厚生労働科学研究費補助金

医療技術実用化総合研究事業

顆粒球コロニー刺激因子(G-CSF)による急性心筋梗塞治療の効果と安全性に関する臨床研究
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顆粒球コロニー刺激因子(G-CSF)による急性心筋梗塞治療の効果と安全性に関する臨床研究

研究代表者　高野 博之　千葉大学医学部附属病院　循環器内科　講師

研究要旨　虚血性心疾患による心不全の病態に心臓リモデリングが重要な役割をはたしている。これまで世界中の研究グループが心臓リモデリングの治療法を検討してきたが、現存の薬物療法では心臓リモデリングおよび心不全を十分抑制することはできない。我が国でも虚血性心疾患の患者数は増加傾向にあることから、強力に心不全の進展を抑制できる新しい抗リモデリング薬の開発が待たれる。申請者のグループは造血性サイトカインである顆粒球コロニー刺激因子 (granulocyte colony-stimulating factor; G-CSF) が白血球数の増加作用だけでなく臓器保護作用、血管新生作用、抗アポトーシス作用なども有することを明らかにし、急性心筋梗塞後の心臓において血管新生や心筋細胞と血管細胞のアポトーシスを抑制することにより心臓リモデリングを強力に抑制することを報告した。また、心臓に対する G-CSF の分子機序を世界で初めて報告した。

急性心筋梗塞後の心不全に対するG-CSF治療を臨床応用させるためには、申請者らがおこなってきた基礎研究の結果をもとに最も効果的なプロトコールで大規模臨床研究をおこないG-CSFの安全性と効果を確認する必要がある。本研究では100症例の急性心筋梗塞患者を対象に6ヵ月後の心臓の機能やサイズを比較検討する。登録開始から解析終了までの期間は2年間を予定している。本研究で目指している治療法は特殊な設備や技術を必要とせず、投与方法も血管への注射ですむため一般病院でも実施が可能である。このような利点から治療を受けられる患者数も膨大なものになると予想される。本研究の成果は、市場における心不全治療薬の製品戦略にも大きな変革をもたらし医療経済にも好影響を及ぼすと期待される。

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A. 研究目的

さまざまな心血管疾患が心不全に進展する際に心臓リモデリングが重要な役割をはたしている。神経液性因子のレニン- アンジオテンシン系やカテコラミンが一部関与していることから、これらの作用を抑制する薬物が心不全の治療薬として用いられているがその効果は十分ではない。より効果的な新規の抗リモデリング薬を開発することは今後、心不全患者数の増加が予想される我が国において急務の課題である。これまで申請者らはマウスやブタを用いた動物実験で、造血性サイトカインである顆粒球コロニー刺激因子 (granulocyte colony-stimulating factor; G-CSF) が急性心筋梗塞後の心臓において血管新生や心筋細胞と血管細胞のアポトーシスを抑制することにより心臓リモデリングを強力に抑制することを明らかにした (Ohtsuka M, et al. FASEB J. 2004; Iwanaga K, et al. Biochem Biophys Res Commun. 2004)。その分子機序として G-CSF は心筋細胞に存在する G-CSF 受容体を介して直接作用し保護効果をもたらすことを世界で初めて報告した (Takano H, et al. Nat Med. 2005)。

申請者は、実際に急性心筋梗塞患者を対象にG-CSFの安全性と効果を確認してきたが40例という少数例での解析結果であり (Takano H, et al. Int. J. Cardiol. 2007)、臨床応用させるためには大規模臨床研究により安全性と効果を再検討する必要がある。海外でも臨床研究がおこなわれているが、治療プロトコールが異なるため結果については一定の見解に至っていない (Takano H, et al. Trends. Pharmacol. Sci. 2007)。申請者らの基礎研究と臨床研究の結果からは、急性心筋梗塞発症後からG-CSF治療を開始するまでの時間と心機能の改善度には逆相関が認められたが、海外での臨床研究ではG-CSF.開始までの時間に注意が払われていない。海外の臨床研究の結果を検討してみると、興味深い事にpositiveな結果が得られた研究ではG-CSF開始までの時間が比較的早く、一方、negativeな結果が得られた研究では開始時間が遅い傾向にある。本研究では100症例の急性心筋梗塞患者を対象に6ヵ月後の心臓の機能やサイズを比較検討する。登録開始から解析終了までの期間は2年間を予定している。

B. 研究方法

1. 被験者を選ぶ方針および目標数

被験者は、千葉県救急医療センターに入院中の急性心筋梗塞患者で、発症後 12 時間以内にインターベンション治療を施行し再開通が得られた患者。80 歳未満の男性および女性。目標症例数は 100 例（G-CSF 群：50 例、コントロール群：50 例）。ただし、以下の項目に該当する場合は適応から除外する。インターベンション治療終了後も心電図の変化を伴う胸痛が持続する場合。急性冠閉塞の可能性がある場合。心原性ショックの場合。問診にて悪性新生物を合併している（または治療中である）ことが確認された場合。問診にて糖尿病性網膜症を治療中であることが確認された場合。問診にて重篤な薬剤アレルギーの既往が確認された場合。インフォームドコンセントが得られない場合。本研究は千葉大学医学部附属病院未来開拓センターを中心に実施し、研究プロトコールは臨床試験部で厳格に検証をおこない医師主導の臨床研究を成功させることを目指す。

2. 研究方法

(1) 実施方法

①投与方法（平成 20-21 年度の計画、千葉県救急医療センターでおこなう）

急性心筋梗塞発症後、24 時間以内に 1 回目の G-CSF を静脈注射する（この日を day1 とする）。1 回の投与量は 5 μg/kg とする。1 日 1 回、計 5 日間（day1 から day5 まで）連続投与する。計 5 回終了する前に白血球数が 40,000/μl を越えた場合は、その時点で G-CSF の投与は中止とする（臨床研究としては継続）。

②前処置および併用薬の有無

a) 前処置は特に必要としない。

b) ヘパリンを急性心筋梗塞発症後、3 日目まで使用（1 日 15,000 U div）。

c) 併用薬として、ACE 阻害薬（レニベース）、バイアスピリンまたは小児用バファリン、パナルジンを服用する。

d) ACE 阻害薬と ARB の併用はしない（レニベースが忍容性の問題で使えない場合はディオバンのみ使用する）。量は設定せず血圧値に応じて増減。

e) 硝酸薬、Ca 拮抗薬、β遮断薬、スピロノラクトン、スタチンに関しては特に制限を設けず、必要に応じ使用する。他の薬剤についても特に制限は設けない。

③臨床検査項目および観察項目（平成 20-21 年度の計画、千葉県救急医療センターでおこなう）

a) 血液、尿検査-血液検査一般、特に血算、血小板凝集能、CK(CK-MB)、LDH、肝機能、腎機能など。
CK の採血時期はピークまでは 3 時間おきに施行。WBC 数および分画は入院時から day7 まで毎朝測定する。$CD34^+$細胞数は day1 と day5 の朝に測定する。

b) 冠動脈造影検査-入院時（インターベンション治療時）と 6 ヵ月後に施行。

c) MRI 検査と RI 検査を急性心筋梗塞発症 5-7 日後と 6 ヵ月後に施行。

d) 心エコー検査。

④有効性評価項目（平成 20-21 年度の計画、千葉大学医学部附属病院臨床試験部でおこなう）

a) 1 次エンドポイント（主要評価項目）
梗塞後の心臓リモデリングの抑制- MRI 検査、左室造影検査にて評価。

b) 2 次エンドポイント
・梗塞領域の縮小-RI 検査による定量的評価。
・心血管イベント (MACE) の減少-心臓死、非致死的心筋梗塞、不安定狭心症、心不全による入院、致死的不整脈。

(2) 研究デザイン

無作為割付臨床試験：最小化法を用いた無作為割付により、2 群（G-CSF 群とコントロール群）に分ける。薬剤の投与は二重盲検法 (double-blind method) により実施する。

（倫理面への配慮）

本研究は千葉大学医学部附属病院未来開拓センターを中心に実施し、研究プロトコールは臨床試験部で厳格に検証をおこない、医師主導の臨床研究を成功させることを目指す。

(1) 予想される結果および危険

これまでの基礎研究の結果から、人においても G-CSF の投与により心筋梗塞後の心臓リモデリングの抑制効果が期待される。G-CSF 製剤はすでに臨床の場で使用されており、これまでに蓄積されたデータから副作用に関する頻度は低いと思われる。危険性も極めて低いと考えられるが、治療中および治療後は G-CSF による副作用の早期発見に努める。また重篤な副作用が発生した場合は、その被験者に対する G-CSF の投与は直ちに中止し、副作用に対する治療に努める。

(2) 個人情報の保護

本研究の成果は医学雑誌や学会などを通じて公表されるが、その際に患者の名前や身元が明らかになることはなく被験者のプライバシーは保護される。

(3) インフォームド・コンセントのための手続きおよび方法

インフォームド・コンセントのための手続きとして、まず文書ならびに口頭で説明を行い、十分理解を得た上で、文書による同意を得る。

C. 研究結果

当初の研究計画では、平成20年度中に臨床研究開始前の準備を全て終了させて患者登録を開始する予定であった。しかし、以下の挙げる理由により若干の遅れが生じている。進捗状況として平成20年度は実施施設の倫理審査委員会に臨床試験計画書を提出し承認された。また、申請者が所属する千葉大学の利益相反委員会に書類を提出し利益相反がないことの承認を得た。登録患者をG-CSF群とコントロール群の2群にランダムに割り当てるソフトを作成しPCにインストールした。試験実施担当者と数回にわたり本研究の打ち合わせをおこなった。本研究で使用するG-CSFと生理食塩水を購入し実施施設に納品した。平成20年度中には目標症例数の1/3 位の登録を予想していたが、倫理審査委員会および利益相反委員会の実施が遅れてしまい承認までに時間がかかった事、実施施設での検査機器（心臓の評価に使用するRI機器）のW修理・点検が1ヶ月ほどあったため承認後から試験開始までに間があいてしまった事、などの理由により登録は少数例しかなかった。平成20年度より症例登録が開始されたが、本格的な登録と治療・検査は平成21年度が中心となる。実施施設では本研究の適応条件を満たす症例は年間300例ほど入院するため、平成21年度中に目標の100例に達するものと思われる。

D. 考察

急性心筋梗塞に対するインターベンション治療法の進歩により急性期の死亡率は減少したが、一方で心筋梗塞後に生じる心臓リモデリングにより心不全を発症する患者が増加傾向にある。心臓リモデリングに対する現存の薬物療法は効果が不十分であり、心不全患者は入退院を繰り返すことが多いため医療経済的にも今後の重要課題となることが予想される。

G-CSFの臓器保護・再生作用を利用した治療法は簡便でかつ侵襲が少なく現実的に実行可能である。本研究で目指している治療法は特殊な設備や技術を必要とせず、投与方法も血管への注射ですむため一般病院でも実施が可能である。我が国でも急性心筋梗塞患者数は増加しており、G-CSF治療を受けられる患者数は膨大なものになると予想される。G-CSFは救命された心筋梗塞患者の心不全発症を予防し、その後のQOLの改善をもたらす新規の治療法となるものと思われる。本研究の成果は、国民の医療や福祉の向上のみならず、市場における心不全治療薬の製品戦略にも大きな変革をもたらし医療行政や経済にも好影響を及ぼすと期待される。

E. 結論

本研究を遂行することにより、G-CSFが急性心筋梗塞後の心不全抑制薬として実用化できるか明らかになる。

F. 健康危険情報

特になし。

G. 研究発表

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H. 知的財産権の出願登録状況
なし。

研究成果の刊行に関する一覧表

雑誌

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Circ J 2009; **73:** 214–220

Peroxisome Proliferator-Activated Receptor γ and Cardiovascular Diseases

Hiroyuki Takano, MD; Issei Komuro, MD

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily and form heterodimers with retinoid X receptor. Three PPAR isoforms have been isolated and termed α, β (or δ) and γ. Although PPARγ is expressed predominantly in adipose tissue and associated with adipocyte differentiation and glucose homeostasis, PPARγ is also present in a variety of cell types. Synthetic antidiabetic thiazolidinediones (TZDs) are well known as ligands and activators for PPARγ. After it was reported that activation of PPARγ suppressed production of pro-inflammatory cytokines in activated macrophages, medical interest in PPARγ has grown and there has been a huge research effort. PPARγ is currently known to be implicated in various human chronic diseases such as diabetes mellitus, atherosclerosis, rheumatoid arthritis, inflammatory bowel disease, and Alzheimer's disease. Many studies suggest that TZDs not only ameliorate insulin sensitivity, but also have pleiotropic effects on many tissues and cell types. Although activation of PPARγ seems to have beneficial effects on cardiovascular diseases, the mechanisms by which PPARγ ligands prevent their development are not fully understood. Recent data about the actions and its mechanisms of PPARγ-dependent pathway in cardiovascular diseases are discussed here. (*Circ J* 2009; **73:** 214–220)

Key Words: Atherosclerosis; Cardiac hypertrophy; Heart failure; PPARγ; Thiazolidinedione

Peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor superfamily that heterodimerize with the retinoid X receptor (RXR) and bind to specific response elements termed PPAR responsive elements (PPREs) in target gene promoters. The PPREs are direct repeats of the hexameric consensus sequence AGGTCA, separated by 1 nucleotide. These nuclear receptors are ligand-dependent transcription factors, and activation of target gene transcription depends on the binding of the ligand to the receptor. PPARs have 3 isoforms, α, β (or δ) and γ. PPAR α regulates genes involved in fatty acid oxidation, whereas PPARγ promotes adipocyte differentiation and glucose homeostasis. The main function of PPARβ/δ has yet to be ascertained, but involvement in the regulation of fatty acid oxidation seems likely. PPAR α is present mainly in the liver, kidney, and muscle, whereas PPARγ is expressed predominantly in adipose tissue. PPAR β/δ is almost ubiquitously expressed. It was recently demonstrated that PPARγ is also expressed in a variety of cell types. After it was reported that activation of PPARγ suppresses production of inflammatory cytokines in activated macrophages, medical interest in PPARγ has grown, along with a huge research effort.

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PPARγ

Peroxisome is a subcellular organelle that plays a crucial role in cellular metabolism. Peroxisome enzymes are implicated in a broad range of catabolic and anabolic enzymatic pathways, such as fatty acid oxidation, biosynthesis of both glycerolipids and cholesterol, and metabolism of reactive oxygen species. Peroxisome proliferation induced in rodents is associated with cellular responses to a range of chemical compounds. In 1990, Issemann and Green reported that peroxisome proliferators activate a member of the steroid hormone receptor superfamily in mouse liver[1] This nuclear receptor was named PPAR. Soon after, 3 major types of PPAR (α, β/δ, and γ) were recognized. PPARγ is associated with adipocyte differentiation and glucose homeostasis. PPARγ is expressed in a variety of cell types, including adipocytes, macrophages, vascular smooth muscle cells (VSMCs), endothelial cells (ECs), and cardiomyocytes[2–7] Several lines of evidence have demonstrated the functional significance of PPARγ in atherosclerotic lesions[8,9]

Activity of PPARγ is depressed by phosphorylation of a serine residue (Ser^{112}) in the N-terminal domain, mediated by a member of the mitogen-activated protein (MAP) kinase family, extracellular signal-regulated protein kinase (ERK). In addition, another member of MAP kinase family, c-Jun N-terminal kinase (JNK) also phosphorylates PPARγ at Ser^{82} and reduces the transcriptional activity of PPARγ. The association of PPARγ polymorphism with metabolic syndrome has also been examined[10,11] In the presence of ligand, PPARγ binds to coactivator complexes, resulting in the activation of target genes. In the absence of ligand, PPARγ binds to the promoters of several target genes and associates with a corepressor complex, leading to active repression of target genes. This process is referred to as active repression (**Fig 1**). The corepressor complex constitutes corepressor proteins, such as nuclear receptor corepressor

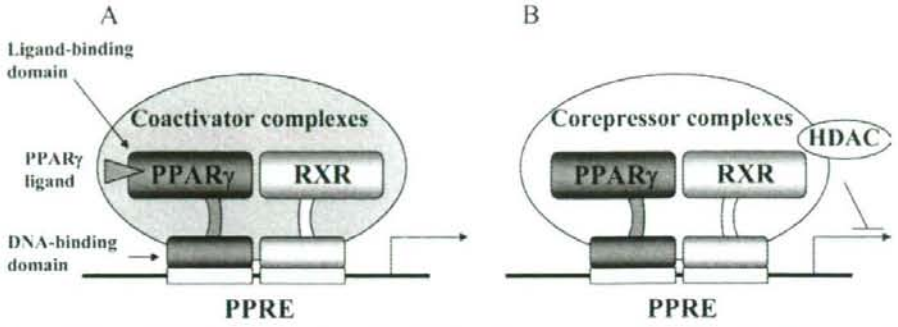

Fig 1. Transactivation and active repression. PPARγ functions as a heterodimer with RXR. (**A**) In the presence of ligand, PPARγ binds to coactivator complexes, resulting in the activation of target genes. (**B**) In the absence of ligand, PPARγ binds to the promoters of several target genes and associates with corepressor complexes, leading to active repression of target genes. HDAC, histone deacetylase; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR responsive element; RXR, retinoid X receptor.

(NCoR) and silencing mediator of retinoid and thyroid hormone receptors, histone deacetylases (HDACs) and transducin β-like protein 1 (TBL1). HDACs are essential in maintaining repressed chromatin structure and TBL1 exchanges a corepressor complex for a coactivator complex in the presence of ligand.[12]

Many nuclear receptors are proposed to sequester inflammatory transcription factors, such as nuclear factor-κB (NF-κB) and AP-1, by inhibiting their DNA-binding activities, resulting in inhibition of inflammatory target genes. In the presence of ligand, PPARγ also interacts with inflammatory transcription factors and inhibits their DNA-binding activities. PPARγ blocks clearance of the corepressor complex in a ligand-dependent manner, and PPARγ stabilizes the corepressor complex bound to the promoter of inflammatory genes.[13] It was demonstrated that PPARγ associates with the protein inhibitor of activated STAT1 (PIAS1), which is a small ubiquitin-like modifier (SUMO)-E3 ligase, in a ligand-dependent manner. PIAS1-induced SUMOylation of the ligand-binding domain of PPARγ enables the receptor to maintain NCoR on the promoter of inflammatory genes.[14] These are the suggested mechanisms of PPARγ transrepression.

PPARγ Ligands

Natural and synthetic ligands bind to PPARγ, resulting in conformational change and activation of PPARγ. The PGD_2 metabolite, 15d-PGJ_2, was the first endogenous ligand for PPARγ to be discovered. Although 15d-PGJ_2 is the most potent natural ligand of PPARγ, the extent to which its effects are mediated through PPARγ in vivo remains to be determined. Two components of oxidized low density lipoprotein (ox-LDL), the 9-hydroxy and 13-hydroxy octadecadienoic acids (HODE), are also potent endogenous activators of PPARγ.[15,16] Activation of 12/15-lipoxygenase induced by interleukin (IL)-4 also produced endogenous ligands for PPARγ;[17] however, whether these natural ligands act as physiological PPARγ ligands in vivo remains unknown. The antidiabetic thiazolidinediones (TZDs), such as troglitazone, pioglitazone, ciglitazone and rosiglitazone, which are used to control glucose concentration in patients with diabetes mellitus (DM), are pharmacological ligands of PPARγ. They bind PPARγ with various affinities and it is conceivable that their insulin-sensitizing and hypoglycemic effects are exerted by activating PPARγ. However, the molecular mechanisms by which TZDs affect insulin resistance and glucose homeostasis are not fully understood. They seem to mediate their effects primarily through adipose tissue, because TZDs alter the expression level of genes that are involved in lipid uptake, lipid metabolism and insulin action in adipocytes. TZDs enhance adipocyte insulin signaling and reduce the release of free fatty acids. TZDs also decrease the inflammation of adipose tissue that is induced by obesity and contributes to increased insulin resistance. There is a possibility that TZDs improve insulin sensitivity in skeletal muscle and liver, the main insulin-sensitive organs, through these multiple adipocentric actions. PPARγ has been demonstrated to have an antiinflammatory effect, leading to initiation of treatment trials for patients with inflammatory diseases. RXR, which interacts with the PPARs, is activated by 9-cis retinoic acid. When combined as a PPAR:RXR heterodimer, the PPAR ligands and 9-cis retinoic acid act synergistically on PPAR responses.

PPARγ and Atherosclerosis

Atherosclerosis is a complex process to which many different factors contribute. Injury of the endothelium, proliferation of VSMCs, migration of monocytes/macrophages, and the regulatory network of growth factors and cytokines are important in the development of atherosclerosis. In addition, chronic inflammation of the vascular wall is also involved. As mentioned earlier, PPARγ has antiinflammatory effect. PPARγ ligands have been shown to reduce production of inflammatory cytokines, such as IL-1β, IL-6, inducible nitric oxide synthase and tumor necrosis factor-α (TNF-α), by inhibiting the activity of transcription factors such as activator protein-1 (AP-1), signal transducers and activators of transcription (STAT), and NF-κB in monocytes/macrophages.[2,3] Those findings suggest that PPARγ activation may have beneficial effects in modulating inflammatory responses in atherosclerosis. Interestingly, expression of PPARγ has been demonstrated in atherosclerotic plaques.[8] Macrophages affect the vulnerability of plaque to rupture and they are implicated in the secretion of matrix metalloproteinases (MMPs), enzymes that are important in the degradation of extracellular matrix. In macrophages and VSMCs, PPARγ

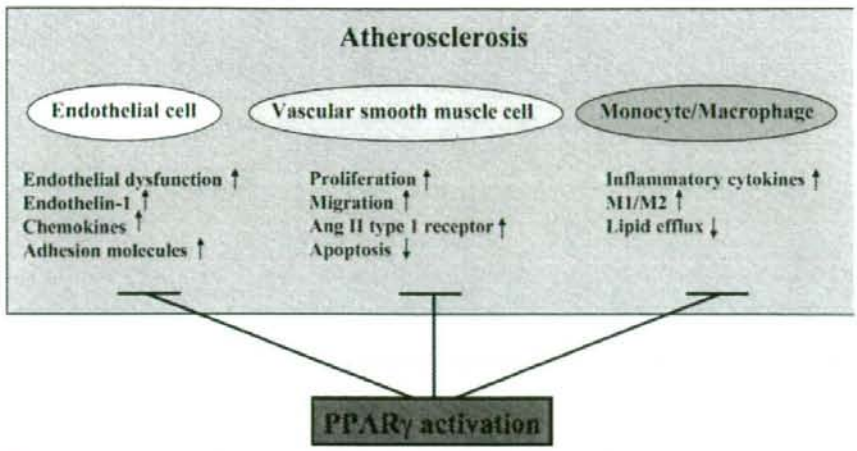

Fig 2. In atherosclerosis, PPARγ inhibits progression of the atherosclerotic lesion. PPAR, peroxisome proliferator-activated receptor.

ligands have been shown to reduce the expression of MMP-9, resulting in the inhibition of migration of VSMCs, and plaque destabilization.[3,4] Although activation of T lymphocytes represents a critical step in atherosclerosis, PPARγ ligands also reduce the activation T lymphocytes.[18] Recently, it was reported that PPARγ is a key regulator of M1/M2 polarization.[19] Classically activated macrophages (M1) express a high level of pro-inflammatory cytokines and reactive oxygen species, whereas alternatively activated macrophages (M2) play an antiinflammatory role in atherosclerosis. PPARγ agonists prime monocytes into M2 and PPARγ expression is enhanced by M2 differentiation.[20]

VSMC proliferation and migration are also critical events in atherosclerosis and vascular-intervention-induced restenosis. TZDs inhibit both these changes in the VSMCs and neointimal thickening after vascular injury.[21–24] Furthermore, TZDs induce apoptosis of VSMCs via p53 and Gadd45.[25,26] Angiotensin II (AngII) plays an important role in vascular remodeling via the AngII type 1 receptor (AT1R) and accelerates atherosclerosis. Although AngII induces transcriptional suppression of PPARγ, activation of PPARγ inhibits AT1R gene expression at a transcriptional level in VSMCs.[27–29] Expression of adhesion molecule by ECs, leading to adhesion of leukocytes, is a critical early step in atherosclerosis. PPARγ ligands inhibit the expression of vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 and decreased production of chemokines, such as IL-8 and monocyte chemotactic protein-1 (MCP-1) via suppressions of AP-1 and NF-κB activities in ECs.[30–32] PPARγ ligands also inhibit MCP-1-induced monocytes migration.[33] Endothelin-1 (ET-1) is involved in the regulation of vascular tone and endothelial functions, and induces proliferation of VSMCs. In bovine aortic ECs, PPARγ ligands suppressed transcription of the ET-1 promoter by interfering with AP-1.[34]

PPARγ activation by major oxidized lipid components of ox-LDL, 9-HODE and 13-HODE has an important role in the development of lipid-accumulating macrophages through transcriptional induction of CD36, a scavenger receptor.[35] These findings suggest that atherogenic ox-LDL particles could induce their own uptake through activation of PPARγ and expression of CD36, leading to atherosclerosis. However, several studies have demonstrated that activation of PPARγ does not promote lipid accumulation in either mouse or human macrophages.[36–38] Liver X receptor α (LXRα) is an oxysterol receptor that promotes cholesterol excretion and efflux by modulating expression of ATP-binding cassette transporter 1 (ABCA1).[37,38] LXRα was recently identified as a direct target of PPARγ in mouse and human macrophages.[39,40] Although the PPARγ-induced increase in CD36 expression might accelerate lipid uptake in macrophages, subsequent activation of LXRα and upregulation of ABCA1 appear to induce lipid efflux.

Diep et al have demonstrated that rosiglitazone and pioglitazone attenuate the development of hypertension and structural abnormalities, and improve endothelial dysfunction in AngII-infused rats.[41] These TZDs also prevented upregulation of AT1R, cell cycle proteins, and inflammatory mediators. Rosiglitazone, but not the PPARα ligand fenofibrate, prevented hypertension and endothelial dysfunction in DOCA-salt hypertensive rats.[42] It has been reported that serum levels of the soluble CD40 ligand are elevated in acute coronary syndrome and associated with increased cardiovascular risk. Treatment with rosiglitazone decreased the serum levels of soluble CD40 and MMP-9 in type 2 diabetic patients with coronary artery disease.[43] Taking all the evidence together, PPARγ ligands may prevent the progression of atherosclerotic lesions, particularly in patients with DM (**Fig 2**).

PPARγ and Ischemic Heart Disease

As the effects of PPARγ on the heart are not fully understood, we and others have examined whether PPARγ is involved in various heart diseases. Although the expression of PPARγ in cardiac myocytes is low compared with adipocytes, PPARγ ligands seem to act on cardiac myocytes.[7,44] We demonstrated that PPARγ ligands inhibited the cardiac expression of TNF-α at the transcriptional level, in part by antagonizing NF-κB activity.[7] Because TNF-α expression is elevated in the failing heart and has a negative inotropic effect on cardiac myocytes, treatment with PPARγ ligands may prevent the development of congestive heart failure. Diabetic cardiomyopathy, which is characterized by

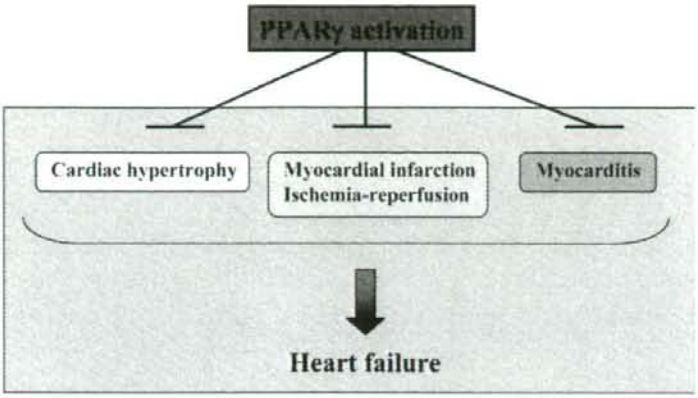

Fig 3. Actions of PPARγ in heart diseases. PPARγ inhibits the progression of heart failure following cardiac hypertrophy, myocardial infarction, ischemia–reperfusion injury, and myocarditis. PPAR, peroxisome proliferator-activated receptor.

systolic and diastolic dysfunction, is a major complication of DM, and therefore TZDs seem to be beneficial for the impaired cardiac function in patients with DM. Following our study, the role of PPARγ in myocardial ischemia–reperfusion (IR) injury has been elucidated[45–48] In animal models, PPARγ ligands reduced the size of the myocardial infarct and improved contractile dysfunction after IR through inhibition of the inflammatory response. IR injury activates JNK, and subsequently JNK induces increases in both AP-1 DNA-binding activity and apoptotic cells. It has been shown in rats that rosiglitazone inhibits the activation of JNK and AP-1 after myocardial IR[46] Furthermore, pioglitazone has been reported to attenuate left ventricular remodeling and heart failure after myocardial infarction (MI) in mice[49] Both of these effects of TZDs ligands were associated with decreases in inflammatory cytokines and chemokines[49,50]

PPARγ and Cardiac Hypertrophy

The PPARγ ligands, troglitazone, pioglitazone and rosiglitazone, inhibited AngII-induced hypertrophy of neonatal rat cardiac myocytes[51–53] Because generalized PPARγ gene deletion causes embryonic lethality, we examined the role of PPARγ in the development of cardiac hypertrophy in vivo using heterozygous PPARγ-deficient ($PPAR\gamma^{+/-}$) mice[53] Pressure overload-induced cardiac hypertrophy was more prominent in heterozygous $PPAR\gamma^{+/-}$ mice than in wild-type (WT) mice. Treatment with pioglitazone strongly inhibited the pressure overload-induced cardiac hypertrophy in WT mice and moderately in $PPAR\gamma^{+/-}$ mice[53] Thereafter, 2 other groups examined the role of PPARγ in the heart by using cardiomyocyte-specific PPARγ knockout mice[54,55] Duan et al reported that these mice develop cardiac hypertrophy through elevated NF-κB activity[54] and unexpectedly, rosiglitazone induced cardiac hypertrophy in both the WT mice and cardiomyocyte-specific PPARγ knockout mice through activation of p38 MAP kinase independent of PPARγ. Ding et al reported that cardiomyocyte-specific PPARγ knockout mice displayed cardiac hypertrophy from approximately 3 months of age and then progress to dilated cardiomyopathy;[55] most mice died from heart failure within 1 year after birth. Mitochondrial oxidative damage and reduced expression of manganese superoxide dismutase were recognized in the cardiomyocyte-specific PPARγ knockout mice[55] These mice models demonstrate that PPARγ is essential for protecting cardiomyocytes from stress and oxidative damage, although the expression level of PPARγ in cardiomyocytes is low. On the other hand, Son et al demonstrated that cardiomyocyte-specific PPARγ transgenic mice develop dilated cardiomyopathy associated with increased uptake of both fatty acid and glucose[56] Rosiglitazone increased this glucolipotoxicity in cardiomyocyte-specific PPARγ transgenic mice. If PPARγ in the heart is expressed at a high level, rosiglitazone may cause cardiotoxic effects; however, as noted earlier the expression level of PPARγ in the heart is quite low.

PPARγ and Myocarditis

Experimental autoimmune myocarditis (EAM) is a T-cell-mediated disease characterized by infiltration of T cells and macrophages, leading to massive myocarditis necrosis, which develops into heart failure in the chronic phase[57] The onset of EAM in rats occurs approximately 2 weeks after the first immunization with porcine cardiac myosin. At this time, small numbers of $CD4^+$ T cells and macrophages start to infiltrate into the myocardium and various cytokines are expressed. Macrophage inflammatory protein-1α (MIP-1α) is a C-C chemokine that induces leukocyte accumulation in tissue sites of inflammation. We previously demonstrated that MIP-1α mRNA and protein are highly expressed in the hearts of rats with EAM from day 11 after first immunization[57] Th1 cells produce interferon-γ (IFN-γ), which is mainly involved in cell-mediated immune responses, whereas Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13, which participate in humoral responses. Immune dysfunction associated with autoimmune disease is known to involve an imbalance between Th1 and Th2 cells.

It has been reported that pioglitazone treatment markedly reduces the severity of myocarditis in a rat model of EAM[58,59] Pioglitazone suppressed expression of inflammatory cytokines and activation of myocardiogenic T cells in the myocardium of EAM rats[58] The mRNA levels of MIP-1γ were upregulated in the hearts of EAM rats, but not in the hearts of those in the pioglitazone group. Furthermore, treatment with pioglitazone decreased the expression levels of pro-inflamatory cytokine (TNF-α and IL-1β) genes and Th1 cytokine (IFN-γ) genes, and increased the expression levels of Th2 cytokine (IL-4) gene[59] These results suggest that PPARγ ligands may have beneficial effects on myocarditis by inhibiting MIP-1α expression and modulating the Th1/Th2 balance (**Fig 3**).

Efficacy and Safety of TZD Treatment in the Clinical Setting

Despite the beneficial effects of TZDs in the basic experiments, their propensity to cause fluid retention is a serious side-effect. Clinical studies report TZD-induced peripheral fluid retention, and an increase in plasma volume in 2–5% of patients on monotherapy[60] Fluid retention was more likely to occur with concomitant insulin use, and in patients with underlying cardiac dysfunction or renal insufficiency. The exact mechanisms for TZD-induced fluid retention are not well understood, and it remains unclear whether TZDs directly cause the development of de novo congestive heart failure. It is known that the level of vascular endothelial growth factor is increased in the patients who develop fluid retention with TZD therapy[61] and this may lead to peripheral edema through increased vascular permeability. The insulin-sensitizing action of TZDs also induces water and salt retention. PPARγ is highly expressed in the kidney and collecting-duct-specific PPARγ knockout mice demonstrated no effects of TZD on fluid retention or the expression level of sodium channel ENaC-γ[62,63] These findings suggest that activation of the sodium channel in the collecting duct cells expressing PPARγ may be a mechanism of fluid retention. In patients without evidence of heart failure, careful examination did not reveal any worsening of left ventricular function by TZDs[64] There are very few studies investigating the safety of TZDs in patients with preexisting heart failure. Although a recent study demonstrated that there is not a direct association between the risk of fluid retention and the baseline degree of severity of heart failure in diabetic patients treated with TZDs, the prescription of TZDs for patients with established heart failure should be avoided at present[60,65]

The PROactive (Prospective Pioglitazone Clinical Trial in Macrovascular Events) study has shown that pioglitazone significantly decreases the occurrence of all-cause mortality, nonfatal MI, and nonfatal stroke in patients with type 2 DM and macrovascular diseases[66] Pioglitazone significantly reduced the occurrence of fatal and nonfatal MI by 28% in the PROactive study[66] Although there was a 1.6% absolute increase in heart failure hospitalizations in the pioglitazone group compared with the placebo group, the number of heart-failure-related deaths was almost identical. In contrast to the PROactive study, it has been recently reported that rosiglitazone treatment is associated with increased incidence of MI by meta-analysis[67,68] Although meta-analysis has a number of limitations and the increased risk in MI is still controversial, those results attracted the attention of many clinicians. There are some differences in the actions of pioglitazone and rosiglitazone. Pioglitazone has more beneficial effects on the lipid profile than rosiglitazone[69] As mentioned earlier, rosiglitazone, but not pioglitazone, induced cardiac hypertrophy by a non-PPARγ-mediated pathway[54] Pioglitazone represses NF-κB activation and VCAM-1 expression in a PPARα-dependent manner[70] Pioglitazone was recently reported to increase the number and function of endothelial progenitor cells (EPCs) in patients with stable coronary artery disease and normal glucose tolerance[71] Pioglitazone may induce angiogenesis by modulating EPC mobilization and function. In the future, more mechanistic studies are required to investigate the differences in action between pioglitazone and rosiglitazone.

Conclusions

The American Heart Association (AHA) and American Diabetes Association (ADA) have released a consensus statement that advises caution regarding the use of TZDs in patients with known or suspected heart failure[72] Because there is a possibility that TZDs may unmask asymptomatic cardiac dysfunction by increasing plasma volume, they should be avoided in patients with congestive heart failure of New York Heart Association (NYHA) class III or IV. The data from in vitro studies suggest that TZDs exert direct actions on vascular cells and cardiomyocytes, independent of their glucose-mediated mechanisms. Further studies using tissue-specific gene targeting mice are necessary to address in vivo the pleiotropic effects of PPARγ on the cardiovascular system. If the beneficial roles of PPARγ can be solved, modulation of PPARγ may become a promising therapeutic strategy for cardiovascular diseases. Because cardiac hypertrophy can be seen even in normotensive diabetic patients, and diabetic cardiomyopathy is a major complication of DM, antidiabetic agents such as the TZDs would be expected to have beneficial effects on cardiac hypertrophy and dysfunction in patients with DM. It has been already clarified that 3-hydroxy-3-methyglutaryl-coenzyme A reductase inhibitors, statins, have pleiotropic effects in cardiovascular diseases. The effects of PPARγ ligands are similar to those of statins in many respects. A recent study demonstrated that statins activate PPARγ through ERK and p38 MAP-kinase-dependent cyclooxygenase-2 expression in macrophages[73] Further studies are needed to elucidate the molecular mechanisms of the pleiotropic effects of PPARγ ligands in cardiovascular disease.

Acknowledgments

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Right-Sided Heart Wall Thickening and Delayed Enhancement Caused by Chronic Active Myocarditis Complicated by Sustained Monomorphic Ventricular Tachycardia

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An asymptomatic healthy 65-year-old man was referred to a hospital for inverted T waves in the precordial leads (Figure 1) with paroxysmal advanced atrioventricular block in the ECG. Chest x-ray showed mild cardiac enlargement (Figure 2), and an echocardiogram showed right ventricular (RV) wall thickening (arrow in Figure 3). Five months later, the patient was referred to another hospital complaining of chest discomfort. Coronary angiogram was normal, but sustained monomorphic ventricular tachycardia (VT) occurred. Suffering from incessant VT, the patient was transferred to our hospital. The ECG and echocardiogram were almost the same as in previous studies. Enhanced multislice computed tomography revealed isolated right atrial, RV, and partial left ventricular (LV) wall thickening with extensive delayed enhancement (Figure 4) but no other organic diseases, which was confirmed by cardiac magnetic resonance (Figure 5).

In an electrophysiological study, 2 sustained monomorphic VTs (Figure 6) were induced, located in the RV midseptum by endocardial ventricular mapping. Radiofrequency ablation was performed at both sites; subsequently, neither VT could be induced. Because of the multislice computed tomography and cardiac magnetic resonance findings, endocardial biopsies were obtained from the RV (Figure 7) that showed interstitial edema, fibrosis, and myocyte destruction with a dense infiltrate of lymphocytes, suggesting chronic active myocarditis, which was consistent with his clinical course. Presumably, the thickening of the right atrial and RV free walls is related to lymphocytic infiltration and edema in the multislice computed tomography and cardiac magnetic resonance. A cardioverter-defibrillator was implanted, and the patient was given 40 mg/d prednisolone. He had no recurrent VTs after discharge.

Disclosures

None.

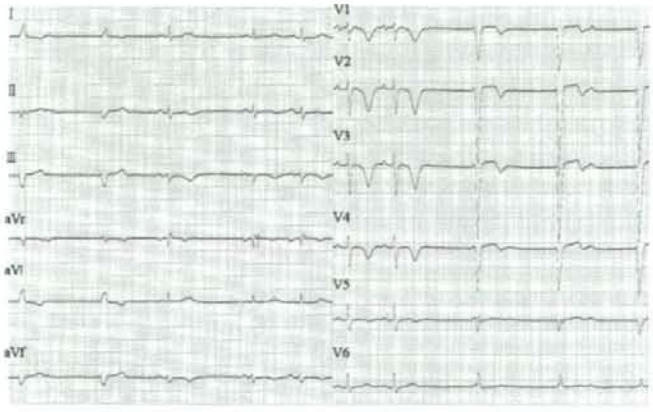

Figure 1. ECG acquired when the subject was referred to hospital showed inverted T waves in the precordial leads.

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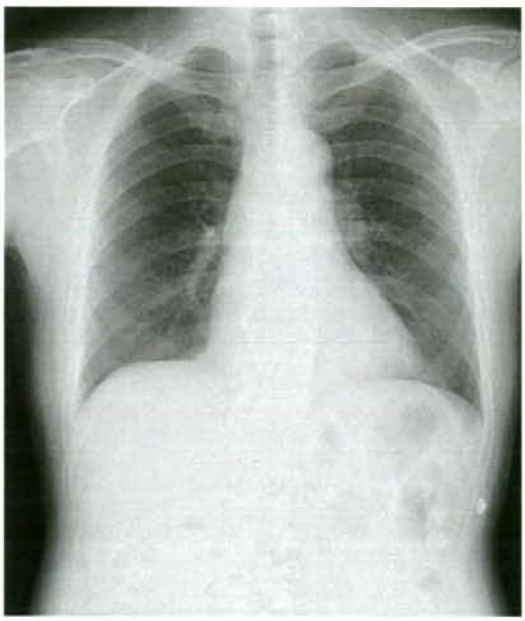

Figure 2. Chest x-ray showed mild cardiac enlargement.

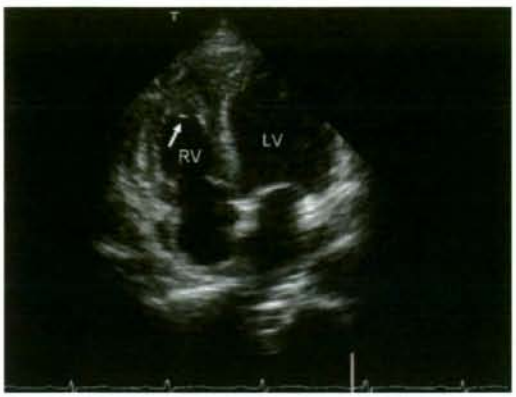

Figure 3. Transthoracic echocardiogram showed pericardial effusion and RV wall thickening (arrow) with no LV hypertrophy and normal systolic function of both ventricles.

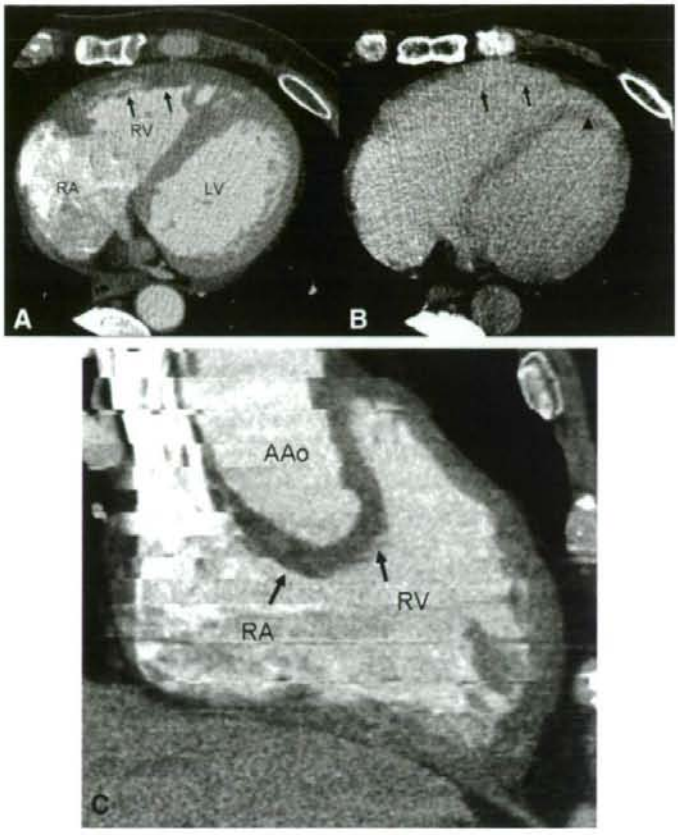

Figure 4. Axial source (A and B) and multiplanar reconstruction images (C) of enhanced multislice computed tomography revealed thickening of the right atrial (RA) and RV free walls (arrows in A and C) and part of the LV wall, which were abnormally enhanced in the later phase, as well as part of LV (arrows in B), suggesting lymphocytic infiltration and edema. AAo indicates ascending aorta.

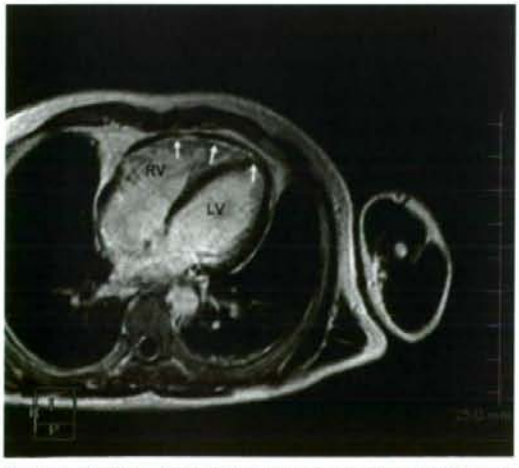

Figure 5. Cardiac magnetic resonance image revealed delayed enhancement (arrows) in the RV and part of the LV that was also observed in multislice computed tomography.

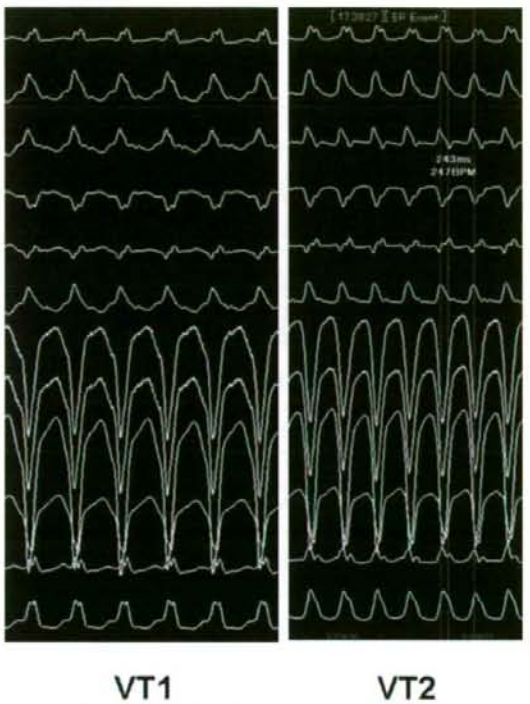

Figure 6. In an electrophysiological study, 2 clinical sustained monomorphic VTs were induced.

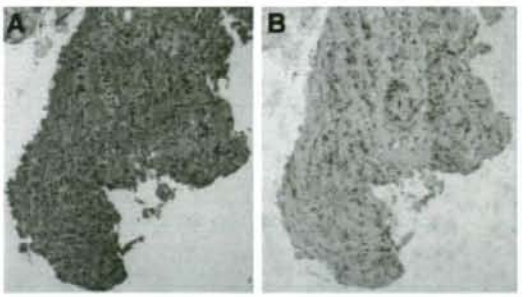

Figure 7. Histological results of endomyocardial biopsies. A, Hematoxylin and eosin staining demonstrated active myocarditis with focal lymphocytic infiltration with adjacent myocytolysis (×100). B, Immunohistological staining of T cells with focal infiltration pattern (×100).

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Letter to the Editor

G-CSF therapy for acute myocardial infarction: Studies of animal experiments give valuable hints to clinical trials

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Abstract

Granulocyte colony-stimulating factor (G-CSF) is a hematopoietic cytokine that promotes proliferation and differentiation of neutrophil progenitors. Clinically, hematopoietic stem cells mobilized by G-CSF are widely used for transplantation. G-CSF has been reported to mobilize bone marrow stem cells and regenerate infarcted hearts of mice. Besides mobilization, G-CSF has activated various signaling pathways such as Akt and Janus family kinase-2/signal transducer and activator of transcription-3 through G-CSF receptors in cardiac myocytes and has markedly prevented left ventricular remodeling after acute myocardial infarction by decreasing cardiomyocyte death and increasing the number of vessels. Although several clinical trials have been performed, the efficacy of G-CSF therapy in patients with acute myocardial infarction is still controversial.

Keywords: Angiogenesis; G-CSF; Heart failure; Myocardial infarction; Remodeling

To the Editor,

We are very grateful to Dr Celik et al. [1] for their comments concerning our article [2]. We agree that the clinical consequences of G-CSF therapy and cell therapy on left ventricular (LV) dysfunction and remodeling after acute myocardial infarction (AMI) are yet uncertain. Especially, the efficacy of G-CSF therapy in patients with AMI remains controversial because the protocols are different among the trials. It was reported that the subcutaneous injection of both G-CSF and stem cell factor (SCF) improved cardiac function and reduced mortality after AMI in mice [3]. G-CSF treatment started immediately after AMI (permanent ligation of left coronary artery) was as effective as the treatment started before AMI and that the treatment with G-CSF alone also has beneficial effects by similar degree as the combination treatment of G-CSF and SCF in mice [4]. The number of apoptotic cells was decreased in the border area of the G-CSF-treated hearts after AMI. Although many bone marrow-derived cells were recognized in the border area of the treatment group but not the control group, most of the bone marrow-derived cells were infiltrated blood cells and some cells were endothelial cells [4]. The number of capillaries in the border area after AMI was much greater in the treatment group than in the control group. Beneficial effects of G-CSF were significantly larger when the G-CSF treatment was started earlier after AMI [5]. The treatment started at 3 days after AMI was less effective than that started immediately after AMI and the treatment started at 7 days after AMI had almost no effects [5]. Therefore, we think that the timing of starting G-CSF treatment is very important. Other groups have also reported the beneficial effects of G-CSF on the prevention of LV remodeling and dysfunction after AMI in various animal models [6,7].

Based on the experimental results in animal models, clinical trials evaluating feasibility and safety of G-CSF in patients with AMI have been carried out [2,8–14]. However, the efficacy of G-CSF therapy for patients with AMI is still controversial

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because the protocols are different among those trials. Unexpectedly, it was reported that high rate of in-stent restenosis in patients with AMI or old MI, who were subjected to subcutaneous injections of G-CSF for 4 days before percutaneous coronary intervention (PCI) [8]. In the study, patients did not receive primary PCI during the golden time of AMI treatment and were treated with G-CSF for 4 days prior to PCI and only a few patients were assessed by coronary angiography at 6-months follow-up [8]. G-CSF treatment for 5 days induced serious adverse events in high-risk patients with severe coronary artery disease in a non-randomized study [9]. Since all 16 patients had Canadian Cardiovascular Society (CCS) functional class 3 or 4 angina despite prior revascularization, there is a possibility that they had many destabilized plaques in their coronary arteries [9]. However, the safety of G-CSF treatment for patients with coronary artery disease such as AMI and angina pectoris has been proven by clinical trials performed afterwards [2,10–14]. Occurrence of restenosis was not significantly different between the G-CSF group and the control group and no severe adverse effects were observed in the G-CSF group [2,10–14].

There were some differences in infarct-related artery, TIMI flow grade and the time from AMI to G-CSF administration in the randomized trials [2,10–14]. The rate of left anterior descending coronary artery-related MI was equal between the G-CSF-treated group and the control group, and Thrombosis in Myocardial Infarction (TIMI) flow grade 3 was documented in all patients after PCI in the trials that showed positive results [2,11]. Many investigators thought that G-CSF inhibits LV remodeling and dysfunction after AMI by accelerating cardiac regeneration in the infarcted hearts and did not pay much attention to the timing of G-CSF administration. The trials in which G-CSF treatment was started early after MI seemed to show positive results [2,10,11], whereas the trials in which G-CSF treatment was started late after MI showed negative results [12–14]. Interestingly, our clinical data demonstrated that there were inverse correlations between time from AMI to G-CSF administration and absolute change in LV ejection fraction (ΔLVEF), and between time from PCI to G-CSF administration and ΔLVEF [2,15]. The beneficial effects of G-CSF were significantly reduced when the G-CSF treatment was started late (after 3 days) in AMI mice model as mentioned above [5] and the experimental results are consistent with our clinical data. In most animal studies, the administration of G-CSF was indeed started immediately or within several hours after AMI [4–7]. These results suggest that the timing of the treatment should be very critical to obtain the most beneficial effects of G-CSF, because the cardioprotective effects of G-CSF might be mainly attributable to the direct action on myocardium.

The appropriate protocol should be strictly determined to assess the feasibility and safety of novel therapies such as G-CSF therapy and cell therapy for coronary heart diseases. It has not yet been determined how much dose of G-CSF should be used and when the treatment should be started. And the duration of therapy and the method of administration (e.g. subcutaneous, intravenous or intracoronary) have also yet to be determined. It remains unknown which modality is most appropriate to evaluate the effects of G-CSF and when the evaluation should be performed. Further studies with more rational designs are needed to conclude on the efficacy of G-CSF therapy for AMI. Studies of animal experiments will give valuable hints to clinical trials.

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