMSC mobilization with resulting benefits to cardiovascular functions. For example, AMD3100, one of the most potent and selective CXCR4 antagonists, triggers stem cell mobilization by interfering with the binding of SDF-1 with its receptor CXCR4.[21-24] Not only was AMD3100 shown to mobilize BMSC into the blood. stream, it was also proven to increase migration of BMSC into sites of blood vessels formation after heart attack.[25,26]

The benefits obtained from statin drugs on overall cardiovascular health have also been linked to statin's ability DS support the release of stem cells from the bone marrow. In one study involving 15 patients, daily treatment with

atorvastatin led to an increase in the number of circulating stem cells - 30% after 1 week and 300% after 4 weeks. Furthermore, atorvastatin also increased the migratory capacity of isolated stem cells.[27,28]

Therefore, aside from their effect on cholesterol, statin drugs appear beneficial by increasing both the number of circulating stem cells and their migrating ability. These results were confirmed in animal studies.[29,30]

The problem with statin drugs, however, is the growing concern linked to their side effects. Two of the main side effects are muscle pain and muscle weakness. While muscle pain and/or weakness may seem a normal experience for older people (the age group for whom statin drugs are commonly prescribed), any experience of muscle pain after ingesting such medication must be taken seriously. Statin drugs can lead to the breakdown of muscle tissue, which produces waste products that can then overload the kidneys, causing more serious problems that can even be fatal. Because of the seriousness of this situation, it is now mandatory to put the following statement on statin drug bottles: "Unexplained muscle pain and weakness could be a sign of a rare but serious side effect and should be reported to your doctor right away."

Statin drugs have also been reported to affect cognitive abilities. Many consumers have reported memory loss, a reduced ability to concentrate, and the feeling that they are developing Alzheimer's disease. The memory loss may be so extreme as to be qualified as amnesia lasting up to 12 hours. Other people have reported mood swings and other behavioral changes. Although statin drugs could potentially bring overall benefits through their ability to support stem cell release from the bone marrow, much like G-CSF they carry significant health risks.

Another BMSC mobilizer, this time natural, is an extract from the plant Aphanizomenon flos-aquae (AFA) which was recently shown to contain an L-selectin blocker. Consumption of one gram of this extract was shown to trigger BMSC mobilization, increasing the number of circulating stem cells by an average of 25-30% (up to 1,600 %). Given the safety of its daily use for extended periods of time, this natural mobilizer offers another paradigm in the use of one's own stem cells for the treatment of AMI. The argument can be made that the relatively small benefits obtained by treatments with G-CSF are linked to the fact that G-CSF triggers a massive release of stem cells (2,000% increase in the number of circulating SC), but only for a few days. The large number of newly released stem cells could easily saturate the SC migration machinery at the level of the post-

capillary venules, leading to relatively little actual migration with significant re-homing of SC to the bone marrow. Therefore, such treatment depends on the chance that the relatively small number of BMSC reaching the tissue will be able to proliferate and form enough new tissue to significantly improve organ function. On this basis, mild daily mobilization (such as that obtained with AFA extract) could be more effective, providing a daily supply of BMSC to the recovering tissue. Preliminary studies with AFA extract support this view.

Indeed, a naturally higher number of circulating stem cells (no induced mobilization) has been linked to greater cardio vascular health. When the number of circulating stem cells wee determined in patients suffering from coronary artery disease (CAD), it was observed that CAD patients have approximately 48% fewer stem cells in their blood compared to age-matched healthy volunteers. Furthermore, when tested in a migration chamber, stem cells isolated from CAD patients showed a reduced ability to migrate compared with stem cells isolated from healthy age-matched volunteers. Overall, cardiovascular risk factors were significantly correlated with lower levels of circulating stem cells as well as reduced migratory capability. In other words, fewer SC were available in the blood of CAD patients to migrate and perform repair, and the available stem cells had a reduced ability to migrate to the heart tissue.

Interestingly, one of the strongest correlations observed was the link between smoking and the number of circulating stem cells: smoking is associated with significantly fewer stem cells in the blood. A lower level of stem cells was also linked to hypertension and diabetes.

These observations were confirmed in another study where the level of circulating stem cells was correlated with cardiovascular risks. In brief, the number of stem cells was determined using flow cytometry in 519 CAD patients. After 12 months, the investigators evaluated the association between baseline levels of stem cells and cardiovascular events (first major cardiovascular event, heart attack, hospitalization, revascularization, or death from cardiovascular causes). After adjustment for age, sex, vascular risk factors, and other relevant variables, higher levels of circulating stem cells were associated with reduced risks of death from cardiovascular causes, a first major cardiovascular event, revascularization, and hospitalization.[33]

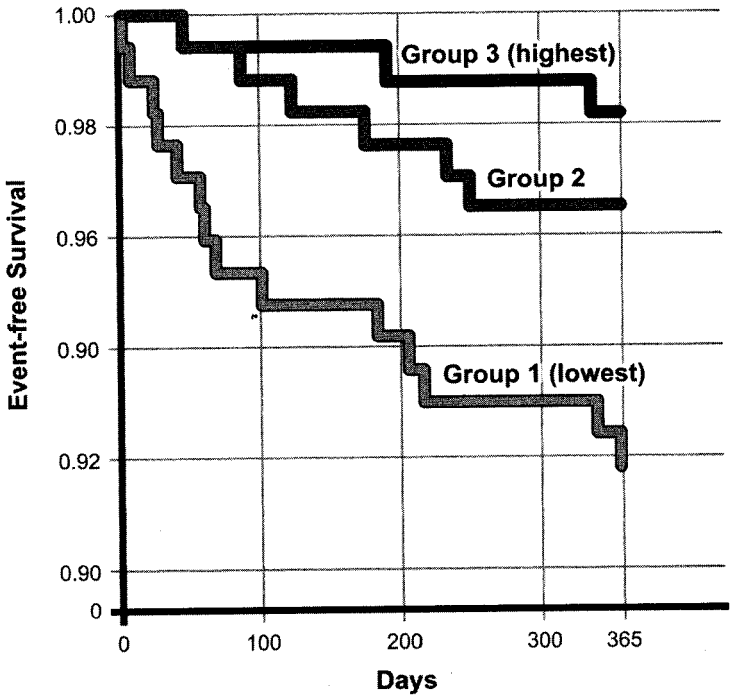

Figure 3. Incidence of cardiovascular problems in three groups of individuals at risk for cardiovascular disease, grouped according to the number of stem cells circulating naturally in their blood. The group with the highest number of stem cells showed the least number of cardiovascular problems, and conversely the group with the smallest number of stem cells showed the greatest number of cardiovascular problems.

Two conclusions can be drawn from these studies. First, the level of circulating stem cells can help evaluate the overall cardiovascular health and predicts the occurrence of cardio vascular events and death from cardiovascular causes, and may help to identify patients at risk for cardiovascular problems. Second, increasing the number of circulating stem cells can prevent cardiovascular events by supporting the constant regeneration of blood vessels and cardiac tissue.

As discussed earlier, lack of oxygen in a tissue triggers the release of SDF-1 and VEGF, which leads to the formation of capillaries. As they migrate into the cardiac tissue, stem cells participate in the formation of new blood vessels that restore proper blood flow. In addition, as seen in the brain and other tissues, migration of BMSC to the heart also supports the proliferation of local cardiac stem cells.[34] Therefore, a higher number of stem cells in the bloodstream helps maintain optimal cardiovascular health by: 1) providing more stem cells available for migration and differentiation into functional cardiac cells, 2) providing more stem cells for the development of new blood vessels, and 3) providing stem cells which will migrate into the heart and promote the proliferation of local cardiac stem cells.

Therefore, mild mobilizers that can be used daily, such as AFA extract or even statin drugs, could constitute one of the safest, easiest and most effective approaches in the prevention and even treatment of CAD and other cardiovascular problems.

References

1. Kajstura I, Leh A, Finato N, et al. (1998) Myocyte proliferation in end-stage cardiac failure in humans. *Proc. Natl. Acad. Sci. USA* 95:8801-8805.
2. Soonpaa MH and Field LJ. (1998) Survey of studies examining mammalian
3. cardiomyocyte DNA synthesis. *Am. Heart Assoc.* 83:15-26.
4. Beltrami AP, Urbanek K, Kajstura J, et al. (2001) Evidence that human cardiac myocytes divide after myocardial infarction. *N. Engl. J Med,* 344:1750-1757.
5. Schwartz Y and Kornowski R. (2003) Autologous stem cells for functional myocardial repair. *Heart Fail. Rev.* 8(3):237-45.
6. Orlic, D, Kajstura, J, Chimenti, S, et al. (2001) Bone marrow cells regenerate infracted myocardium. *Nature* (London) 410, 701-705.
7. Orlic D, Kajstura J, Chimenti S, et al. (2001) Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc. Natl. Acad. Sci. USA.* 98(18):10344-9.
8. Orlic D, Kajstura J, Chimenti S, et al. (2003) Bone marrow stem cells *regenerate* infarcted myocardium. *Pediatr. Transplant.* 7 Suppl. 3:86-8.
9. Kan I, Melamed E, and Offen D. (2005) Integral Therapeutic Potential of Bone Marrow Mesenchymal Stem Cells. *Current Drug Targets* 6: 31-41.
10. Strauer BE, Brehm M, Zeus T, et al. (2002) Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 106: 1913-1918.
11. Kang HJ, Lee HY, Na SH, et al. (2006) Differential effect of intracoronary infusion of mobilized peripheral blood stem cells by granulocyte colony-stimulating factor on left ventricular function and remodeling in patients with acute myocardial infarction versus old myocardial infarction: the MAGIC Cell-3-DES randomized, controlled trial. *Circulation* 114(1 Suppl): 1145-51.
12. Quaini F, Urbanek K, Beltrami AP, et al. (2002) Chimerism of the transplanted heart. *N. Engl. J Med.* 346(1):5-15.
13. Ince H, Petzsch M, Kleine HD, et al. (2005) Prevention of left ventricular remodeling with granulocyte colony-stimulating factor after acute myocardial infarction. Final 1-year results of the Front-Integrated Revascularization and Stem Cell Liberation in Evolving Acute Myocardial Infarction *by* Granulocyte Colony-Stimulating Factor (FIRSTLINE-AMI) trial. *Circulation,* 112 [suppl 11:I-73-I-80.
14. Seiler C, Pohl T, Wustmann K, et al. (2001) Promotion of collateral growth by granulocyte-macrophage colony-stimulating factor in patients with coronary artery disease: a randomized, double-blind, placebo-controlled study. *Circulation.* 104:2012-2017.
15. Ellis SG, Penn MS, Bolwell B, et al. (2006) Granulocyte colony stimulating
16. factor in patients with large acute myocardial infarction: results of a pilot dose-escalation randomized trial. *Am. Heart J.* 152(6):1051.e9-14.
17. Zohlnhöfer D, Dibra A, Koppara T, et al. (2008) Stem cell mobilization by granulocyte colony-stimulating factor for myocardial recovery after acute myocardial infarction: A meta-analysis. *JACC* 51: 1429-37.
18. Ripa RS and Kastrup J. (2008) G-CSF therapy with mobilization of bone marrow stem cells for myocardial recovery after acute myocardial infarction - a relevant treatment? *Exp. Hematol.* 36(6):681-6.
19. Abdel-Latif A, Bolli R, Zuba-Surma EK, et al. (2008) Granulocyte colony-stimulating factor therapy for cardiac repair after acute myocardial infarction: a systematic review and meta-analysis of randomized controlled trials. *Am. Heart J.* 156(2):216-226.e9.
20. Wojakowski W, Tendera M, Zebzda A, et al. (2006) Mobilization of CD34(+), CD117(+), CXCR4(+), c-met(+) stem cells is correlated with left ventricular ejection fraction and plasma NT-proBNP levels in patients with acute myocardial infarction. *Eur. Heart J.* 27(3):283-9.
21. Leone AM, Galiuto L, Garramone B, et al. (2007) Usefulness of granulocyte colony-stimulating factor in patients

with a large anterior wall acute myocardial infarction to prevent left ventricular remodeling (the Rigenera study). *Am J Cardiol.* 100:397- 403.
22. Kang S, Yang Y, Li CJ, et al. (2007) Effectiveness and tolerability of administration of granulocyte colony-stimulating factor on left ventricular function in patients with myocardial infarction: a meta-analysis of randomized controlled trials. *Clin. Ther.* 29(11): 2406-18.
23. Broxmeyer HE, Orschell CM, Clapp DW, et al. (2005) Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp. Med.* 201(8):1307-18.
24. Larochelle A, Krouse A, Metzger M, et al. (2006) AMD3100 mobilizes hematopoietic stem cells with long-term repopulating capacity in nonhuman primates. *Blood* 107(9):3772-8.
25. Cashen AF, Nervi B, and DiPersio J. (2007) AMD3100: CXCR4 antagonist and rapid stem cell-mobilizing agent. *Future Oncol.* 3(1):19-27.
26. Fricker SP, Anastassov V, Cox J, et al. (2006) Characterization of the molecular pharmacology of AMD3100: a specific antagonist of the G-protein coupled chemokine receptor, CXCR4. *Biochem. Pharmacol.* 72(5):588-96.
27. De Clercq E. (2005) Potential clinical applications of the CXCR4 antagonist bicyclam AMD3100. *Mini Rev. Med. Chem.* 5(9):805-24.
28. Shepherd RM, Capoccia BJ, Devine SM, et al. (2006) Angiogenic cells can be rapidly mobilized and efficiently harvested from the blood following treatment with AMD3100. *Blood* 108(12):3662-7.
29. Vasa M, Fichtlscherer S, Adler K, et al. (2001) Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. *Circulation* 103(24):2885-90.
30. Walter DH, Dimmeler S, and Zeiher AM. (2004) Effects of statins on endothelium and endothelial progenitor cell recruitment. *Semin. Vasc. Med.* 4(4):385-93.
31. Landmesser U, Engberding N, Bahlmann FH, et al. (2004) Statin-induced improvement of endothelial progenitor cell mobilization, myocardial neovascularization, left ventricular function, and survival after experimental myocardial infarction requires endothelial nitric oxide synthase. *Circulation* 110(14):1933-9.
32. Walter DH, Rittig K, Bahlmann FH, et al. (2002) Statin therapy accelerates reendothelialization: a novel effect involving mobilization and incorporation of bone marrow-derived endothelial progenitor cells. *Circulation* 105(25):3017-24.
33. Kumar AJ, Wong SK, and Andrew G. (2008) Statin-induced muscular symptoms: a report of 3 cases. *Acta Orthop. Belg.* 74(4):569-72.
34. Vasa M, Fichtlscherer S, Aicher A, et al. (2001b) Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ. Res.* 89:e1-e7.
35. Werner N, Kosiol S, Schiegl T, et al. (2005) Circulating endothelial progenitor cells and cardiovascular outcomes. *N Engl. J Med.* 353(10):999-1007.
36. Klocke R, Kuhlmann MT, Scobioala S, et al. (2008) Granulocyte colony-stimulating factor (G-CSF) for cardio- and cerebrovascular regenerative applications. *Curr. Med. Chem.* 15(10):968-77.