となるような状態を指す。末梢動脈疾患の症状で最も多いのは，この間歇性跛行である。間歇性跛行が軽度の場合，運動療法や抗血小板剤などによる薬物療法を行うが，重症例では狭窄した血管をカテーテルによって拡張する経皮的血管形成術（percutaneous transluminal angioplasty：PTA）や外科手術（バイパス手術）が必要となる。Fontaine Ⅲ～Ⅳ度を重症下肢虚血（critical limb ischemia：CLI）と呼ぶが，このような状態にまで進行すると，安静時にも下肢疼痛が出現し，皮膚の潰瘍や壊疽もみられるようになる。重症下肢虚血を呈する患者では，痛みや壊疽のために運動療法を施行するのは困難で，薬物治療も無効のことが少なくない。また，重症下肢虚血をきたすような血管は動脈硬化性変化が強く，血管形成術やバイパス手術もしばしば困難である。このような重症例に対する治療法として考えられたのが血管新生療法（therapeutic angiogenesis）[1]である。

Ⅱ 血管新生療法とは

血管新生療法は，血管増殖因子やその遺伝子あるいは骨髄や末梢血細胞を用いて血管新生を促進させ，組織虚血の改善を図る治療法で，1994年，米国のIsnerらにより初めて臨床応用された[2]。Isnerらが行ったのは，vascular endothelial growth factor（VEGF）[3]遺伝子を用いた血管新生療法であり，循環器領域における初の遺伝子治療としても知られている。以後，今日までに10年以上が経過し，遺伝子以外にも増殖因子蛋白，骨髄細胞，末梢血細胞などを用いたさまざ

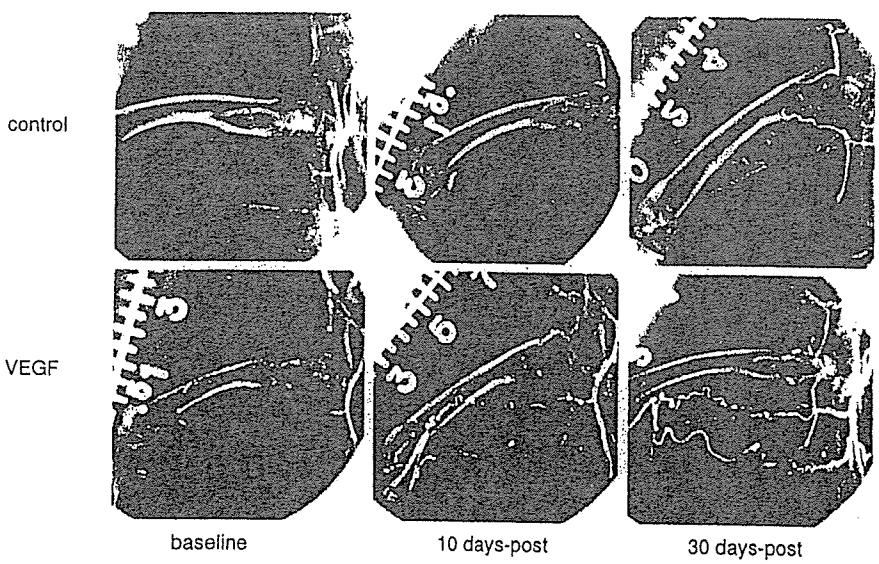

図1　VEGF蛋白投与後の血管造影所見
生理食塩水または組み換えVEGF蛋白を家兎虚血肢モデルの内腸骨動脈内へ選択的に投与し，側副路の発達を比較した。上段の対照群では，治療後30日間において側副血行路に大きな変化は認められない。これに対し，下段のVEGF治療群においては治療から10日間で側副路の著明な改善が認められる。

（文献1より引用）

まな血管新生療法が開発されてきた。各々の治療法の有効性が臨床試験により明らかにされつつある中，その対象疾患も，末梢動脈疾患から虚血性心疾患や虚血性脳疾患などへと拡大されてきている。

III 血管新生療法の臨床応用まで

血管新生療法のコンセプトは決して新しいものではない。1980年代後半には，ネコの虚血肢モデルに対して大網の脂肪分画を投与し，虚血を改善させる試みが行われている。大網や脂肪細胞の再生医療への応用は最近のトピックであり，このような研究がすでに20年以上前に存在したことは興味に値する。これらの血管新生療法とIsnerらが行ったそれとの違いは，後者がVEGFという血管内皮細胞に特異的な増殖因子を用いた点にある。1990年代初頭，Isnerらは家兎の虚血肢モデルにVEGF蛋白を投与することにより下肢の側副血行発達を促進できないか検討を行った（図1）[1]。VEGF蛋白の動脈投与，静脈投与，繰り返し静脈投与，ヘパリン併用などのさまざまな投与法が検討されたが，投与法の如何にかかわらず，側副血行の促進には100～1,000 μgのVEGF蛋白が必要なことが明らかとなった。しかしながら，大量のVEGF蛋白を投与すると，投与した蛋白が全身を循環し，非目的部位へと到達するのは避け難い。血管増殖因子の全身への拡散は，糖尿病患者においては網膜症を悪化させ，癌患者では腫瘍血管の発達を促進させ得る。また，一部の血管増殖因子は一酸化窒素（NO）を介した血管拡張作用を有しており，遷延性低血圧を惹起させ得る。事実，VEGF蛋白を用いた血管新生療法の臨床試験では，低血圧を避けるためにその投与量が制限された。

大量の蛋白投与に伴う副作用を回避するために行き着いた結論が，遺伝子を用いたローカルドラッグデリバリーであった。Isnerらはカテーテルを用いてVEGF遺伝子を経皮的に血管細胞へと導入し，それらの細胞からVEGF蛋白を分泌させることに成功した。ここでは，表面が親水性ゲルでコーティングされた冠動脈形成術用バルーンカテーテル（ハイドロゲル・バルーンカテーテル）を用いて下肢血管への遺伝子導入が行われた。ハイドロゲルは，狭窄部位におけるバルーンの通過性を改善するために施されたコーティングであるが，Isnerらはこのゲルにプラスミドの水溶液をしみ込ませ，遺伝子キャリアとして使用したのである。通常のPTAテクニックを用いてバルーンを目的部位へと進め，4～8気圧で1分間バルーンを拡張させることで遺伝子を血管壁へと導入する。その遺伝子導入効率はリポゾームによる遺伝子導入に比し100倍以上の高効率ではあったが，βガラクトシダーゼ遺伝子を用いた組織所見の検討では，導入部位のわずか0.1%以下の細胞にしか遺伝子発現が認められなかった[4]。このわずかな細胞によって血管新生を促進することが可能なのか疑問なわけだが，遺伝子の導入効率（transfection efficiency）と治療効率（therapeutic efficiency）とは同義ではない。遺伝子産物である増殖因子が細胞外へと分泌されれば，たとえ導入効率は低くとも，パラクリン効果が期待できる[5]。この仮説は動物実験によって検証された。すなわち，ハイドロゲル・バルーンカテーテルを用いて家兎虚血肢モデル

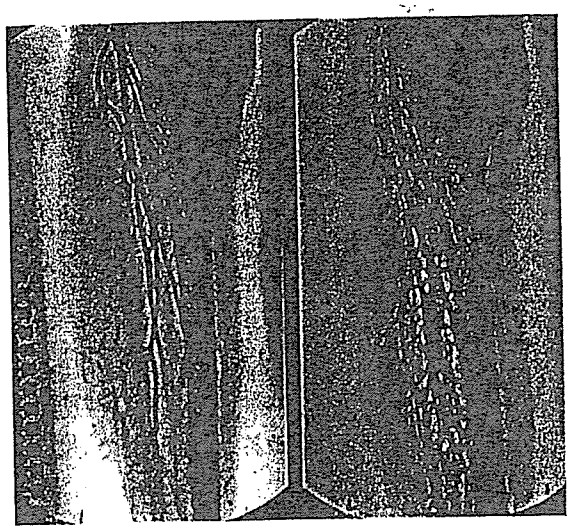

図2　遺伝子治療前後におけるDSA（digital subtraction angiography）所見
左：遺伝子治療前，右：遺伝子治療1ヵ月後
VEGFによる遺伝子治療の1ヵ月後，下肢側副血行の著明な発達を認める。
（文献2より引用）

にVEGF遺伝子の導入を行うと，約3週間にわたりその発現が認められ，VEGF蛋白の動脈内投与と同等以上の側副路発達効果が得られたのである。一方，末梢血中のVEGF蛋白の濃度はELISAによる測定限界付近にあり極めて低値であった。つまり，遺伝子の導入効率は低くとも，局所では治療効果を得るに十分な組織濃度が維持され，逆に血中濃度は希釈効果によって低く抑えられるわけである。ここで忘れてならないのは，本法がプラスミドDNA以外には何のベクターも用いない遺伝子導入法であった点である（naked DNAアプローチ）。この研究によって，臨床応用における本法の高い安全性が裏づけられた。

Ⅳ　VEGFを用いた血管新生療法の臨床応用

1994年，Isnerらは血管新生療法の臨床試験を開始した[2]。前述のように，この試験は循環器領域における初の遺伝子治療としても知られており，内科治療や外科治療が無効な重症末梢動脈疾患患者を対象に行われた。遺伝子治療から1～2ヵ月で，血管造影上，新生血管の出現が認められ，これに伴い下肢疼痛や難治性潰瘍が消失した（図2）。副作用は下腿浮腫や良性血管腫など，一過性の軽微なものだけであった。しかしながら，バルーンカテーテルを用いた遺伝子導入は，動脈穿刺が不可能な例，動脈硬化が高度でカテーテルの標的血管へのアクセスが困難な例，遺伝子導入に際し解離などの血管損傷リスクが高い例には施行できない。そこで考案されたのが，虚血筋への遺伝子導入である。Baumgartnerらは，VEGFプラスミドの虚血下肢への筋注を行い，7～8割の症例において血管造影上の側副路発達や臨床症状改善を得ることに成功した[6]。筋注

法の導入は，遺伝子治療の手技を単純化させるだけでなく，それまでカテーテルのアクセスが困難であった症例さえも治療可能とし，その適応症例を大きく拡大させることにつながった。また，筋注法は，心筋への遺伝子導入にも応用可能であり，虚血性心疾患に対する血管新生療法の臨床応用への契機ともなった。

Ⅴ 血管新生療法の問題点

末梢動脈疾患に対する血管新生療法は，今から約10年前，VEGFを用いた遺伝子治療として幕を開けた。重症下肢虚血に対する本法の治療成績は良好である。安静時疼痛や難治性潰瘍を有する患者の少なくとも6～7割において，臨床所見の改善が期待可能である。しかしながら，本法のメカニズムに関しては不明な点が少なくない。臨床症状の改善にもかかわらず血管造影での改善が明らかでないことも多く，はたして血管新生療法によって血管新生が本当に促進されるのか，その治療メカニズムの基本的な部分でさえ，解明されていないのが実情である。また，遺伝子のパテント問題，遺伝子を用いることの倫理的問題など，一般臨床の場に普及するに至るまでに解決されるべき問題も決して少なくない。

●文　献

1) Takeshita S, Zheng LP, Brogi E, et al : Therapeutic angiogenesis ; a single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. J Clin Invest 93 : 662-670, 1994
2) Isner JM, Pieczek A, Schainfeld R, et al : Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patients with ischemic limb. Lancet 348 : 370-374, 1996
3) Ferrara N, Henzel WJ : Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun 161 : 851-855, 1989
4) Takeshita S, Weir L, Chen D, et al : Therapeutic angiogenesis following arterial gene transfer of vascular endothelial growth factor in a rabbit model of hindlimb ischemia. Biochem Biophys Res Commun 227 : 628-635, 1996
5) Takeshita S, Losordo DW, Kearney M, et al : Time course of recombinant protein secretion after liposome-mediated gene transfer in a rabbit arterial organ culture model. Lab Invest 71 : 387-391, 1994
6) Baumgartner I, Pieczek A, Manor O, et al : Constitutive expression of phVEGF165 after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia. Circulation 97 : 1114-1123, 1998

●参考：海外の研究施設

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Intravenous injection of phagocytes transfected ex vivo with FGF4 DNA/ biodegradable gelatin complex promotes angiogenesis in a rat myocardial ischemia/reperfusion injury model

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Abstract Conventional gene therapies still present difficulties due to poor tissue-targeting, invasiveness of delivery, method, or the use of viral vectors. To establish the feasibility of using non-virally ex vivo transfected phagocytes to promote angiogenesis in ischemic myocardium, gene-transfection into isolated phagocytes was performed by culture with positively charged gelatin impregnated with plasmid DNA. A high rate of gene transfection was achieved in rat macrophages and human monocytes, but not in mouse fibroblasts. The efficiency was 68 ± 11 % in rat macrophages and 78 ± 8 % in human monocytes. Intravenously injected phagocytes accumulated predominantly in ischemic tissue (13 ± 8 %) and spleen (84 ± 6 %), but negligibly in other organs in rodents. The efficiency of accumulation in the target ischemic tissue reached more than 86 % on direct local tissue injection. In a rat model of myocardial ischemia-reperfusion, intravenous injection of fibroblast growth factor 4 (FGF4)-gene-transfected macrophages significantly increased regional blood flow in the ischemic myocardium (78 ± 7.1 % in terms of flow ratio of ischemic/non-ischemic myocardium) compared with intravenous administration of saline (36 ± 11 %) or non-transfected macrophages (42 ± 12 %), or intramuscular administration of naked DNA encoding FGF4 (75 ± 18 %). Enhanced angiogenesis in the ischemic tissue we confirmed histologically. Similarly, intravenous injection of FGF4-gene-transfected monocytes enhanced regional blood flow in an ischemic hindlimb model in mice (93 ± 22 %), being superior to the three other treatments described above (38 ± 12, 39 ± 15, and 55 ± 12 %, respectively).

Phagocytes transfected ex vivo with FGF4 DNA/gelatin promoted angiogenesis. This approach might have potential for non-viral angiogenic gene therapy.

Key words angiogenesis – cells – gene therapy – growth substances – ischemia

Abbreviations and acronyms

ANOVA = analysis of variance
FGF4 = fibroblast growth factor-4
GFP = green fluorescent protein
pI = isoelectric point

Introduction

Conventional gene therapies still require improvement with regard to transfection efficiency and safety [1, 2], as well as tissue targeting [3], despite recent advances. Achievement of a high transfection rate often requires a viral vector, but the safety of the viruses has not yet been

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established [4–6]. Conventional non-viral vectors seem to be inferior to viral ones in transfection efficiency, except for nucleofection [7, 8]. Conventional gene therapy using a viral vector can induce inflammation in the gene-transduced tissue [9]. Moreover, in vivo gene-delivery to the localized target tissue usually necessitates invasive approaches. For example, direct gene-transfection to cardiomyocytes requires surgical operation [10] or cardiac catheterization [11, 12]. On the other hand, ex vivo gene-transfection is less invasive, but tissue-targeting by intravenous injection is difficult to achieve [3].

Macrophages accumulate in ischemic tissue based on the mechanism of immune response (chemotaxis) [13]. This suggests that intravenous transplantation of macrophages may target the ischemic tissue in vivo. Tabata et al. previously reported that gelatin particles are phagocytized by macrophages [14, 15]. The isoelectric point (pI) of gelatin can be changed by modification of its residues, and positively charged gelatin can be impregnated with negatively charged substances [16] such as nucleic acid [17]. Thus, gelatin may be suitable as a vector for transfecting phagocytes ex vivo.

We describe here a study aimed at examining the feasibility of a new concept for less invasive, cell-based gene therapy, by means of ex vivo gene transfection into isolated phagocytes (macrophages and monocytes) using a non-viral vector, gelatin, followed by intravenous injection of the transfected phagocytes. The present method has significant advantages over conventional cell-based gene delivery [18, 19], in that the intravenously injected cells (phagocytes) not only produce protein from the transfected gene, but have a tissue-targeting ability.

Methods

This study was performed in accordance with the Guideline of Tokai University School of Medicine on Animal Use, which conforms to the NIH Guide for the Care and Use of Laboratory Animals (DHEW publication No. (NIH) 86-23, Revised 1985, Offices of Science and Health Reports, DRR/NIH, Bethesda, MD 20205).

Animals

A total of 121 Fisher rats (male, 10 weeks old, Clea Japan Inc., Tokyo) and 61 nude SCID mice (male, 6 weeks old, Shizuoka Animal Center, Shizuoka, Japan) were used. Rats were anesthetized by inhalation of diethyl ether for harvesting macrophages and with isofluren (1.5–3%) for thoracotomy, after which they were mechanically ventilated with a mixture of oxygen and nitrous oxide. Mice were anesthetized by intraperitoneal injection of sodium pentobarbital (40 mg/kg).

A model of myocardial ischemia-reperfusion injury was prepared in 41 rats. The remaining 80 rats were used for collecting activated macrophages. The heart was exposed via thoracotomy, and the proximal left anterior descending coronary artery was ligated [20] for 180 min, followed by reperfusion. A model of hindlimb ischemia was prepared in 61 mice. The left femoral artery was ligated and resected [21].

Cells

Macrophages were obtained from 80 rats. Thioglycolate (4%, 8 ml) was injected into the peritoneal cavity, and after 4 days, peritoneal macrophages were collected [22]. Monocytes were obtained from peripheral blood of healthy volunteers. Leukocyte-rich plasma was obtained by dextran 500 sedimentation and layered onto Nycoprep 1.068 (Nycomed, Birmingham, UK). The monocyte-containing layer was aspirated, washed twice and allowed to adhere to the dish for 90 minutes. Fibroblasts (NIH 3T3, Invitrogen Corporation, Carlsbad, CA) were also used. The cells were resuspended in RPMI 1640 medium (Sigma) containing 5% heat-inactivated fetal calf serum and cultured for 7–14 days. The cell viability and type were determined by trypan blue exclusion and by immunostaining using anti-macrophage antibody up to 14 days.

Genes and vector

Complementary DNA (cDNA) of green fluorescent protein (GFP), Renilla luciferase or human hst1/FGF4 (FGF4) [17] was inserted into the expression vector pRC/CMV (Invitrogen Corporation, Carlsbad, CA) and the constructs were designated as pRC/CMV-GFP, pRC/CMV-luciferase and pRC/CMV-HST1-10, respectively. Preparation and purification of the plasmid from cultures of pRC/CMV-GFP-, pRC/CMV-luciferase-, or pRC/CMV-HST1-10-transformed *Escherichia coli* were performed by equilibrium centrifugation in cesium chloride-ethidium bromide gradients.

Gelatin was prepared from porcine skin [14]. After swelling in water the gelatin particles used in this study were spheroids with a diameter of approximately 5–30 μm, water content of 95%, and pI of 11. Gelatin (2 mg) was incubated with 50 μg of the plasmid for 7 days at 4 °C to make a gelatin-DNA complex [14].

Experimental protocols

Ex vivo gene transfection Macrophages, monocytes, and fibroblasts (1×10^6) were cultured with the gelatin-DNA complex (2 mg of gelatin plus 50 μg of DNA) for 14 days on a culture dish (100 mm in diameter). Gene ex-

pression of GFP was evaluated by fluorescence microscopy and fluorescence-activated cell sorting. Luciferase activity in the cell lysate was evaluated with a photon counter system after cell lysis [23].

Organ distribution of phagocytes injected intravenously and directly into ischemic muscle To examine tissue-targeting by intravenous injection of transfected phagocytes, the distribution of the cells into organs was evaluated by immunohistochemistry. In the rat model of myocardial ischemia-reperfusion injury, the GFP-gene-transfected macrophages (1.0×10^6 each) were injected into the superficial dorsal vein of the penis at the initiation of reperfusion (n = 7 and 5, respectively). In the mouse model of hindlimb ischemia, the GFP-gene-transfected monocytes (1.0×10^6) were injected into the caudal vein 14 days after induction of ischemia (n = 5). To examine the tissue-targeting by direct local injection of transfected phagocytes, the distribution of the cells into organs was also evaluated. In the rat model of myocardial ischemia-reperfusion injury (n = 7) and the mouse model of hindlimb ischemia (n = 5), the same numbers of transfected macrophages and monocytes were directly injected into ischemic myocardium and ischemic skeletal muscle, respectively. Tissue samples were obtained 24 hours after cell administration. Each tissue was homogenized and cytospin was performed. Immunohistochemical analysis was done with anti-GFP antibody (CLONTECH, USA. GFP-monoclonal antibody). GFP positive macrophages were counted in each tissue and expressed as a percentage of total GFP-positive cells.

Amelioration of ischemia by intravenous injection of angiogenic gene-transfected phagocytes The angiogenic effect of intravenously injected FGF4-gene-transfected phagocytes on the ischemia models was evaluated. In the rat model of myocardial ischemia-reperfusion injury, FGF4-gene-transfected macrophages (n = 5), non-transfected macrophages (1.0×10^6 each) (n = 5), or saline (n = 5) were injected into the superficial dorsal vein of the penis, or naked FGF4-DNA (50 μg) was injected directly into the ischemic myocardium (n = 5), at the initiation of reperfusion. Fourteen days after the cell administration, blood flows in the ischemic and non-ischemic regions in the heart were evaluated with a noncontact laser Doppler flowmeter (FLO-N1, Omegawave Corporation). Then, tissue samples were obtained and histological analysis was performed. In a mouse model of hindlimb ischemia, just after induction of ischemia, FGF4-gene-transfected monocytes (n = 15), non-transfected monocytes (n = 8) (1.0×10^6 each), or saline (n = 10) were injected into the caudal vein, or naked FGF4-DNA (50 μg) was injected directly into the ischemic muscle (n = 12). Fourteen days after induction of ischemia, blood flows in the limbs were evaluated with the noncontact laser Doppler flowmeter (FLO-N1, Omegawave Corporation).

Histology

Ten micrometer sections were cut from formalin-fixed, paraffin-embedded tissue. Two sections were used for H.E. staining and azan staining, and eight sections were used for immunohistochemical staining. Immunohistochemical staining was performed by an indirect immunoperoxidase method. Anti-GFP antibody, anti-Mac1 antibody (BMA Biomedicals Ag, Switzerland), and anti-CD31 antibody (Serotec, UK) were used as primary antibodies. Mac1-antigen is specific to macrophages/monocytes. Anti-Ig, peroxidase-linked species-specific F(ab')2 fragments (Amersham Pharmacia Biotech UK Ltd., UK), were used as a secondary antibody. Double staining was performed with alkaline staining and peroxidase staining. The vessel density stained with von Willebrand factor-antibody was calculated by morphometric assessment in one 16 randomly selected fields of each heart and expressed as number/mm^2.

Statistical analysis

Data are presented as mean values ± SD. Differences were assessed by using ANOVA (analysis of variance) with the Scheffe's multiple comparisons test. A value of $P < 0.05$ was considered statistically significant.

Results

Ex vivo gene transfection

We studied whether genes could be transfected into isolated rat macrophages, human monocytes, and mouse fibroblasts ex vivo by using gelatin. Transfection of the GFP gene into isolated rat macrophages (Figs. 1A and B) and human monocytes (Figs. 1C and D), but not into mouse fibroblasts (data not shown), was achieved by culture with gelatin-DNA complex for 14 days. The gene transfection efficiency into rat macrophages was 68 ± 11 % (30 experiments, Fig. 2A) and that into human monocytes was 78 ± 8 % (30 experiments) as determined with a fluorescence activated cell sorter. Sequential analysis after luciferase-gene transfection into rat macrophages revealed high expression after 14 days of culture (Fig. 2B).

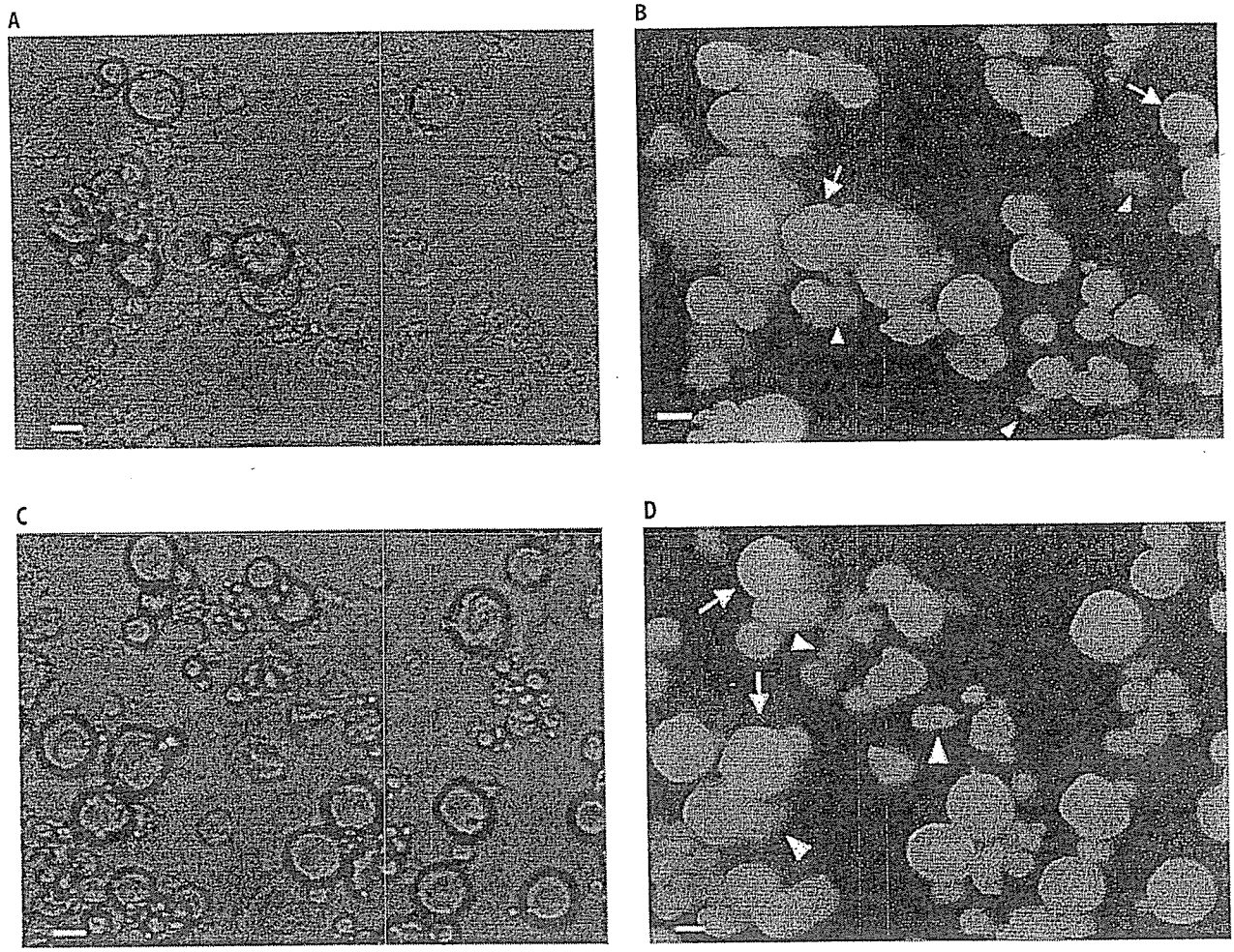
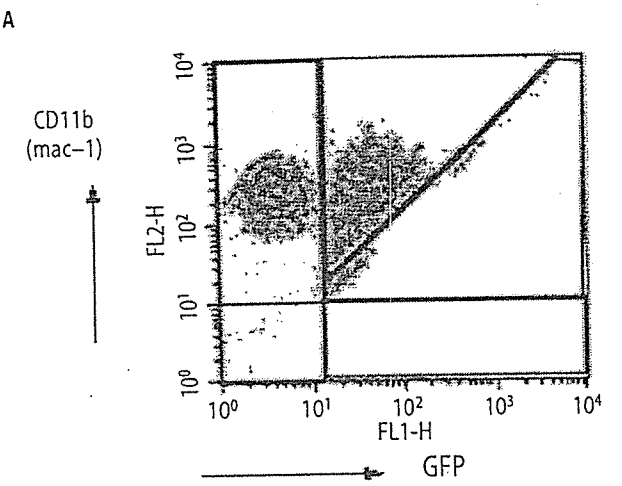


Fig. 1 Fluorescent presentation of ex vivo gene transfection with gelatin-DNA complex in macrophages/monocytes as well as fibroblasts. Rat macrophages (**A** and **B**) and human monocytes (**C** and **D**) were cultured with gelatin-GFP-gene complex for 14 days. Transmittance microscopic images (**A** and **C**) and fluorescence images (**B** and **D**) of the cells are shown. Macrophages (**B**) and monocytes (**D**) show fluorescence due to GFP. Arrowheads indicate GFP-expressing cells. Arrows indicate gelatin particles themselves. Bars = 20 μm

Organ distribution of phagocytes injected intravenously or directly into ischemic muscle

We studied quantitatively whether intravenously injected luciferase-gene-transfected phagocytes could target ischemic tissues (the third and fifth columns from the left in Table 1). In non-ischemic rats, the injected macrophages were recognized almost exclusively in the spleen (98 ± 4 %) (n = 7, the second column in Table 1). In non-ischemic mice, similar results were observed (n = 7, data not shown). In a rat with myocardial ischemia-reperfusion injury, some of the intravenously injected macrophages were incorporated into the heart (the third column in Table 1). The incorporation into the post-ischemic pericardium amounted to 13 ± 6 % (n = 7) (non-ischemic rats 0 ± 0 %, n = 7, Table 1). The incorporated cells expressed GFP (Fig. 3). Fibrosis with inflammatory infiltrates was recognized in the anterior wall of the left ventricle, extending to the interventricular septum (Figs. 3A and B). These infiltrates were mainly polymorphonuclear leukocytes and macrophages (Figs. 3C and D). Approximately 20 % of the macrophages showed GFP-positivity in this area (Figs. 3E and F). Similar tissue-targeting by intravenously injected monocytes was confirmed in a mouse model with hindlimb ischemia (13 ± 7 %, n = 7, the fifth column in Table 1). Furthermore, we studied whether local intramuscular injection increased the degree of tissue targeting (the fourth and sixth columns from the left in Table 1). After direct injection of phagocytes into ischemic muscle, 86 ± 10 % and 88 ± 6 % of the cells remained in the target tissue in the two models. Thirteen and 11 % of phagocytes in-



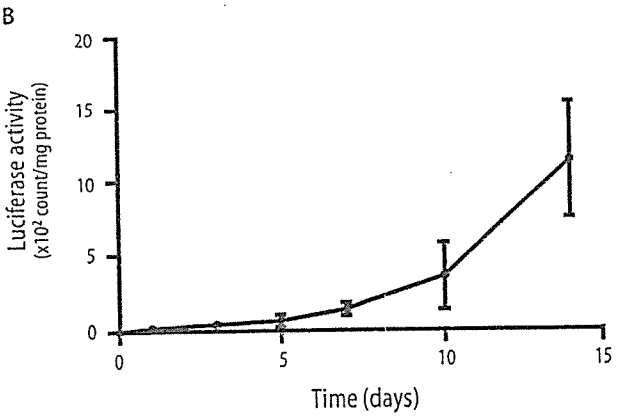


Fig. 2 Quantitative assessment of gene transfection into rat macrophages. (A) Fluorescence-activated cell sorting analysis of transfected macrophages done on day 14 of culture with reference to GFP-positive and Mac1-positive cells. (B) Sequential changes of luciferase activity in cultured macrophages in the presence of luciferase-gene-gelatin complex. Values are mean ± SD. The number of experiments is shown in parentheses

jected into the cardiac or hindlimb muscle migrated to the spleen. In the other organs, accumulation of phagocytes were negligible.

Amelioration of ischemia by intravenously injected angiogenic-gene-transfected phagocytes

In the rat model with myocardial ischemia-reperfusion injury, we studied the angiogenic effect of intravenously injected macrophages transfected with fibroblast growth factor 4 (FGF4) gene by using gelatin. Intravenous injection of these macrophages (1.0×10^6) significantly increased the regional blood flow in the ischemic myocardium (78 ± 7.1 %, n = 8, in terms of flow ratio of ischemic/non-ischemic myocardium) compared with the other three treatments ($P < 0.05$, ANOVA), that is, intravenous administration of saline (35 ± 10 %, n = 8), intramuscular administration of naked DNA encoding FGF4 (50 μg, direct intramyocardial injection after thoracotomy) (58 ± 5.3 %, n = 8), and intravenous administration of the same number of non-transfected macrophages (42 ± 12 %, n = 8) (Fig. 4A). Histological analyses revealed angiogenesis in the ischemic tissue after the administration of transfected cells (Figs. 4B and C). Similar results were observed in the mouse model with hindlimb ischemia. Intravenous injection of FGF4-gene-transfected monocytes (1.0×10^6) enhanced regional blood flow in the ischemic leg (Fig. 4D). The increase of blood flow in the mice with transfected monocytes (93 ± 22 % in terms of flow ratio of ischemic/non-ischemic leg) was significantly larger than those obtained with the other three treatments described above (38 ± 12, 55 ± 12, and 39 ± 15 %, $P < 0.05$, ANOVA). Neither lymph node swelling in any part of the body nor pathologic change in the spleen or lung, such as angioma or abnormal immune response, was found in any of the animals.

Table 1 Organ distribution of phagocytes injected into the vein and into local tissue

Organ	Normal i.v. (7 rats)	Myocardial injury i.v. (7 rats)	Myocardial injury i.m. (7 rats)	Hindlimb ischemia i.v. (7 mice)	Hindlimb ischemia i.m. (7 mice)
Heart	0 ± 0	13 ± 6	86 ± 10	0 ± 0	0 ± 0
Hindlimb muscle	0 ± 0	0 ± 0	0 ± 0	13 ± 7	88 ± 6
Spleen	98 ± 4	84 ± 6	13 ± 10	84 ± 6	11 ± 6
Lung	1 ± 2	1 ± 1	1 ± 2	1 ± 2	1 ± 1
Liver	1 ± 2	1 ± 1	1 ± 1	1 ± 2	1 ± 1
Brain	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Kidney	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Intestine	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Each value shows a distribution ratio (%) into organs of transfected macrophages/monocytes (mean ± SD). *i.v.* intravenous injection into the vein; *i.m.* direct injection into the jeopardized muscle

Discussion

The advantages of the present method are as follows. First, genes can easily be transfected into phagocytes (macrophages/monocytes). In preliminary experiments, we found that genes can also be transfected into endothelial progenitor cells [25]. Compared with other transfection method, the transfection efficiency was high (68 ± 11 %) and it is not necessary to use a potentially hazardous viral vector [2, 26, 32]. Second, the phagocytes can target the pathologic tissues by chemotaxis even after intravenous injection, and higher tar-

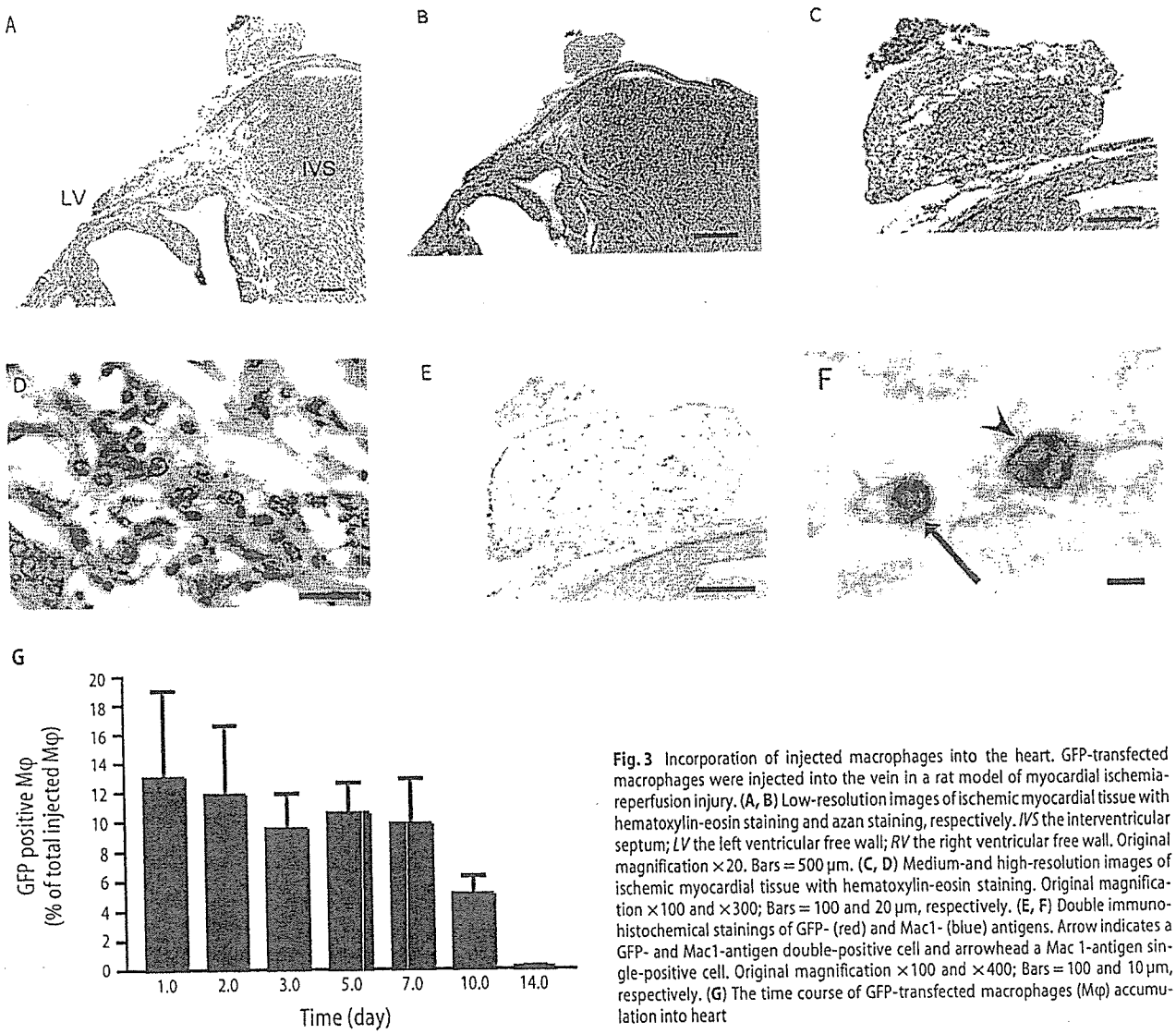


Fig. 3 Incorporation of injected macrophages into the heart. GFP-transfected macrophages were injected into the vein in a rat model of myocardial ischemia-reperfusion injury. (**A, B**) Low-resolution images of ischemic myocardial tissue with hematoxylin-eosin staining and azan staining, respectively. *IVS* the interventricular septum; *LV* the left ventricular free wall; *RV* the right ventricular free wall. Original magnification ×20. Bars = 500 µm. (**C, D**) Medium-and high-resolution images of ischemic myocardial tissue with hematoxylin-eosin staining. Original magnification ×100 and ×300; Bars = 100 and 20 µm, respectively. (**E, F**) Double immunohistochemical stainings of GFP- (red) and Mac1- (blue) antigens. Arrow indicates a GFP- and Mac1-antigen double-positive cell and arrowhead a Mac 1-antigen single-positive cell. Original magnification ×100 and ×400; Bars = 100 and 10 µm, respectively. (**G**) The time course of GFP-transfected macrophages (Mφ) accumulation into heart

geting is available if they are administered locally. The injection is repeatable. We confirmed that the angiogenic gene-transfected phagocytes enhanced angiogenesis after ischemia-reperfusion injury in rat heart and ameliorated ischemia in a mouse hindlimb model.

The injected phagocytes migrated into pathologic tissues, presumably in response to the release of cytokines such as monocyte chemoattractant protein 1 by injured endothelial cells [27]. Adhesion molecules such as P-selectin [28] are probably involved in the recruitment of phagocytes to the vessel wall. The injected phagocytes also migrated to the spleen, but no pathologic change was found in the spleen.

The present method has several advantages over conventional methods of cell-based gene therapy such as fibroblast-based and smooth muscle cell-based approaches [18, 19, 33, 34]. For example, monocytes do not aggregate in vessels, while fibroblasts or smooth muscle cells cannot be injected intravenously because of aggregation. The transfected phagocytes not only synthesize protein from the transfected gene, but also are partially targeted to the impaired tissue. In addition, the transfection rate was better than those of methods such as lipofection, viral vectors and electroporation [26, 29]. The newly developed technique of nucleofection has a transfection efficiency of 40–70 % [30], which is similar to that of our method, but our procedure is easier to use [30, 31]. Further, the therapeutic effect obtained here was superior to that of conventional gene therapy which we reported previously, i.e., intramuscular injection of

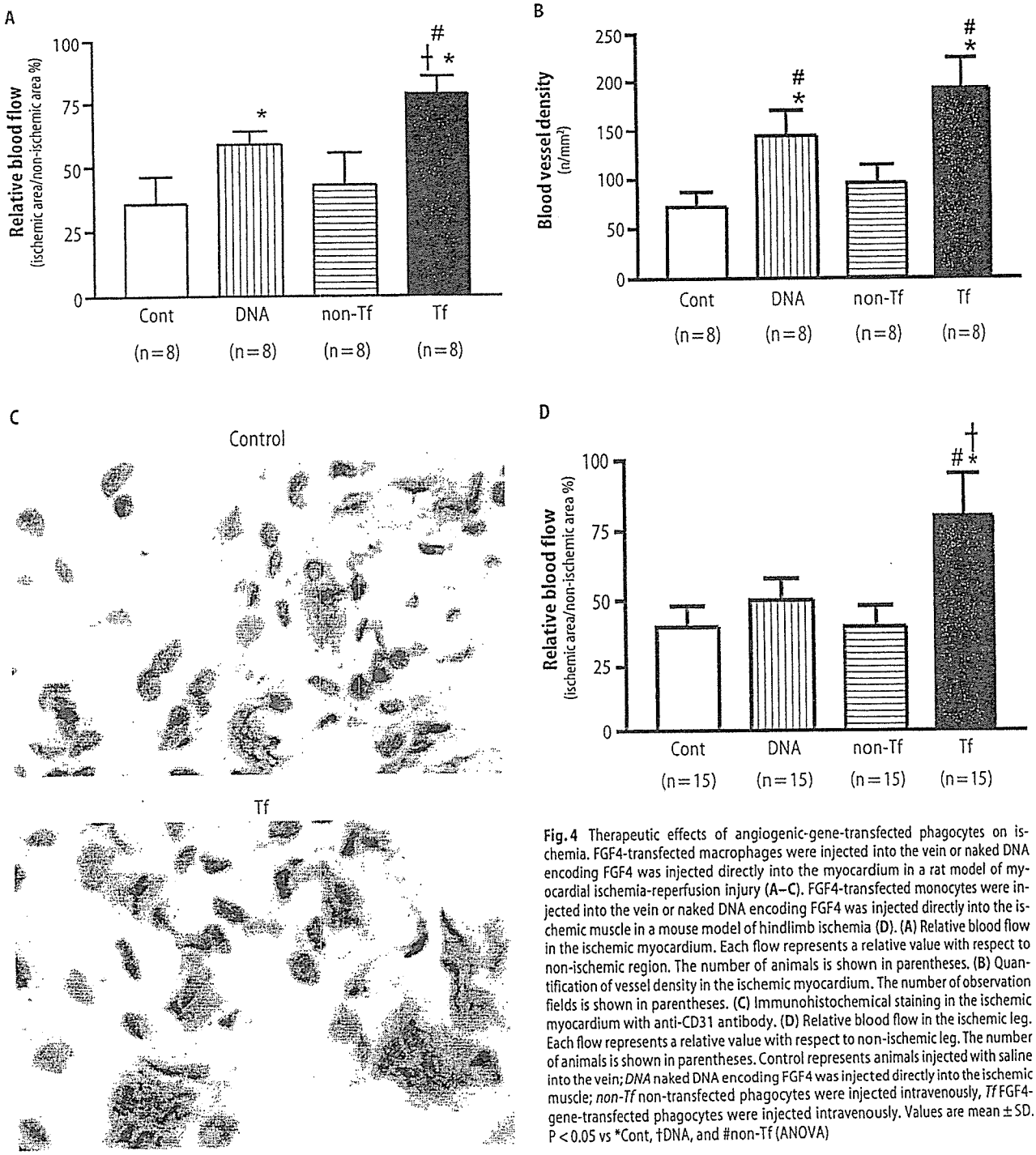


Fig. 4 Therapeutic effects of angiogenic-gene-transfected phagocytes on ischemia. FGF4-transfected macrophages were injected into the vein or naked DNA encoding FGF4 was injected directly into the myocardium in a rat model of myocardial ischemia-reperfusion injury (**A–C**). FGF4-transfected monocytes were injected into the vein or naked DNA encoding FGF4 was injected directly into the ischemic muscle in a mouse model of hindlimb ischemia (**D**). (**A**) Relative blood flow in the ischemic myocardium. Each flow represents a relative value with respect to non-ischemic region. The number of animals is shown in parentheses. (**B**) Quantification of vessel density in the ischemic myocardium. The number of observation fields is shown in parentheses. (**C**) Immunohistochemical staining in the ischemic myocardium with anti-CD31 antibody. (**D**) Relative blood flow in the ischemic leg. Each flow represents a relative value with respect to non-ischemic leg. The number of animals is shown in parentheses. Control represents animals injected with saline into the vein; *DNA* naked DNA encoding FGF4 was injected directly into the ischemic muscle; *non-Tf* non-transfected phagocytes were injected intravenously, *Tf* FGF4-gene-transfected phagocytes were injected intravenously. Values are mean ± SD. $P < 0.05$ vs *Cont, †DNA, and #non-Tf (ANOVA)

naked DNA, in ischemia models of heart and leg [17]. The major disadvantage of our method is the cell preparation time of 2 weeks before therapy can be started, and further work is needed to speed up this process.

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References

1. Pfeifer A, Verma IM (2001) Gene therapy: promises and problems. Annu Rev Genomics Hum Genet 2:177–211
2. Watson DJ, Kobinger GP, Passini MA, Wilson JM, Wolfe JH (2002) Targeted transduction patterns in the mouse brain by lentivirus vectors pseudotyped with VSV, Ebola, Mokola, LCMV, or MuLV envelope proteins. Mol Ther 5:528–537
3. Li Q, Bolli R, Qiu Y, Tang XL, Guo Y, French BA (2001) Gene therapy with extracellular superoxide dismutase protects conscious rabbits against myocardial infarction. Circulation 103: 1893–1898
4. Ferber D (2001) Gene therapy. Safer and virus-free? Science 294:1638–1642
5. Kay MA, Glorioso JC, Naldini L (2001) Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. Nat Med 7:33–40
6. Isner JM (2002) Myocardial gene therapy. Nature 415:234–239
7. Nishikawa M, Hashida M (2002) Nonviral approaches satisfying various requirements for effective in vivo gene therapy. Biol Pharm Bull 25:275–283
8. Schakowski F, Buttgereit P, Mazur M, Mazur M, Marten A, Schottker B, Gorschluter M, Schmidt-Wolf IG (2004) Novel non-viral method for transfection of primary leukemia cells and cell lines. Genet Vaccines Ther 2:1
9. Tomasoni S, Benigni A (2004) Gene therapy: how to target the kidney. Promises and pitfalls. Curr Gene Ther 4: 115–122
10. Losordo DW, Vale PR, Symes JF, Dunnington CH, Esakof DD, Maysky M, Ashare AB, Lathi K, Isner JM (1998) Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. Circulation 98:2800–2804
11. Kornowski R, Leon MB, Fuchs S, Vodovotz Y, Flynn MA, Gordon DA, Pierre A, Kovesdi I, Keiser JA, Epstein SE (2000) Electromagnetic guidance for catheter-based transendocardial injection: a platform for intramyocardial angiogenesis therapy. Results in normal and ischemic porcine models. J Am Coll Cardiol 35:1031–1039
12. Laitinen M, Hartikainen J, Hiltunen MO, Eranen J, Kiviniemi M, Narvanen O, Makinen K, Manninen H, Syvanne M, Martin JF, Laakso M, Yla-Herttuala S (2000) Catheter-mediated vascular endothelial growth factor gene transfer to human coronary arteries after angioplasty. Hum Gene Ther 11:263–270
13. Ramsay SC, Weiller C, Myers R, Cremer JE, Luthra SK, Lammertsma AA, Frackowiak RS (1992) Monitoring by PET of macrophage accumulation in brain after ischaemic stroke. Lancet 339:1054–1055
14. Tabata Y, Ikada Y (1987) Macrophage activation through phagocytosis of muramyl dipeptide encapsulated in gelatin microspheres. J Pharm Pharmacol 39: 698–704
15. Tabata Y, Ikada Y (1988) Macrophage phagocytosis of biodegradable microspheres composed of L-lactic acid/glycolic acid homo- and copolymers. J Biomed Mater Res 22:837–858
16. Ikada Y, Tabata Y (1998) Protein release from gelatin matrices. Adv Drug Deliv Rev 31:287–301
17. Kasahara H, Tanaka E, Fukuyama N, Sato E, Sakamoto H, Tabata Y, Ando K, Iseki H, Shinozaki Y, Kimura K, Kuwabara E, Koide S, Nakazawa H, Mori H (2003) Biodegradable gelatin hydrogel potentiates the angiogenic effect of fibroblast growth factor 4 plasmid in rabbit hindlimb ischemia. J Am Coll Cardiol 41:1056–1062
18. Xie Y, Yang ST, Kniss DA (2001) Three-dimensional cell-scaffold constructs promote efficient gene transfection: implications for cell-based gene therapy. Tissue Eng 7:585–598
19. Panetta CJ, Miyauchi K, Berry D, Simari RD, Holmes DR, Schwartz RS, Caplice NM (2002) A tissue-engineered stent for cell-based vascular gene transfer. Hum Gene Ther 13:433–441
20. Gidh-Jain M, Huang B, Jain P, el-Sherif N (1996) Differential expression of voltage-gated K+ channel genes in left ventricular remodeled myocardium after experimental myocardial infarction. Circ Res 79:669–675
21. Takeshita S, Zheng LP, Brogi E, Kearney M, Pu LQ, Bunting S, Ferrara N, Symes JF, Isner JM (1994) Therapeutic angiogenesis. A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. J Clin Invest 93:662–670
22. Ribeiro RA, Flores CA, Cunha FQ, Ferreira SH (1991) IL-8 causes in vivo neutrophil migration by a cell-dependent mechanism. Immunology 73:472–477
23. Fukuyama N, Ichimori K, Su Z, Ishida H, Nakazawa H (1996) Peroxynitrite formation from activated human leukocytes. Biochem Biophys Res Commun 224:414–419
24. Mori H, Haruyama S, Shinozaki Y, Okino H, Iida A, Takanashi R, Sakuma I, Husseini WK, Payne BD, Hoffman JI (1992) New nonradioactive microspheres and more sensitive X-ray fluorescence to measure regional blood flow. Am J Physiol 263:H1946–H1957
25. Nagaya N, Kangawa K, Kanda M, Uematsu M, Horio T, Fukuyama N, Hino J, Harada-Shiba M, Okumura H, Tabata Y, Mochizuki N, Chiba Y, Nishioka K, Miyatake K, Asahara T, Hara H, Mori H (2003) Hybrid cell-gene therapy for pulmonary hypertension based on phagocytosing action of endothelial progenitor cells. Circulation 108: 889–895
26. Kobinger GP, Deng S, Louboutin JP, Vatamaniuk M, Matschinsky F, Markmann JF, Raper SE, Wilson JM (2004) Transduction of human islets with pseudotyped lentiviral vectors. Hum Gene Ther 15:211–219
27. Leonard EJ, Yoshimura T (1990) Human monocyte chemoattractant protein-1 (MCP-1). Immunol Today 11:97–101
28. Ikeda Y, Young LH, Lefer AM (2002) Attenuation of neutrophil-mediated myocardial ischemia-reperfusion injury by a calpain inhibitor. Am J Physiol Heart Circ Physiol 282:H1421–H1426
29. Veit K, Boissel JP, Buerke M, Grosser T, Meyer J, Darius H (1999) Highly efficient liposome-mediated gene transfer of inducible nitric oxide synthase in vivo and in vitro in vascular smooth muscle cells. Cardiovasc Res 43:808–822
30. Maasho K, Marusina A, Reynolds NM, Coligan JE, Borrego F (2004) Efficient gene transfer into the human natural killer cell line, NKL, using the Amaxa nucleofection system. J Immunol Methods 284:133–140
31. Mertz KD, Weisheit G, Schilling K, Luers GH (2002) Electroporation of primary neural cultures: a simple method for directed gene transfer in vitro. Histochem Cell Biol 118:501–506
32. Lei Y, Haider HK, Shujia J, Sim ES (2004) Therapeutic angiogenesis; Devising new strategies based on past experiences. Basic Res Cardiol 99:121–132
33. Ott HC, McCue J, Taylor DA (2005) Cell-based cardiovascular repair: The hurdles and the opportunities. Basic Res Cardiol 100:504–517
34. Koch KC, Schaefer WM, Liehn EA, Rammos C, Mueller D, Schroeder J, Dimassi T, Stopinski T, Weber C (2006) Effect of catheter-based transendocardial delivery of stromal cell-derived factor 1α on left ventricular function and perfusion in a porcine model of myocardial infarction. Basic Res Cardiol 101:69–77

Circ J 2007; **71**: 173–179

Poor Implementation of Cardiac Rehabilitation Despite Broad Dissemination of Coronary Interventions for Acute Myocardial Infarction in Japan

— A Nationwide Survey —

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for the Japanese Cardiac Rehabilitation Survey Investigators*

Background The implementation of cardiac rehabilitation (CR) after acute myocardial infarction (AMI) has not been fully investigated in Japan, so a nationwide survey of hospitals was conducted.

Methods and Results Questionnaires were sent in 2004 to a total of 1,875 hospitals in Japan, including all the 859 Japanese Circulation Society (JCS)-authorized cardiology-training hospitals (THs), 311 JCS-associated hospitals (AH), and 705 randomly sampled non-THs (NTHs). The response rate was 59% (1,106/1,875). The percentages of hospitals treating hospitalized AMI patients were 97% in 526 TH, 85% in 194 AH, and 20% in 339 NTH. Although the rates of implementation of emergency percutaneous coronary intervention were very high (92%, 56%, and 4%, respectively), the rates of implementation of recovery phase CR were low (20%, 8%, and 2%, respectively). In addition, patient education programs (23%, 13% and 2%) and formulated exercise prescriptions based on exercise testing (16%, 7% and 1%) were poorly implemented. More importantly, only 9%, 2% and 0% of these hospitals had outpatient CR programs. From these data, the nationwide participation rate in outpatient CR after AMI in Japan was estimated to be only 3.8–7.6%.

Conclusion This first nationwide survey demonstrated that, in contrast to the broad dissemination of acute phase invasive treatment for AMI, the implementation of recovery phase CR, especially outpatient CR, is extremely poor in Japan. In addition, patient education programs and exercise prescription based on exercise testing are only poorly implemented. (*Circ J* 2007; **71**: 173–179)

Key Words: Acute myocardial infarction; Cardiac rehabilitation; Exercise prescription; Percutaneous coronary intervention

There is ample evidence showing that cardiac rehabilitation (CR) with exercise training improves functional capacity and quality of life[1–5] and reduces cardiovascular and total mortality[1,6,7] in patients with acute myocardial infarction (AMI). However, the implementation of CR in Japan has been limited to large hospitals, and thought to be insufficient nationwide[8] The fee for CR after AMI is reimbursed by the Japanese health insurance system only to hospitals approved for CR which fulfill the CR facility standards. According to the Japanese Association of Cardiac Rehabilitation, the number of hospitals approved for CR was only 164 in August 2004[9] and 186 in February 2005, which is in sharp contrast to the number of hospitals performing percutaneous coronary intervention (PCI) for coronary artery disease (>1,000 hospitals).[10,11]

A recent study demonstrated that the participation rate in CR programs by hospitalized patients with AMI in 1996–1998 was 34% in CR-approved hospitals and 8% in non-approved hospitals in Japan, and they estimated the nationwide participation rate to be 5–12%.[12] However, that was a small survey of 46 hospitals with cardiology divisions, and there has not been a nationwide large-scale survey of CR in Japan.

Recently, the length of hospital stay for patients with AMI has been substantially shortened, because emergency PCI enables early ambulation and the economic pressure to minimize hospital stay has increased. This shortening of hospitalization has made it difficult for the "traditional in-hospital CR" program with exercise training and patient education to be performed in time, but outpatient CR programs, which should be an alternative for traditional in-hospital CR programs, do not appear to be widely used and the actual implementation of outpatient CR programs in Japan has not been investigated.

Accordingly, the purpose of the present study was to investigate the status of CR for patients with AMI in Japan by conducting a nationwide large-scale survey, with special reference to comparisons of implementation of acute-phase invasive treatment, such as emergency PCI, and recovery-phase CR for AMI.

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**The Japanese Cardiac Rehabilitation Survey Investigators are listed in Appendix 1*

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Table 1 Hospital Size and Cardiac Care According to Hospital Category

	JCS training hospital	JCS associated hospital	Non-training hospital	Total
No. of surveyed hospitals	526 (100%)	194 (100%)	339 (100%)	1,059 (100%)
Hospital data				
No. of hospital beds	467±258	262±133	138±114	324±249
No. of cardiology beds	40±19	25±19	2.4±7.8	27±23
No. of cardiologists (full time + part-time)	8.2±9.4	3.5±2.8	1.0±2.6	5.0±7.6
Coronary care unit	360 (68.4%)	62 (32.0%)	6 (1.8%)	423 (39.9%)
Cardiac surgery section	300 (57.0%)*	23 (11.9%)*	3 (0.9%)	326 (30.8%)
Approved for specific intensive care	240 (45.6%)*	26 (13.4%)*	8 (2.4%)	274 (25.9%)
Approved for CR	65 (12.4%)*	3 (1.5%)*	1 (0.3%)	69 (6.5%)
Status of cardiology care				
Hospitals treating AMI	511 (97.1%)	163 (84.0%)	68 (20.1%)	742 (70.1%)
No. of patients with AMI (per year)	59.5±49.6	19.1±22.6	2.0±6.9	33.7±44.9
Hospitals implementing coronary arteriography	503 (95.6%)	135 (69.6%)	16 (4.7%)	654 (61.8%)
No. of coronary arteriography (procedures/year)	626±709	160±208	11±71	344±583
Hospitals implementing PCI	495 (94.1%)	115 (59.3%)	13 (3.8%)	623 (58.8%)
No. of PCI (procedures/year)	191±223	42±67	3±19	104±183
Hospitals implementing emergency PCI	486 (92.4%)	109 (56.2%)	12 (3.5%)	607 (57.3%)
No. of emergency PCI (procedures/year)	58±56	15±31	1±6	32±49

JCS, Japanese Circulation Society; CR, cardiac rehabilitation; AMI, acute myocardial infarction; PCI, percutaneous coronary intervention.
*$p<0.01$ compared with the implementation rate of emergency PCI in each hospital category.

Methods

This study was conducted by the research group of the "Study on the current status and promotion of cardiac rehabilitation in Japan (Japanese Cardiac Rehabilitation Survey)". There were 8,245 hospitals practicing cardiology or internal medicine in Japan in 2002[13] and of those, 859 with a cardiology section were authorized by the Japanese Circulation Society (JCS) as "Training hospitals for the Board-Certified Member of the JCS" (THs) and 311 were designated as "Associated hospitals" (AHs) at the time of this survey (ie, in 2004). Of the remaining 7,075 hospitals not designated as TH or AH, 10% were randomly sampled, 2 of which had been closed, and therefore 705 hospitals were identified as random-sampled non-THs (NTHs). Questionnaires were sent in February to May, 2004, to a total of 1,875 hospitals including all of the 859 THs and 311 AHs, and random-sampled 705 NTHs. The response rate was 59% (1,106/1,875), with THs 63% (541/859), AHs 66% (204/311) and NTHs 51% (361/705).

The questionnaire surveyed the following: (1) hospital data: number of beds, number of cardiologists, approval as a specific intensive care facility by the specific intensive care unit (ICU) standards, and approval as a CR facility; (2) cardiology practice data in 2003: number of hospitalized patients with AMI, implementation of coronary arteriography, implementation of PCI, and implementation of emergency PCI; (3) implementation of CR: acute phase CR for patients with AMI, recovery phase CR, patient education programs, formulated exercise prescriptions based on exercise testing, cardiopulmonary exercise testing with respiratory gas analysis, and outpatient CR program after hospital discharge. The data sheets were collected and analyzed at the Division of Cardiology, National Cardiovascular Center.

The CR facility standards for CR fee reimbursement at the time of this survey were: (1) attendance of a staff physician with access to facilities of an authorized ICU in case of emergency, (2) an exclusive CR training room equipped with appropriate devices, and (3) at least one full-time CR physician and 1 nurse or physical therapist.

Statistical Analysis

Data were analyzed according to the hospital categories. Numerical data are presented as means ± standard deviation. Chi-square test was used to compare the rate of implementation of emergency PCI (a representative therapeutic procedure for AMI) and that of various types of CR activities in each hospital category. Next, Bonferroni's corrections were used to compensate the compromised statistical certainty by the multiple comparisons. Thus, p-values smaller than the usual cutoff levels divided by the number of comparisons (ie, 0.05/10=0.005 and 0.01/10=0.001) were considered to be statistically significant at the risk levels of 5% and 1%, respectively.

Ethical Considerations

This study did not deal with data from individual patients, and conformed to the 2004 revised version of the Ethical Guidelines of Epidemiological Study by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labor and Welfare of Japan.

Results

Effective replies were obtained from 1,059 hospitals including 526 THs (61% of all THs in Japan), 194 AHs (62% of all AHs), and 339 NTHs (4.8% of all NTHs).

Hospital Data

The hospital data are summarized in Table 1 and indicate that the THs are large, general hospitals equipped with sufficient numbers of total hospital beds (467±258 beds), cardiology beds (40±19 beds), and staff cardiologists (8.2±9.4 including both full-time and part-time staff). Although 32% of THs did not have an independent coronary care unit (CCU), most had an ICU available as a CCU for AMI patients. Approximately half of the THs had a cardiac surgery section and had been approved as a "specific intensive care" facility (ie, equipped with an authorized high-quality ICU). However, only 12% (65/526) of all THs, or only 27% (65/240) of the hospitals approved for specific intensive care, had been approved as a CR facility, which implied that the

Table 2 Use of CR for Patients With AMI According to Hospital Category

	JCS training hospital (n=526)	JCS associated hospital (n=194)	Non-training hospital (n=339)	Total (n=1,059)
Implementation of CR for AMI				
Any CR for AMI	281 (53.4%)*	66 (34.0%)*	16 (4.7%)	363 (34.4%)
Acute-phase CR for AMI	256 (48.7%)*	59 (30.4%)*	10 (2.9%)	325 (30.7%)
Recovery-phase CR for AMI	104 (19.8%)*	16 (8.2%)*	5 (1.5%)	125 (11.8%)
Outpatient CR program after discharge	49 (9.3%)*	3 (1.5%)*	0 (0%)*	52 (4.9%)
Patient education program	123 (23.4%)*	26 (13.4%)*	5 (1.5%)	154 (14.5%)
Exercise prescription based on exercise test	86 (16.3%)*	13 (6.7%)*	3 (0.9%)	102 (9.6%)
Cardiopulmonary exercise test with expired gas analysis	72 (13.7%)*	5 (2.6%)*	0 (0%)*	77 (7.3%)
Number of AMI patients who participated in CR in each hospital				
Patients who participated in any CR (patients/year)	13.0±31.0	4.1±16.6	0.36±3.0	7.2±23.5
Patients who participated in recovery-phase CR (patients/year)	9.2±28.6	1.5±14.6	0.2±2.3	4.9±21.5
Patients who participated in outpatient-CR assuming 100% transfer from recovery-phase CR (patients/year)	5.7±23.4	0.08±0.8	0.0±0.0	2.8±16.7
Patients who participated in outpatient-CR assuming 50% transfer from recovery-phase CR (patients/year)	2.8±11.7	0.04±0.4	0.0±0.0	1.4±8.4
Pooled data in each category				
Patients with AMI (patients/year)	31,366	3,704	669	35,739
Patients who participated in any CR (patients/year)	6,711	793	121	7,624
Patients who participated in recovery-phase CR (patients/year)	4,847	295	69	5,212
Patients who participated in outpatient-CR assuming 100% transfer from recovery-phase CR (patients/year)	2,981	15	0	2,996
Patients who participated in outpatient-CR assuming 50% transfer from recovery-phase CR (patients/year)	1,491	8	0	1,498
Estimated participation rate in CR in each category#				
Participation rate in any CR (% of AMI survivors)	23.8	23.8	20.1	23.7
Participation rate in recovery-phase CR (% of AMI survivors)	17.2	8.9	11.5	16.2
Participation rate in outpatient-CR assuming 100% transfer from recovery-phase CR (% of AMI survivors)	10.6	0.4	0	9.3
Participation rate in outpatient-CR assuming 50% transfer from recovery-phase CR (% of AMI survivors)	5.3	0.2	0	4.2

Abbrebiations see in Table 1.
**p<0.01 compared with the implementation rate of emergency PCI in each hospital category.*
#Estimated participation rate was calculated as the number of participants relative to the number of acute-phase survivors. Acute-phase survival rate was assumed to be 90% according to previous reports (references 15 and 16).

remaining 73% (175/240) of the hospitals approved for specific intensive care did not have approval as a CR facility despite their potential ability to fulfill the CR facility standards, because the presence of an authorized ICU was 1 of the major components of the CR facility standards.

According to the hospital data, AHs are considered to be medium-sized hospitals with 262±133 total hospital beds and 25±19 cardiology beds. Of those, 32% had a CCU and 13% had been approved for specific intensive care. However, again, only 1.5% (3/194) of AHs were approved hospitals for CR.

Random-sampled NTHs are considered to be small-sized hospitals with 138±114 total beds and only a few cardiology beds. Only 1–2% of NTHs are equipped with a CCU and cardiac surgery section, and had been approved for specific intensive care. As anticipated, only 1 hospital (0.3%) had been approved for CR.

Status of Cardiology Care

Of the 526 THs, almost all (97%) were treating hospitalized AMI patients and the rates of implementation of invasive cardiac procedures, such as coronary arteriography, PCI, and emergency PCI, were all higher than 90% (Table 1). Of the 194 AHs, 84% were treating hospitalized AMI patients, 70% were performing coronary arteriography, and more than half were performing PCI and emergency PCI. Of the 339 NTHs, 20% were treating AMI patients, but only a few were performing invasive cardiac procedures. As a whole, 70% of 1,059 hospitals were treating AMI patients, and more than half were performing coronary arteriography, PCI, and emergency PCI.

The number of hospitalized AMI patients in each hospital in the year of 2003 averaged 59.5±49.6 patients/year in THs, 19.1±22.6 patients/year in AHs, and 2.0±6.9 patients/year in NTHs, and the total number of AMI patients hospitalized in the 1,059 hospitals in this survey amounted to 35,665 patients/year. These data estimated that in the year of 2003, 51,111 (59.5×859=51,111), 5,940 (19.1×311=5,940), and 14,150 (2.0×7075=14,150) AMI patients were hospitalized in all THs, AHs, and NTHs, respectively, yielding an estimated total number of hospitalized AMI patients in allover Japan to be 71,201 patients/year. This figure closely agreed with the number of hospitalized AMI patients of 66,459 patients in the year of 2000 in a previous nationwide survey![4] In addition, this estimation indicated that approximately 72% (51,111/71,201) of all hospitalized AMI patients in Japan were treated in THs, and 80% (57,051/71,201) were treated in either THs or AHs.

Implementation of CR for AMI

Implementation of CR for AMI and the numbers of patients who participated in CR are summarized in Table 2. The rates of implementation of any CR and acute-phase CR for AMI patients were approximately 50% for THs, 30% for AHs, and less than 5% for NTHs, which were lower than the rates of invasive procedures for AMI in these hospitals.

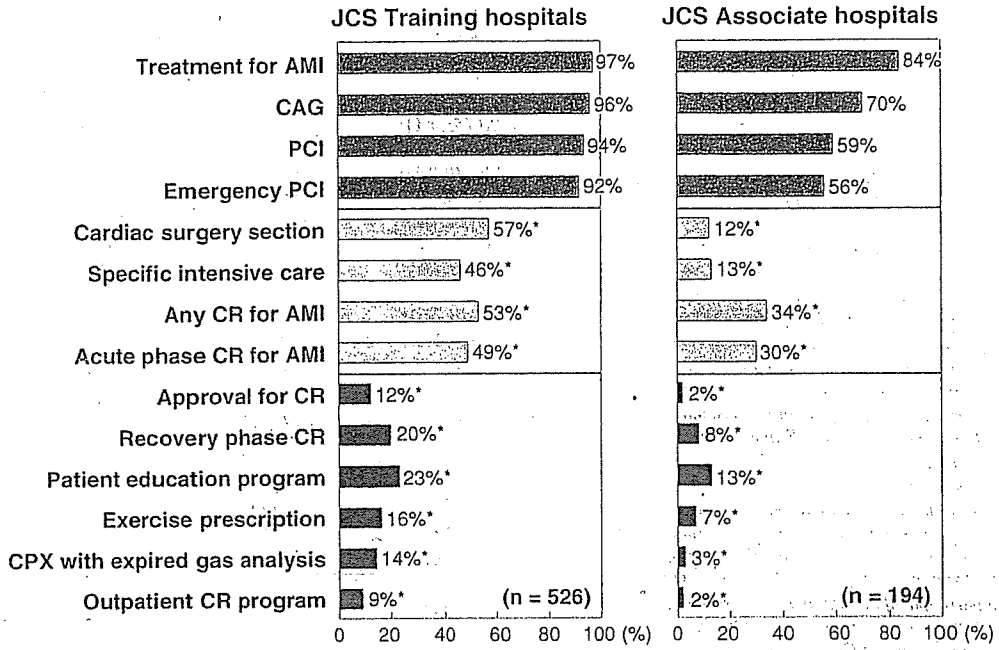

Fig 1. Implementation rates of various types of medical care for AMI in cardiology training hospitals authorized by the JCS. The implementation rates of care related to CR were remarkably low compared with the very high implementation rates of invasive procedures in both training and associate hospitals. JCS, Japanese Circulation Society; AMI, acute myocardial infarction; CAG, coronary arteriography; PCI, percutaneous coronary angioplasty; CR, cardiac rehabilitation; CPX, cardiopulmonary exercise test. *p<0.01 compared with the implementation rate of emergency PCI in each hospital category.

Table 3 Estimation of Number of Patients and Participation Rates in CR in Japan

	*Equation of estimation**	*Estimated total in Japan (patients/year)*	*Estimated participation rate (%)§*
Total number of hospitalized patients with AMI	*59.5×859+19.1×311+2.0×7,075=*	*71,201*	
No. of acute phase survivors‖	*71,201×0.9=*	*64,809*	
Participation in any CR	*13.0×859+4.1×311+0.36×7,075=*	*14,989*	*23.1*
Participation in recovery-phase CR (patients/year)	*9.2×859+1.5×311+0.20×7,075=*	*9,811*	*15.1*
Participation in outpatient-CR in case of 100% transfer from recovery-phase CR	*5.7×859+0.08×311+0.0×7,075=*	*4,896*	*7.6*
Participation in outpatient-CR in case of 50% transfer from recovery-phase CR	*2.8×859+0.04×311+0.0×7,075=*	*2,443*	*3.8*

**Estimated total numbers in Japan were calculated as the sum of patients in 859 JCS training hospitals, 311 JCS associated hospitals, and 7,075 non-training hospitals.*
‖Acute-phase survival rate was assumed to be 90% according to previous reports (references15 and 16).
§Estimated participation rate was calculated as the number of participants relative to the number of acute-phase survivors.

The rates of implementation of recovery-phase CR were 20% for TH, 8% for AH, and 2% for NTH, which were much lower than those for acute-phase CR. More importantly, only 9.3% of THs, 1.5% of AHs, and 0% of NTHs had outpatient CR programs for AMI patients.

Regarding the content of the CR program, patient education programs (23%, 13% and 2%), formulated exercise prescription based on exercise testing (16%, 7% and 1%), and cardiopulmonary exercise testing with expired gas analysis (14%, 3%, and 0%) were also only poorly implemented in each category of hospital (Table 2).

Fig 1 illustrates the rates of implementation of various types of medical care for AMI patients in the 526 THs and 194 AHs that participated in the present survey. In contrast to the very high rates of treatment of hospitalized AMI patients and implementation of invasive procedures such as emergency PCI, the implementation rates of recovery-phase CR and CR activities such as formulated exercise prescription and outpatient CR were all significantly lower in both THs and AHs (all p<0.01). Thus, the implementation rates of all CR activities were consistently and markedly lower than the implementation rates of invasive cardiac procedures in moderate to large-sized cardiology hospitals in Japan.

Participation Rate in CR After AMI

Patient participation rates in CR after AMI were calculated from the numbers of AMI survivors and participants in CR in each category (Table 2). The number of acute-phase hospital survivors of AMI was estimated by assuming the in-hospital mortality rate to be 10%, based on previous multicenter surveys that have reported the in-hospital mortality of AMI patients in Japan in 1998–2003 to be 9–11%[15,16] The number of patients who participated in outpatient CR after hospital discharge was estimated as the number of patients who participated in recovery-phase CR in the hospitals that also provided an outpatient CR program. When we assumed that all patients who participated in the in-hospital recovery-phase CR program also participated in the outpa-

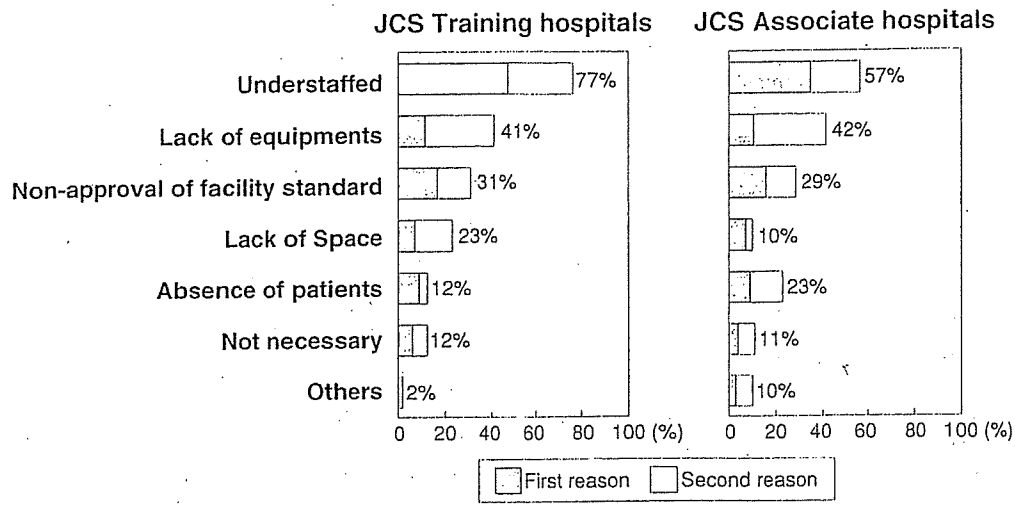

Fig 2. Reasons for not implementing cardiac rehabilitation (CR) in cardiology training hospitals authorized by the JCS. Data were collected from 222 of the 245 JCS training hospitals (Left) and 106 of the 128 JCS associate hospitals (Right) that were not performing any CR. The first and second reasons for non-implementation of rehabilitation were added. JCS, Japanese Circulation Society.

tient CR program of the same hospital when available (ie, a transfer rate of 100%), the participation rates of outpatient CR were only 10.6%, 0.4% and 0% among acute-phase survivors in THs, AHs, and NTHs, respectively. Furthermore, when we assumed the transfer rate from the in-hospital recovery-phase CR to outpatient CR to be 50%, the participation rates fell to 5.3%, 0.2%, and 0%, respectively.

From the data of this nationwide survey, the participation rates in CR after AMI in Japan were estimated. The numbers of patients who participated in any CR and recovery-phase CR after AMI in the whole of Japan in the year of 2003 were calculated from the average number of patients in each hospital in each category and the numbers of hospitals in the 3 categories (Table 3), yielding a total of 14,989 patients and 9,811 patients per year who participated in any CR and recovery-phase CR, respectively, in Japan. When we assumed that the transfer rates from the in-hospital recovery-phase CR to outpatient CR to be 100% and 50%, respectively, the number of participants in outpatient CR programs was estimated to be 4,896 and 2,443 patients/year, respectively, for the whole of Japan. When we assumed the acute-phase survival rate to be 90%[15,16] this yielded the acute-phase survivors (ie, denominator of CR participation rates) in Japan to be 64,809 patients/year. As a result, the estimated nationwide participation rates in any CR and recovery-phase CR were 23.1% and 15.1%, respectively, among the acute-phase survivors. Finally, the nationwide participation rate in outpatient CR was calculated to be only 3.8–7.6%, depending on a transfer rate from the in-hospital recovery-phase CR to outpatient CR after hospital discharge of 50–100% (Table 3).

Reasons for Non-Implementation of CR

In the present survey, 222 of the 245 THs and 106 of the 128 AHs that did not have any CR program for AMI gave reasons for not implementing rehabilitation (Fig 2). When the 1st and 2nd reasons were added, the 3 major reasons for non-implementation in both THs and AHs were lack of staff, lack of equipment and lack of achieving CR facility standards. The 4th reason was lack of CR space in THs, compared with lack of participating patients in AHs.

Discussion

Major Findings

This is the first nationwide survey of the implementation of CR for AMI patients in Japan. The major findings are: (1) in contrast to the broad dissemination of acute-phase invasive procedures for AMI, the implementation of recovery-phase and outpatient CR after AMI is extremely poor in Japan; the implementation rate of outpatient CR was only 9.3%, even in JCS THs, and the nationwide participation rate in outpatient CR was estimated to be only 3.8–7.6%; (2) the quality of CR programs reflected by implementation rates of patient education programs and exercise prescriptions based on exercise testing was also poor; and (3) the major reasons for not implementing CR were lack of staff, equipment and space, and not achieiving the CR facility standards. These data clearly indicate that recovery-phase and outpatient CR for AMI is severely underutilized in Japan.

Hospital Implementation of CR in Japan

The present study has demonstrated that most THs and AHs in Japan are aggressively treating patients with AMI with invasive procedures such as emergency PCI, but that the implementation rates of all types of CR are disproportionately low relative to the high implementation rates of invasive procedures. In particular, THs have sufficient beds and staff cardiologists, an ICU and sufficient numbers of hospitalized AMI patients, so there seems to be no objective reason for not implementing CR for AMI.

A recent survey has reported that there are 2,621 CR programs in the USA[17] whereas according to the Japanese Association of Cardiac Rehabilitation, the number of hospitals approved for CR in Japan was only 186 in February 2005. This number accounted for only 15.9% of the total number (1,170 hospitals) of JCS-authorized THs (THs and AHs) that are treating 80% of all hospitalized AMI patients in Japan, and also accounted for only 15.0% of the 1,240 hospitals performing PCI according to the Japan Coronary Intervention Study.[11] Clearly, this small number of CR-approved hospitals is a major obstacle for the nationwide

spread of CR in Japan[12]

From the present result, the number of hospitals that have an outpatient CR program is estimated to be only 85 in Japan (859×9.3%+311×1.5%), which is even less than half of the CR-approved hospitals (85/186=45.7%). In contrast, almost all of the 2,621 CR programs in the USA are conducted as outpatient programs[17] The length of hospital stay of AMI patients is rapidly decreasing because of early ambulation after aggressive reperfusion therapy (ie, less physical deconditioning) and the socioeconomic pressure on hospitals. Because the shorter hospital stay prevents patients from receiving enough education and instruction on life-style modification for secondary prevention, there is an increasing need for outpatient CR after discharge[2,18] Even when the smaller population (≈1/2) and the lower disease prevalence (≈1/5) in Japan than in the USA are taken into account, the number of the facilities for outpatient CR in Japan (approximately 1/30) is disproportionately small.

Patient Participation Rate in CR in Japan

The patient participation rates in the present survey can be compared with those in a previous report[12] which estimated the participation rate of recovery-phase CR in 1996–1998 to be 12% in JCS THs and 5% in all hospitals in Japan. The present result of a participation rate of 17.2% in THs in 2003 is slightly higher, but largely in accordance with the previous report, indicating that the participation rate in CR is slowly increasing but remains low in JCS THs. On the other hand, the nationwide participation rate in recovery-phase CR of 15.1% is higher than the previous estimation of 5%[12] possibly because of an increase in the proportion of patients hospitalized in THs or an increase in the implementation of recovery-phase CR in NTHs.

The low patient participation rate of 3.8–7.6% in outpatient CR in the whole of Japan (Table 3) is in accordance with the low implementation rate of outpatient CR programs. This is the first assessment of the patient participation rate in outpatient CR in Japan. Because the proportion of patients who subsequently participate in the outpatient CR program among the initial participants in the in-hospital CR program (ie, the transfer rate) is usually less than 50%, the estimated lower rate of 3.8% could even be overestimated. In the United States, the participation rate of AMI patients in phase II (usually outpatient-type) CR has been reported to be 11–47%[19–23] and a recent community survey reported an even higher participation rate of 55% in Omland County, Minnesota[24] Therefore, it is clear that the patient participation rate in outpatient CR is markedly lower in Japan than in the USA. Because the role of post-AMI outpatient CR is rapidly emerging in the era of short hospital stay, it is critically important to urgently increase both the number of CR-approved facilities and the patient participation rates in outpatient CR in Japan.

Quality of Care in CR

The present survey has revealed that the standard procedures in CR, such as patient education programs, exercise prescription based on exercise tests, and cardiopulmonary exercise tests with expiratory gas analysis, are poorly implemented even in JCS THs in Japan (Fig 1). All these activities and procedures are important components of a comprehensive CR program[1,2,7,25] Therefore, not only an increase in the implementation rate but also an enhancement of the quality of care in CR should be aimed for in Japan. Thus, future surveys should assess not only the implementation of exercise training but also the implementation of these comprehensive activities in CR.

Reasons for Non-Implementation of CR

The reasons for not implementing CR in THs were lack of staff, lack of equipments, lack of achieving approval for a CR facility, and lack of CR space. Before this survey, the difficulty in fulfilling the CR facility standards had been thought to be the main reason for the low implementation rate of CR in Japan. However, THs are usually large, general hospitals that would be expected to have sufficient staff, equipment and space. In addition, the present result that 73% (175/240) of THs that had been approved for specific intensive care did not have approval for CR despite their ability to fulfill the CR facility standards indicates that there are reasons other than the CR facility standards for the non-implementation of CR in these hospitals.

Ades et al reported that according to multivariate analysis, the strength of the physician's recommendation for participation was the most powerful predictor of entry into CR by patients after AMI or coronary bypass surgery[20] Thus, physicians' reluctance or ignorance regarding CR after AMI might be a reason for the low implementation rate of CR in Japan. Because the CR facility standards in Japan have been loosened in 2004 and 2006, the motivation of both physicians and the hospitals would be a critically important factor for the implementation of CR.

Because the beneficial effects of CR on exercise capacity, coronary risk factor reduction, quality of life, and prognosis (cardiovascular mortality and total mortality) in patients after AMI have been established[1–7,26] the low implementation rate of CR implies that patients are not participating in CR for reasons unrelated to their physical conditions. Thus, efforts should be made urgently to increase the implementation rate of CR in Japan. To achieve this goal, it appears necessary to increase the number of hospitals approved for CR and to enhance physicians' understanding of the benefits of CR after AMI.

Study Limitations

This was a hospital-based survey using a questionnaire, so the reliability of data depends on the accuracy of diagnosis and the patient statistics in the surveyed hospitals. However, the close agreement of the estimated total number of hospitalized AMI patients in the present survey in 2003 (71,201 patients) and that of a the previous nationwide survey in 2000 (66,459 patients)[14] suggests that the data collected in the present survey are reliable.

The relatively low response rate (59%) in the present survey compared with the previous survey[14] might have yielded a potential statistical bias. However, similar or even lower response rates have been reported in other nationwide surveys[27,28] In addition, when the hospitals that replied and those that did not reply were compared, there were no significant differences in the numbers of total hospital beds (THs: Reply 467±258 beds vs No-reply 446±241 beds, NS; AHs : Reply 262±133 beds vs No-reply 275±141 beds, NS; NTHs: Reply 138±114 beds vs No-reply 143±111 beds, NS) or in the regional distribution (ie, northeast or southwest Japan, urban or rural areas) between the 2 hospital groups, suggesting that a statistical bias caused by the low reply rate should be negligible.

Because the present survey did not investigate the actual numbers of acute-phase survivors and participants in outpatient CR in each hospital, the participation rate in outpatient

CR had to be estimated on the basis of some assumptions. However, because we used assumptions that would lead to higher participation rates, the results should be biased, if anything, toward overestimation, rather than underestimation of participation rates. Even with the possible overestimation, the participation rates in all CR in Japan were extremely low.

Conclusion

This first nationwide survey of CR demonstrated that, in contrast to the broad dissemination of acute-phase PCI for AMI, the implementation of recovery-phase CR, especially outpatient CR, is extremely poor in Japan. In addition, patient education programs and exercise prescriptions based on exercise testing are only poorly implemented. Considering the established benefits of CR in patients with AMI, urgent efforts should be made to improve this marked underutilization of recovery-phase and outpatient CR in Japan.

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References

1. Wenger NK, Froelicher ES, Smith LK, Ades PA, Berra K, Blumenthal JA, et al. Clinical practice guideline No. 17: Cardiac rehabilitation: US Department of Health and Human Services, AHCPR Publication No. 96–0672, 1995.
2. Ades P. Cardiac rehabilitation and secondary prevention of coronary heart disease. *N Engl J Med* 2001; **345:** 892–901.
3. Yoshida T, Yoshida K, Yamamoto C, Nagasaka M, Tadaura H, Meguro T, et al. Effects of a two-week, hospitalized phase II cardiac rehabilitation program on physical capacity, lipid profiles and psychological variables in patients with acute myocardial infarction. *Jpn Circ J* 2001; **65:** 87–93.
4. Iwanaga Y, Nishi I, Ono K, Takagi S, Tsutsumi Y, Ozaki M, et al. Angiotensin-converting enzyme genotype is not associated with exercise capacity or the training effect of cardiac rehabilitation in patients after acute myocardial infarction. *Circ J* 2005; **69:** 1315–1319.
5. Suzuki S, Takaki H, Yasumura Y, Sakuragi S, Takagi S, Tsutsumi Y, et al. Assessment of quality of life with 5 different scales in patients participating in comprehensive cardiac rehabilitation after acute myocardial infarction. *Circ J* 2005; **69:** 1527–1534.
6. Taylor RS, Brown A, Ebrahim S, Jolliffe J, Noorani H, Rees K, et al. Exercise-based rehabilitation for patients with coronary heart disease: Systematic review and meta-analysis of randomized trials. *Am J Med* 2004; **116:** 682–697.
7. Leon AS, Franklin BA, Costa F, Balady GJ, Berra KA, Stewart KJ, et al. Cardiac rehabilitation and secondary prevention of coronary heart disease: American Heart Association scientific statement. *Circulation* 2005; **111:** 369–376.
8. Goto Y, Sumida H, Ueshima K, Adachi H, Nohara R, Itoh H. Safety and implementation of exercise testing and training after coronary stenting in patients with acute myocardial infarction. *Circ J* 2002; **66:** 930–936.
9. Nagayama M. A view on the approval standard for cardiac rehabilitation facility. *Shinzou Rehab (J Jpn Assoc Cardiac Rehab)* 2005; **10:** 145–147 (in Japanese).
10. Shihara M, Tsutsui H, Tsuchihashi M, Shigematsu H, Yamamoto S, Koike G, et al. Coronary revascularization in Japan. Part 3: Percutaneous coronary intervention during 1997. *Circ J* 2002; **66:** 10–19.
11. Nishigaki K, Yamazaki T, Fujiwara H for the Japanese Coronary Intervention Study (JCIS) group. Assessment of coronary intervention in Japan from the Japanese Coronary Intervention Study (JCIS) group: Comparison between 1997 and 2000. *Circ J* 2004; **68:** 181–185.
12. Goto Y, Itoh H, Adachi H, Ueshima K, Nohara R. Use of exercise cardiac rehabilitation after acute myocardial infarction: Comparison between health insurance-approved and non-approved hospitals in Japan. *Circ J* 2003; **67:** 411–415.
13. Kosei-Mondai-Kenkyukai: Zenkoku-Byoin-Nenkan [CD-ROM]. Institute on Health and Welfare Affairs, Tokyo, 2002.
14. Nishigaki K, Yamazaki T, Fukunishi M, Tanihara S, Fujiwara H for the Japanese Coronary Intervention Study (JCIS) group. Assessment of acute myocardial infarction in Japan by the Japanese Coronary Intervention Study (JCIS) group. *Circ J* 2004; **68:** 515–519.
15. Kinjo K, Sato H, Nakatani D, Mizuno H, Shimizu M, Hishida E, et al. Predictors of length of hospital stay after acute myocardial infarction in Japan. *Circ J* 2004; **68:** 809–815.
16. Kusuoka H, Koretsune Y, Chino M, Nishiyama K, Shiraki T, Nakamura K, et al. Disparity in the process and outcome of the treatment for acute myocardial infarction in Japan: CAMPAIGN study in the national hospital network. *Circ J* 2005; **69:** 1447–1453.
17. Curnier DY, Savage PD, Ades PA. Geographic distribution of cardiac rehabilitation programs in the United States. *J Cardiopulm Rehab* 2005; **25:** 80–84.
18. Ades PA, Balady GJ, Berra K. Transforming exercise-based cardiac rehabilitation programs into secondary prevention centers: A national imperative. *J Cardiopulm Rehab* 2001; **21:** 263–272.
19. Leon AS, Certo C, Comoss P, Franklin BA, Froelicher V, Haskell WL, et al. Scientific evidence of the value of cardiac rehabilitation services with emphasis on patients following myocardial infarction. Section I: Exercise conditioning component [Position Paper]. *J Cardiopulm Rehab* 1990; **10:** 79–87.
20. Ades PA, Waldmann ML, McCann WJ, Weaver SO. Predictors of cardiac rehabilitation participation in older coronary patients. *Arch Intern Med* 1992; **152:** 1033–1035.
21. Mark DB, Naylor DC, Hlatky MA, Califf RM, Topol EJ, Granger CB, et al. Use of medical resources and quality of life after acute myocardial infarction in Canada and the United States. *N Engl J Med* 1994; **331:** 1130–1135.
22. Thomas RJ, Miller NH, Lamendola C, Berra K, Hedback B, Durstine JL, et al. National survey on gender differences in cardiac rehabilitation programs: Patient characteristics and enrollment patterns. *J Cardiopulm Rehab* 1996; **16:** 402–412.
23. Evenson KR, Rosamond WD, Luepker RV. Predictors of outpatient cardiac rehabilitation utilization: The Minnesota Heart Surgery Registry. *J Cardiopulm Rehab* 1998; **18:** 192–198.
24. Witt BJ, Jacobsen SJ, Weston SA, Killian JM, Meverden RA, Allison TG, et al. Cardiac rehabilitation after myocardial infarction in the community. *J Am Coll Cardiol* 2004; **44:** 988–996.
25. Fletcher GF, Balady GJ, Amsterdam EA, Chaitman B, Eckel R, Fleg J, et al. Exercise standards for testing and training: A statement for healthcare professionals from the American Heart Association. *Circulation* 2001; **104:** 1694–1740.
26. Antman EM, Anbe DT, Armstrong PW, Bates ER, Green LA, Hand M, et al. ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1999 Guidelines for the Management of Patients With Acute Myocardial Infarction). *Circulation* 2004; **110:** e82–e293.
27. Matsumori A, Furukawa Y, Hasegawa K, Sato Y, Nakagawa H, Morikawa Y, et al. Epidemiologic and clinical characteristics of cardiomyopathies in Japan: Results from nationwide surveys. *Circ J* 2002; **66:** 323–336.
28. Niwa K, Nakazawa M, Miyatake K, Tateno S, Yoshinaga M. Survey of prophylaxis and management of infective endocarditis in patients with congenital heart disease: Japanese nationwide survey. *Circ J* 2003; **67:** 585–591.

Appendix 1

The Japanese Cardiac Rehabilitation Survey Investigators

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Enhanced cardiac production of matrix metalloproteinase-2 and -9 and its attenuation associated with pravastatin treatment in patients with acute myocardial infarction

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ABSTRACT

Previous experimental studies have demonstrated that MMPs (matrix metalloproteinases) contribute to LV (left ventricular) remodelling. We hypothesized that cardiac MMPs are activated in patients with AMI (acute myocardial infarction) and, if so, MMP production may be attenuated by statins (3-hydroxy-3-methylglutaryl-CoA reductase inhibitors) through their cardiovascular protective actions. We studied 30 patients, ten control patients with stable angina pectoris and 20 patients with AMI, in whom LV catheterization at the chronic stage was performed 22 ± 12 days (value is mean ± S.D.) after the onset of AMI. Blood samples were collected from the CS (coronary sinus) and a peripheral artery. In patients with AMI, the levels of MMP-2 and MMP-9 were significantly ($P < 0.05$) higher in the CS than the peripheral artery (MMP-2, 853 ± 199 compared with 716 ± 127 ng/ml; MMP-9, 165 ± 129 compared with 98 ± 82 ng/ml), whereas no significant differences were observed in the patients with angina pectoris. The CS–arterial concentration gradients of MMP-2 and MMP-9 correlated positively with BNP (brain natriuretic peptide) levels (MMP-2, $R = 0.68$, $P < 0.01$; MMP-9, $R = 0.59$, $P < 0.05$) and LV end-diastolic volume index (MMP-2, $R = 0.70$, $P < 0.01$; MMP-9, $R = 0.70$, $P < 0.01$). When patients with AMI treated with 10 mg of pravastatin or without ($n = 10$ in each group) were compared, this statin therapy significantly ($P < 0.05$) decreased the CS–arterial concentration gradients of MMP-2 (69 ± 43 compared with 213 ± 185 ng/ml) and MMP-9 (14 ± 27 compared with 119 ± 84 ng/ml). In conclusion, the enhanced production of cardiac MMP-2 and MMP-9 is associated with LV enlargement and elevated BNP levels in patients with AMI. A pleiotropic effect of statins appears to be associated with the modulation of cardiac MMP activation, which may be potentially beneficial in the attenuation of post-infarction LV remodelling.

Key words: acute myocardial infarction, angina pectoris, brain natriuretic peptide (BNP), metalloproteinase (MMP), remodelling, statin, tissue inhibitor of metalloproteinases (TIMP).

Abbreviations: ACE-I, angiotension-converting enzyme inhibitor; AMI, acute myocardial infarction; Ang II, angiotensin II; AP, angina pectoris; BNP, brain natriuretic peptide; CK, creatine kinase; CRP, C-reactive protein; CS, coronary sinus; LDL, low-density lipoprotein; LV, left ventricular; LVEDVI, LV end-diastolic volume index; LVEF, LV ejection fraction; MMP, matrix metalloproteinase; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinases; WBC, white blood cell.

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