CHAPTER I

INTRODUCTION

1.1 Statement and significance of the problem

Ischemic heart disease is a major health problem in most countries around the world.[1] Pharmacological interventions in ischemic heart disease is one of several means that has been under extensive investigation with an attempt to decrease myocardial damage, infarct size, cardiac dysfunction and fatal arrhythmia. Ischemic heart disease is known to cause an increase in reactive oxygen species (ROS)[2] and mitochondria is the principal source of ROS production. Cardiac ischemia has been demonstrated to cause mitochondrial dysfunction, leading to increased oxidative stress.[3] In the cardiac cell, oxidative stress is one of the several causes of cell death. Oxidative stress is caused by an imbalance between the production of reactive oxygen species and a biological system's ability to defend cell damage. This defense mechanism is preserved by enzymes that decrease the produced ROS in mitochondria. Disturbances in normal oxidative phosphorylation of electron transport chain can cause toxic effects through the production of ROS that damage all components of the cells. Although most of the O_2 is reduced to water through the process of mitochondrial electron transport chain (ETC). Under the normal conditions, 1-2% of electron flowing within ETC can leak out and react with O_2 to form O_2^-.[4]

Growing body of evidence has demonstrated the roles of mitochondria in the heart during ischemic condition. It is likely that the characteristics of myocardial

ischemia have been attributed to the consequence of mitochondrial disturbance in the heart.[5] During ischemia, it has been shown that the energy production is decreased due to reduced oxidative phosphorylation rate at the inner mitochondrial membrane, and this event also rapidly induces ROS production.[6] These events lead to mitochondrial membrane potential change ($\Delta\Psi m$) and cell death, respectively.[7] Recently, the mitochondrial permeability transition pore (mPTP) is proposed to have an important role in the processes of cell apoptosis and necrosis.[8] The mitochondrial permeability transition (MPT) is caused by the opening of mPTP of cells (trigger by oxidative stress, growth factor removal, or exposures to cytokines), which determines not only whether cells live or die, but also whether cell death occurs by apoptosis or necrosis.[9, 10] Several pieces of evidence suggested that the burst of ROS production resulted in the inhibition of oxidative phosphorylation [11] and the increase of permeability of the inner mitochondrial membrane through the opening of mPTP.[12] Such events are responsible for the uncoupling of oxidative phosphorylation, mitochondrial membrane potential collapse and mitochondrial swelling, leading to the release of cytochrome *c*, activation of caspase pathways and cell death by necrosis and/or apoptosis.[13] Although the exact molecular composition of mPTP has not been definitively established, proteins such as hexokinase, mitochondrial benzodiazepine receptor (mBZR) and cyclophilin D have been suggested to be implicated in mPTP formation or regulation.[14-16] From these deleterious effects of oxidative stress, investigators have been attempting to investigate *novel* drugs for oxidative stress treatment.

Granulocyte-colony-stimulating factor (G-CSF) has recently been shown to have various beneficial effects in promoting the proliferation and differentiation of

myeloid progenitor cells, including the mobilization of bone marrow stem cells.[17, 18] In addition, many studies revealed that G-CSF can improve cardiac function, increase blood vessel as well as reduce mortality after cardiac injury under several conditions such as ischemic heart.[19-21] Despite these finding, the roles of G-CSF on cardiac mitochondria under oxidative stress is unclear. In the present study, we tested the hypothesis that G-CSF can (1) protect mitochondrial swelling, (2) decrease the change of mitochondrial membrane potential and (3) reduce ROS production under hydrogen peroxide-induced oxidative stress condition in isolated cardiac mitochondria. The information obtained from this study should provide novel knowledge for the research community in the field to better devise the strategies to intervene/attenuate the worsening cascades during ischemia and to improve the therapeutic approach by pharmacological interventions in ischemic hearts in the near future.

1.2 Literature review

Acute myocardial infarction (AMI) is one of the major causes of death in most countries around the world.[1] When a coronary artery is occluded by thrombotic blood clot or lipid accumulation, acute myocardial ischemia occurs. If the occlusion continues for more than approximately 20-40 minutes, the ischemic heart tissue was irreversibly damaged and resulted in the infarction. However, the definite time for the development of infarction varies between species and gender.[22] The only way to stop the progressing irreversible damage during myocardial ischemia is to remove the occlusion and allow tissue reperfusion as soon as possible.[23] However,

reperfusion itself can cause severe and also irreversible damage to the myocardium similar to that found during the ischemic phase.

1.2.1 The structure of granulocyte colony-stimulating factor

G-CSF is a hematopoietic cytokine that has been used in patients receiving chemotherapy to increase the number of neutrophil. In addition, it regulates the proliferation of hematopoietic stem cells (HSCs). Evidences suggested that non-hematopoietic cell types (i.e. heart, platelet, endothelial cell, neuronal cell) are also differentiated from stem cell.[24-27] Therefore, hematopoietic (HSCs) and non-hematopoietic stem cell (non-HSCs) can be differentiated into several tissues. Several studies have suggested that G-CSF acts as cardioprotection, which markedly improves cardiac function after MI condition.[28, 29] G-CSF, produced by endothelium and macrophages, is a 19.6-kDa glycoprotein consisting of 174 amino acid residues.[30] The receptor of G-CSF, which was recently discovered on cardiomyocyte [20, 31], have been predicted to share some common structures with that of granulocyte-macrophage colony stimulating factor (GM-CSF) [32] and growth hormone (GH) [33]. The binding of G-CSF to its receptor activates intracellular signaling pathways and induces several effects. The mature GCSF receptor (GCSF-R) is composed of an extracellular region, which consists of an Ig-like domain, a cytokine receptor homologous (CRH) domain, three fibronectin type III-like domains, a transmembrane region, and a cytoplasmic domain (Figure 1-1).[34] Binding of GCSF to the extracellular Ig-like and the CRH domain of its receptor triggers the activation of G-CSF effect. The binding of complex 2:2 crystal structure is formed by means of cross-over interactions between the hGCSF and the Ig–CRH domains of

GCSF-R.[35] In contrast, Haniu *et al*. found single binding between G-CSF and a GCSF receptor.[36] Cardioprotective effects on physiological and pathological conditions are made possible, owing to the location of G-CSF receptor in the heart. Evidence has been suggested that the expression of G-CSF receptor is augmented not only in post-MI hearts but also in G-CSF effect.[37] Apart from the effect on proliferation and differentiation of HSCs into cardiomyocyte in infarcted area, the presence of G-CSF receptor on cardiac cell reveals its direct effect in repairing cardiomyocyte in MI condition. Therefore, G-CSF has been proposed as the *novel* drug for MI treatment.

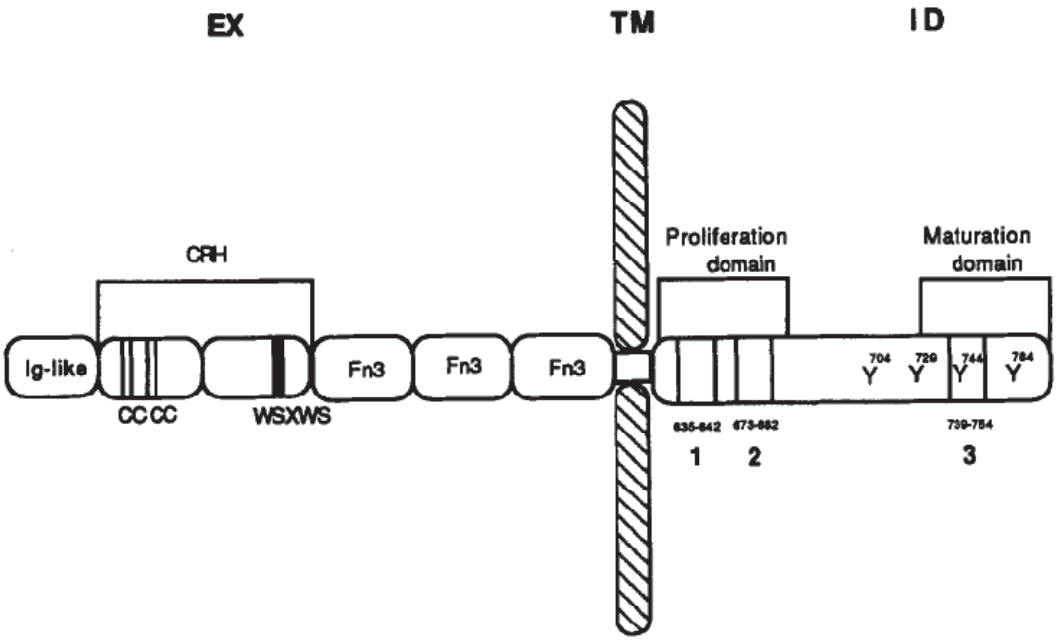

Figure 1-1

The structure of granulocyte colony-stimulating factor

1.2.2 G-CSF-induced bone marrow mobilization

G-CSF is a cytokine, which stimulates the survival, proliferation, differentiation and function of HSCs. Additionally, G-CSF stimulates bone marrow to release stem cells into the blood.[38] The mechanism of HSCs mobilization is described as the mobilization of HSCs into the blood by G-CSF, and homing is described as the movement of stem cells to the targeted tissue such as the heart. Recent studies have identified that chemokine stromal cell-derived factor-1 (SDF-1), and its receptor (CXCR-4) have been implicated in the mobilization and homing of human $CD34^+$ stem cells to the targeted organ.[39] CXCR-4 is the receptor on cell surface of $CD34^+$, which mediates the attachment between bone marrow and stem cell. The binding of SDF-1to CXCR-4 on $CD34^+$ cell leads to a cleaving of stem cell from bone marrow. This study reported that a possible role for SDF-1 in post-MI is the recruitment of extra-cardiac stem cells, since SDF-1 mRNA in the infarct zone was increased while the level of SDF-1 serum was decreased during MI. Therefore, SDF-1/CXCR4 interactions play a crucial role in the recruitment of stem cells to the heart after MI. In addition, stem cell recruitment is a 2-step process that begins with the binding of stem cell to adhesive complexes, followed by the engraftment of the vessel and cardiomyocyte on the infarcted heart. Therefore, SDF-1 is served as an adhesive molecule for binding of stem cells to the vessel.

Regarding the cell mobilization, it was found that G-CSF acts as the mobilizer of stem cell to the infarcted heart and induces differentiation into cardiomyocyte.[40] It is possible that due to the diminished level of SDF-1 in MI model, the repair of the injured cardiac cell is deranged. This evidence leads to a continued release of several

cytokines such as G-CSF, followed by an increased mobilization of $CD34^+$ stem cells from bone marrow into the circulation.[17] Misao *et al*. found that, in the heart, stem cells are mobilized and recruited into infarcted myocardial tissue to stimulate the post-MI healing process via myocardial regeneration.[41] The study revealed that the CXCR4/SDF-1 axis plays an important role in the G-CSF-mediated recruitment of stem cells into the infarcted myocardium. Similarly, the number of $CD34^+$ stem cell is increased by the treatment of G-CSF in ischemia model [42], while its mobilization into the peripheral blood of patients with AMI is significantly correlated to the increased endogenous G-CSF.[43] Nevertheless, Misao *et al*. suggested that the effective effect of SDF-1 was limited within 7 days after MI. Therefore, the level of SDF-1 after this period could be increased by the treatment of G-CSF, allowing mobilization and differentiation of stem cells into cardiomyocyte.[30] After the mobilization, the stem cells were differentiated into mature cardiomyocytes.[44] Moreover, it has been reported that, in MI condition, the level of proliferation in cardiomyocyte is greater than that in the physiological condition, leading to cardiomyocyte regeneration.[45] In addition to the fact that bone marrow stem cell can be differentiated by G-CSF into cardiomyocyte, Harada *et al*.[50] recently found that G-CSF has a direct effect on cardiomyocyte due to the presence of G-CSF receptor on cardiac cell. Therefore, G-CSF has a direct effect in improving the MI condition.[8]

1.2.3 Relationship between G-CSF and myocardial infarction condition

In recent years, G-CSF) has been identified as the novel treatment for under myocardium infarction (MI) (Figure 1-2). This section will review the roles of G-

CSF in different models; *in vitro*, *in vivo*, pre-clinical study and clinical study, respectively. The *in vitro* experimental study suggested that G-CSF has an anti-arrhythmic effect during ischemia through sustaining gap junction in cardiac tissue.[46] G-CSF can be treated with other therapies, for example, myelosuppressives, where such therapy has been considered to play a role in the mobilization of stem cells into the peripheral circulation. It was suggested that the combination of G-CSF and myelosuppressives might enhance myocardial repair by increasing the number of bone marrow cell-derived cardiomyocytes and endothelial cells, and reducing fibrosis and remodeling in the left ventricle.[42] The recruitment of stem cells into the myocardium was achieved by up-regulation of SDF-1. It was observed that embryonic stem cell transplantation, co-treatment of the transplantation with G-CSF significantly increased the reduction of infarcted area and cell apoptosis, compared to the transplantation alone.[47, 48] Therefore, G-CSF treatment is able to enhance the efficacy of cardiomyocyte transplantation in the infarcted myocardium.[49]

While the effects of G-CSF include the reduction of apoptosis and the increase of vascularization in the infarcted hearts,[50] Okada *et al*. reported that G-CSF did not affect the reduction of apoptosis.[51] Disagreement in these findings could be due to the differences in methodology, for example in the regimen of G-CSF treatment and the timing of the treatment after MI. Li *et al.* have elucidated, in regards of the differentiation of stem cells into cardiac cells, that cardiac remodeling and functioning could be improved by adult stem cell transplantation after MI.[52] Stem cell is differentiated into endothelium and cardiomyocyte. Therefore, the

increased amount of endothelium enhances neovascularization of infarcted area, these improving in cardiac function in MI model.[42]

In addition to myocardial regeneration effected by G-CSF treatment, Shinya *et al*. have demonstrated that G-CSF accelerates myocardial healing process after MI.[53] The impaired myocardium and limited ventricular expansion might be replaced by the accumulation of collagen fibers, known as reparative fibrosis.[54] Recently, the novel effect of G-CSF showed that G-CSF has anti-arrhythmic effect through sustaining the gap junction, both in *in vivo* and *in vitro* studies. Apart from such effect, Kuwabara *et al*. revealed that protein levels and protein localization were regulated by G-CSF through the gap junction.[46] The effect of G-CSF, both *in vitro* and *in vivo*, were applied in preclinical study to confirm the result of such treatments. In a preclinical study, cardiac dysfunction and remodeling after ischemic condition as well as the reduction of fibrosis and apoptosis in infarcted swine heart model could be prevented by the treatment of G-CSF.[28]

The transforming growth factor (TGF)-β1 plays a crucial role in reparative fibrosis.[55] Sugano *et al*. demonstrated that G-CSF has a positive effect in increasing TGF-β1 mRNA expression to promote the healing process after MI.[54] The healing process for MI is identified as three phases [56]: acute stage (within several days after MI), where the absorption of necrotic tissues and increased macrophage occur; subacute stage (1 to 3 weeks after MI), where the granulation and increase of collagen occur; and chronic stage (more than 1 month), where fibrosis and scar occur. Minatoguchi *et al.* reported that absorption of necrotic tissues via the increase of macrophages, and the reduction of granulation and scar tissues via the

expression of matrix metalloproteinases (MMPs), are accelerated by G-CSF. Although the increase of MMP has a harmful effect through collagen degradation, the study reported that increase of MMP could be beneficial for the healing process.[53] Because, in the chronic stage of the healing process, the contractility of the heart and cardiac function are decreased due to the formation of scar tissues. Hence, an increase in MMP may be one of the protective mechanisms via proteolysis of excessive collagen to accelerate the healing process. Previous studies reported that an MMP family can induce heart failure – only in the condition of permanent occlusion and large infarction via collagen degradation.[57, 58] In Minatoguchi *et al.*, their method was designed as transient ischemia and none-large infarcted area, therefore, it is believed that, MMP, which is up-regulated by G-CSF, can improve cardiac function in MI model.[10] Similarly, another study reported that MMP-1 is beneficial for post-MI heart failure via its anti-fibrotic action.[59] Therefore, different methods, for example, in the induction of MI period and the size of MI area, yield different results of MMP effect. Additionally, the effect of G-CSF depends on aging. Lehrke, *et al*. suggested that G-CSF fails to decrease apoptosis in aging animals and did not benefit MI.[60]

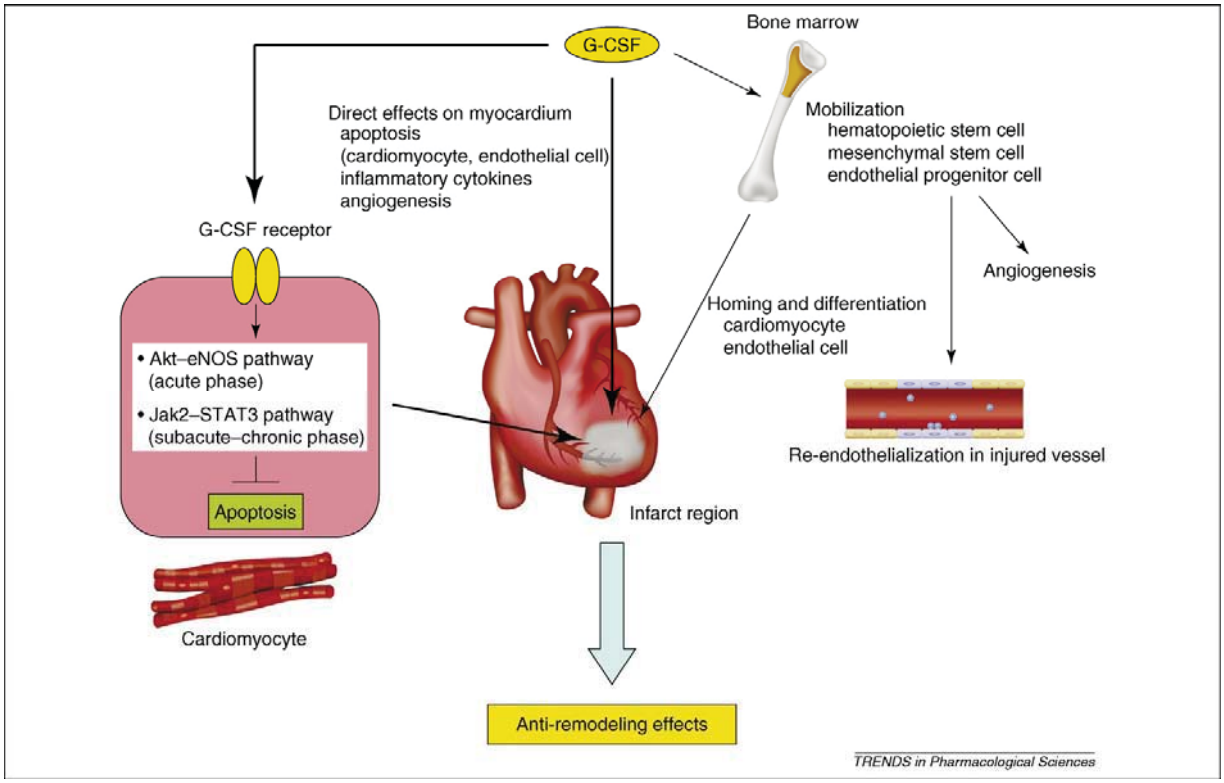

Figure 1-2

Hypothetical scheme demonstrating G-CSF effects on myocardium infarction

1.2.4 The relation between oxidative stress condition and cardiac cell death

Growing body of evidence has demonstrated the roles of mitochondria in the heart during ischemia-reperfusion injury.[61] The characteristics of myocardial ischemia have been proposed to attribute to the consequence of mitochondrial disturbance in the heart.[5] During ischemia, it has been shown that the energy production is decreased due to reduced oxidative phosphorylation rate at the inner mitochondrial membrane, and this event also rapidly induces ROS production.[6] This events lead to mitochondrial membrane potential ($\Delta\Psi m$) change and cell

death.[7] ROS produced by the electron-transport chain accumulates and cause to a threshold level, triggering the opening of the inner membrane anion channel (IMAC). The result of IMAC opening is caused to release of O_2^- from mitochondrial matrix. This situation leads loss of negative anion within mitochondrial matrix, which is called membrane depolarization. Recently, the mitochondrial permeability transition pore (mPTP) is proposed to have an important role in the processes of cell apoptosis and necrosis.[8] The mitochondrial permeability transition (MPT) is caused by the opening of mPTP of cells (such as oxidative stress, growth factor removal, or exposures to cytokines), which determines not only cells live or die, but also cell death by apoptosis or necrosis.[9, 10] Several pieces of evidence suggested that the burst of ROS production resulted in the inhibition of oxidative phosphorylation [11] and the increase of permeability of the inner mitochondrial membrane through the opening of mPTP.[12] Such events are responsible for the uncoupling of oxidative phosphorylation, mitochondrial membrane potential changes ($\Delta\Psi_m$) and mitochondrial swelling, leading to the release of cytochrome *c*, activation of caspase pathways and cell death by necrosis and/or apoptosis.[13]

1.2.5 Relationship between G-CSF and oxidative stress condition

It is known that mitochondria, the source of energetic pool in the cell during oxidative phosphorylation, are also the main source of reactive oxygen species (ROS) production. In cardiac cell, the number of mitochondria in cytoplasm is greater than 50% of the cell volume. ROS has been shown to play an important role as the determinant of cell survival in ischemia.[46] It is also suggested that the released

ROS during ischemia is able to protect cardiac function through mitochondrial K_{ATP} channel activation.[62] One study disagreed and suggested that the excessive ROS level may lead to mitochondrial dysfunction, apoptotic cell death and persistent contractile dysfunction.[63]

Under physiological condition, there is a balance between ROS production and the intracellular ROS scavenging capacity. $O_2^{\cdot}$ is reduced oxygen into water through several steps in mitochondria, in the process of mitochondrial electron transport chain. Superoxide radicals ($O_2^{\cdot}$) are dismutated to H_2O_2 by mitochondrial superoxide dismutase (MnSOD) [64] and H_2O_2 is detoxified by glutathione peroxidase.[65] Aon *et al.* showed that ROS production triggers the collapse of mitochondrial inner membrane potential in isolated cardiomyocytes.[7] In reperfused ischemic hearts, increased oxidative stress leads to cardiac dysfunction.[66] The pro-apoptotic proteins are activated by the increased ROS to form protein complex. Consequently, cytochrome *c* is released from this protein complex to induce apoptosis. Therefore, current research focuses on investigating the novel process for eliminating the excessive ROS level. Zhu *et al*. found the relationship between the effect of G-CSF and this oxidative stress condition. Under physiological condition, intracellular ROS acts as a second messenger to a variety of growth factors, including G-CSF, which induces the activation of signaling molecules to survival effect.[67] Recently, it was suggested that G-CSF has a protective effect against the excessive oxidative stress.[68]

1.2.6 Mechanism of G-CSF on myocardial infarction

In different models of heart disease, G-CSF signaling is activated through different mechanisms. In myocardial infarction model, the mechanism of G-CSF was shown in Figure 1-3. In this model, the survival effect of cardiomyocyte is activated via the Jak/Stat pathway[50], while it is the ERK signaling that is responsible for this effect in doxorubicin (DOX)-induced non-ischemic cardiomyopathy model.[69] In addition**,** during ischemia-reperfusion injury (IR), it was suggested that G-CSF has direct effects against IR injury by the activation of Akt–eNOS pathway.[70] A recent study reported that G-CSF actions through different mechanisms could be affected by the timing of the occurrence of MI. In acute infarcted myocardium, effects of G-CSF are activated via the Jak/Stat pathway.[50] However, in the chronic myocardial ischemia, G-CSF strongly activates Akt signaling.[28] The cause of these different results occurs from the chronic stage, where the heart is reperfused by the increase of blood flow in the heart. Therefore, during IR model, G-CSF has direct effects on myocardium against IR injury by activating Akt–eNOS pathway.[70] In addition to Jak/Stat and Akt pathway, G-CSF signaling was found to sustain the gap junction via the Wnt signaling pathway.

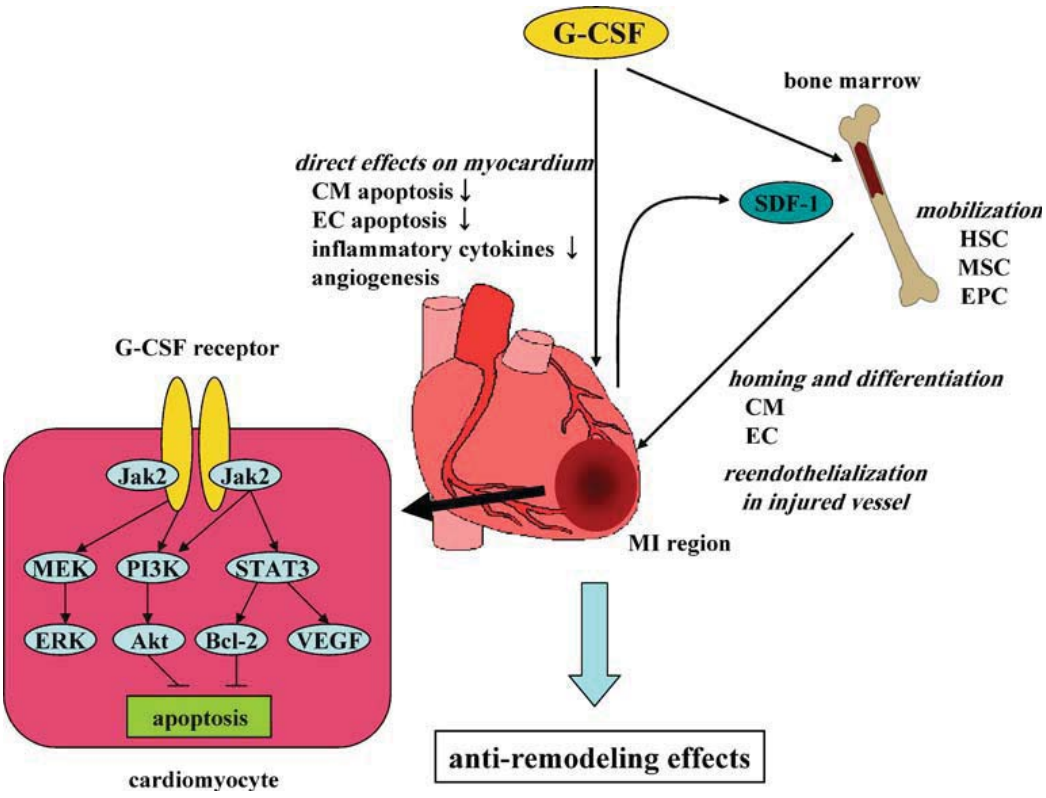

Figure 1-3

Mechanism of G-CSF on myocardial infarction

1.2.7 Clinical study

The evidence suggested that an increase of circulating CD34+ cells after AMI is potentially influencing LV function in the post-infarction setting.[17, 71] The correlation between spontaneous mobilization of CD34+ and endogenous G-CSF in patients with AMI was shown by Leone Am *et al*.[43] G-CSF is also synthesized and released from the heart in the early phase of AMI, probably as a natural defense

mechanism. Consequently, CD34+ is increased in infarcted tissue by the mobilization of G-CSF effect. Recently, Ince H *et al*. suggested that in the Front-Integrated revascularization and Stem Cell Liberation in Evolving Acute Myocardial infarction (FIRSTLINE-AMI) study to determine safety and functional impact of G-CSF in the setting of human myocardium infarction in conjunction with primary percutaneous coronary intervention (PCI) and abciximab were tested in a randomized protocol with serial assessment of LV function after 1 and 4 months and coronary morphology at 6 months.[72] FIRSTLINE-AMI was set up to recruit 50 consecutive patients with acute ST-elevation myocardium infarction (STEMI) subjected to primary PCI with stenting and abciximab administration. After successful PCI successful reperfusion, patients were randomized in 1:1 allocation (with 25 subjected per group) to 10 μg/kg G-CSF over a period of 6 days in addition to standard care or to standard post-interventional care alone. At baseline, all parameters of LV function were similar in both the G-CSF-treated and the control groups. Interestingly, with G-CSF, LV end-diastolic diameter (LVEDD) showed no enlargement over a period of 4 months, whereas LVEDD increased to 58±4 min in control subjects and was worse than with G-CSF. Recovery of LV ejection fraction (EF) with G-CSF was documented at 35 days and over 4 months, whereas no longitudinal improvement was present in control subjects. Similar to LVEF, resting wall motion score index revealed partial recovery with G-CSF, from 1.71±0.22 at baseline to 1.41±0.25 after 4 months, but no change in control subjects.

Moreover, in a recent prospective, non randomized, open label study by Kuethe et al. suggested that no severe side effects of G-CSF treatment were observed, whereas significant improvement of the regional wall motion and perfusion was seen

with G-CSF. Moreover, EF in the treatment group increased from 0.4±0.11 to 0.48±0.13, whereas in the control group, EF was changed only from 0.4±0.13 to 0.43±0.13. The angiography of one patient in the treatment group showed one in-stent restenosis.[73] However, the promising effect was not observed in patient with old MI.[74] It was suggested that the most infarcted tissue was replaced by scar and followed by the loss of myocardial contractility.

Despite a lot of clinical trials showed a promising effect of G-CSF in acute myocardial infarction, there are some conflicts on the benefit of its function and a complication of G-CSF to increase restenosis rate as indicated by a randomized clinical trial. Kang *et al*. evaluated the potential value of G-CSF in comparision to and/or in combination with stem cell infusion.[75] All 27 patients were randomized into 3 groups as stem cell infusion with G-CSF, G-CSF alone and control group. After 6 months, the improvement of LVEF in stem cell infusion group but not the improvement in stem G-CSF group. Administration of G-CSF was associated with an unexpectedly high rate of restenosis. In contrast, the study in patients with ST-segment elevation AMI who had successful reperfusion by PCI within 12 hours after onset of symtoms. All patients were randomized to receive subcutaneously either a daily dose of 10 μg/kg of G-CSF or placebo for 5 days. Despite circulating stem cells increased, there was no difference between the groups. This study revealed that stem cell mobilization by G-CSF in patients with AMI and successful reperfusion has no influence on infarct size, LV function and coronary restenosis. Similarly, patients with STEMI who underwent successful primary PCI were assigned to G-CSF (10 μg/kg) or placebo daily for 6 days. At 6 months, there was no difference between the two groups in the change of systolic wall thickening and LVEF.[76] The difference

of time in G-CSF administration after reperfusion could be contributed to the discrepancy of the results of G-CSF on both studies.[72, 75] Therefore, in the future study need to investigate optimal dose and procedure for myocardial infarction treatment.

1.3 Hypotheses of the study

1. G-CSF can (1) protect mitochondrial swelling, (2) prevent mitochondrial membrane potential change and (3) reduce ROS production under hydrogen peroxide-induced oxidative stress condition in isolated cardiac mitochondria.

2. G-CSF prevents mitochondrial damage via mitochondrial permeability transition pore (mPTP).

1.4 Objectives of the study

1. To study the effect of G-CSF on hydrogen peroxide-induced oxidative stress in isolated cardiac mitochondria

In this aim, G-CSF was investigated for its roles in isolated mitochondria under hydrogen peroxide-induced oxidative stress condition. It has been shown in previous studies that the treatment of G-CSF immediately after acute myocardium infarction (AMI) is as effective as the treatment that starts before AMI. The treatment with G-CSF alone also has beneficial effects in a similar degree to the combination treatment of G-CSF and stem cell factor (SCF) in ischemic model [77], which leads

to the reduction of apoptotic cells in the border area of the G-CSF-treated hearts after AMI. Similarly, Harada *et al.* reported that postinfarction administration of G-CSF decreases apoptotic cell death both *in vivo* and *in vitro* models and improves cardiac function by activating the Jak-Stat pathway.[50] Additionally, mitochondria are known not only for supplying 95% of the used energy by the cell through oxidative phosphorylation [78], but also for its role in the induction of cardiomyocyte cell death through apoptosis.[79, 80] Although G-CSF has been shown to be contributed to the improvement in the doxorubicin (DOX)-induced cardiac mitochondrial damage in culture cardiomyocyte [81], the effect of G-CSF in isolated mitochondria with hydrogen peroxide-induced oxidative stress has not been investigated. Therefore, G-CSF was investigated for its roles in protecting isolated mitochondria damage in a model of hydrogen peroxide-induced oxidative stress.

2. To investigate the mechanism underlying G-CSF effect on isolated cardiac mitochondria

In this aim, we investigate the mechanism underlying G-CSF effect on isolated cardiac mitochondria. Recently, the mitochondrial permeability transition pore (mPTP) was proposed to have an important role in the processes of cell apoptosis and necrosis.[8] The mitochondrial permeability transition (MPT) is caused by the opening of mPTP of cells (such as oxidative stress, growth factor removal, or exposures to cytokines), which determines not only cells live or die, but also cell death by apoptosis or necrosis.[9, 10] Several pieces of evidence suggested that the burst of ROS production resulted in the inhibition of oxidative phosphorylation [11] and the increase of permeability of the inner mitochondrial

membrane through the opening of mPTP.[12] The evidence of the uncoupling of oxidative phosphorylation, mitochondrial membrane potential collapse and mitochondrial swelling, leading to the release of cytochrome *c*, activation of caspase pathways and cell death by necrosis and/or apoptosis.[13] Therefore, we investigate whether the mechanism of G-CSF effects on cardiac mitochondria is mediated via mPTP.