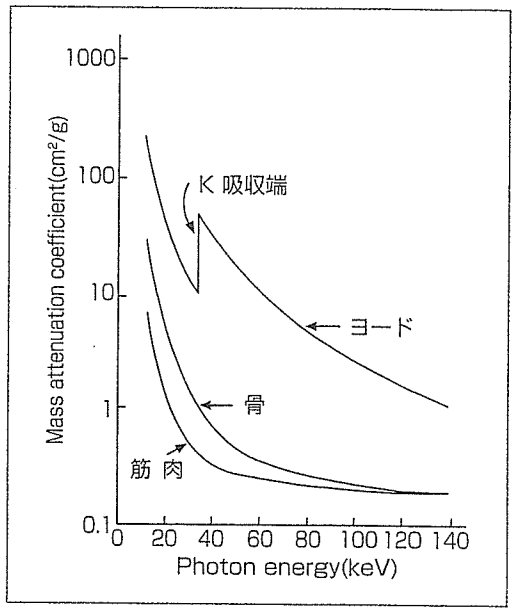


筋肉，骨およびヨードのK吸収端の関係を示す．33.3keV直上でヨードK吸収端は上昇し，人体との組織吸収係数の差が最大となる．

図Ⅲ-71　X線エネルギーと質量吸収係数の関係

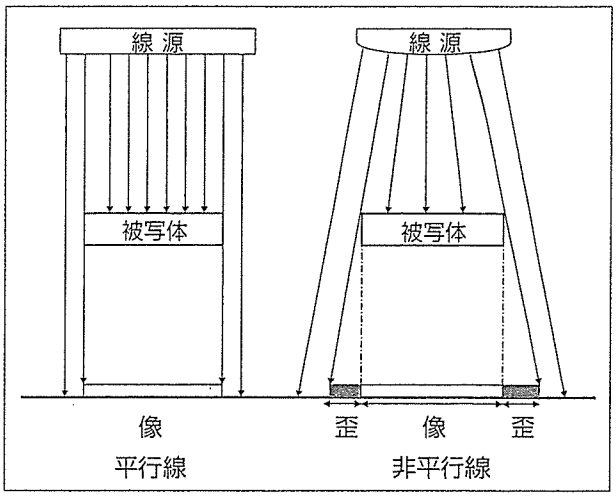
少を伴う過程を経た後でも，通常の白色X線と同等のフォトン数を撮像系前面で確保することができる．この要素は，微小血管造影には必要不可欠であるといってよい．

b．単色化

血管造影には通常ヨード含有造影剤が使用されている．ヨードは33.3 keVのエネルギーレベルで質量吸収係数が不連続に上昇するため（K吸収端），X線のエネルギーをヨードのK吸収端の直上のエネルギーの直上に変換（シリコン結晶によるBragg反射を応用する）すると，ヨードと周囲組織との質量吸収係数の差（コントラスト）が最大となり，微量ヨードの検出ができる（図Ⅲ-71）．また，MRIなどに使用されているガドリニウム（Gd）がX線による血管造影時の造影剤として使用可能となれば，単色X線のエネルギーを50.5 keV（Gd吸収端直上）にしたときに最適の吸収の差を得られる．50.5 keVのX線の人体による吸収は33.3 keVのヨードK吸収端のそれと比較して著しく少ない．すなわち，Gd使用により患者被曝量を軽減した血管造影が実現できる．

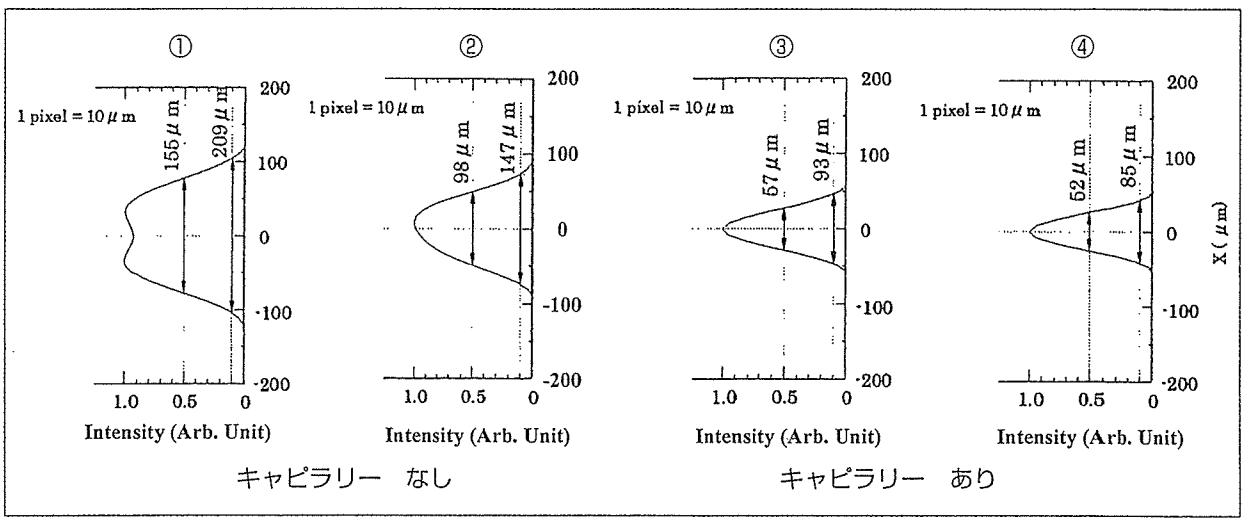
c．平行化

X線源が平行であれば，被写体とその像は理論的にいえば等大の大きさになる（正確には，X線が平行であっても散乱・回折するので本当の等大にはならない）．平行でない光線は被写体から像との距離が離れるほど像は拡大し，辺縁は歪んでしまう（図Ⅲ-72）．こうした変化はミクロの血管を評価するには，その血管の径を正確に検出するのに影響を与えることはいうまでもない．放射光は平行に限りなく近い性質を持ち，微小血管造影に理想的な線源といえるが，通常の医療用X線源は平行化されておらず，微小血管径の計測には不向きである．浜松ホトニクスが開発した微細な孔を多数有するキャピラリープレートは，そのキャピラリー（孔）にX線を通過させると平行化することができ，低コストで通常のX線源を平行化できる有効な手法と考えられる．実際にその効果をコンピューターシミュレーションにおいて評価した．X線源



線源が平行の場合，被写体と像は等大になるが，平行でない場合には像は拡大し，辺縁が歪む．

図Ⅲ-72　X線の平行化の影響



図Ⅲ-73　キャピラリーによる平行化の評価

検出器から10cm離した場所に50 μmのスリットを設置し，線源とスリットの距離を変化させ平行化の効果を検討した．①②は線源とスリットとの距離がそれぞれ50cmと80cmで，キャピラリーによる平行化がない状態である．この時に検出器に入るX線幅は，それぞれ155 μm，98 μmに拡大される．③④も同じく距離を50cmと80cmとし，キャピラリーで平行化した状態である．平行化した場合は，52～57 μm程度の誤差しかない．

と被写体の距離を近づけた場合，平行化しないと像が拡大するが，平行化すると像の拡大を防ぐことができる（図Ⅲ-73）．すなわち，X線の平行化は解像度を確保するのに有用であることがわかる．

d. 高解像度化・高感度化

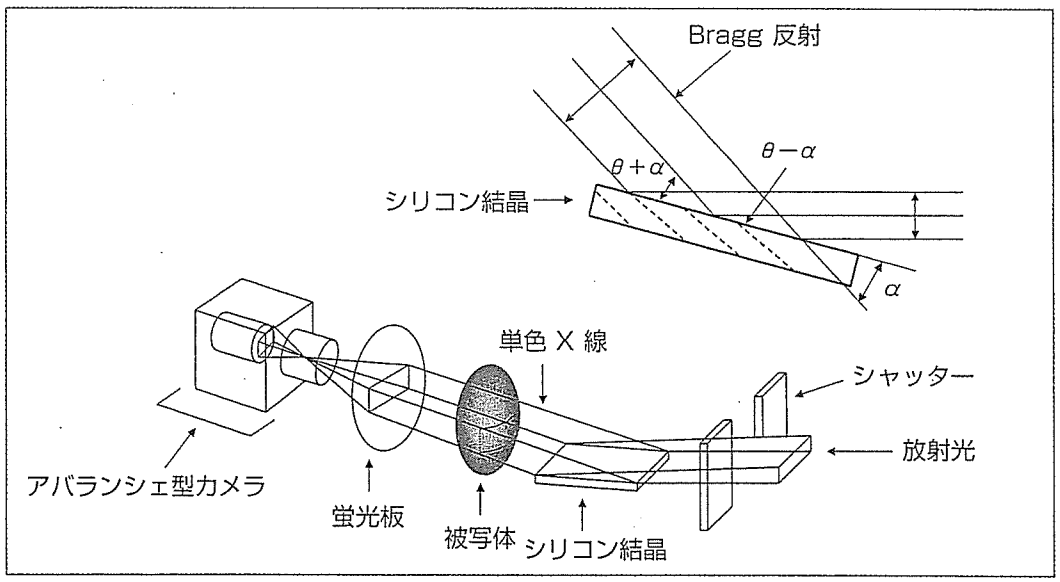
高輝度で単色化され，かつ平行化を有する理想的なX線源を実現できたとしても，検出器の解像度・感度が低ければ微小血管像は劣化してしまう．解像度を表すには通常チャート撮影が用いられる．1ミリ幅に40本のラインを引き（20ラインペア），それを識別できれば25 μm（1mm/40 = 25 μm）の解像度が得られたことになる．通常の血管造影装置では2ラインペア程度が普通であるが（空間分解能250 μm），高解像度撮影装置であれば25 μmレベルの解像度が可能である．また，感度が高くな

いと微量のX線を検出することができないため，イメージングプレートとしては高感度蛍光板が使用される．装置の原理・構造については後述する．

3．線源の種類

a．放射光

放射光とは光速で直進する電子が磁石によって進行方向を変えられた際に発生する電磁波であり，1947年に電子シンクロトロンで観測された．負の電荷をもつ電子は周囲に電場があり，高エネルギーの電子が磁場で曲げられると光子となって放出される．その性質は，高エネルギーの電子を持ち，進行方向の変化が大きいほど高輝度となり，紫外線からX線などの短い波長の光を含む広いスペクトルを有している．放射光施設は，電子ビームを発生させ光速近くまで加速する入射系加速器と，電子ビームを円形の軌道に留めておくための蓄積リングを必要とする．代表的な大型放射光施設はSPring－8（日本），APS（米国），ESRF（仏）があり，それぞれ8，7，6 GeVの電子ビームの加速エネルギーを有している．SPring－8放射光の原理と方法は，電子銃から電子ビームを発射し，線形加速器で1 GeVまで加速し，さらにシンクロトロンに導入して8 GeVまで加速する．それを蓄積リングに導入し，8 GeVのエネルギーで偏向電磁石や挿入光源により放射光を発生させる．発生した放射光は，ビームラインを通して，数カ所に設置してある蓄積リング棟に導かれ医学利用，地球科学，生命科学，環境科学，材料科学などの分野で応用される．特に，医学分野にて放射光は微小血管を造影し再生医療における新生血管治療の効果判定，悪性腫瘍による栄養血管の



図Ⅲ－74　放射光施設での微小血管造影システムの概略

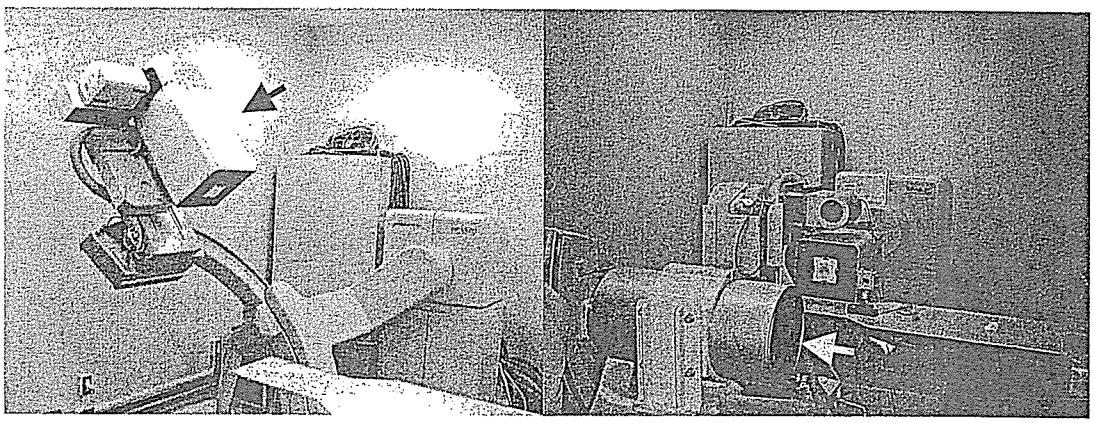
白色放射光をシリコン結晶に反射させ単色化する．被写体を通過したX線は蛍光板に像を作り，高感度高解像度アバランシェ型カメラで撮影する．シリコン結晶の格子面の角度（Bragg angle:θ）により単色化エネルギーが決定され，格子面と結晶表面の角度（α）でZ軸への拡大率が決定される．

早期発見・早期治療，脳血管系や循環器系の微小循環障害などの評価への応用が検討されている．

放射光はその非常に高い輝度を有し，限りなく平行で指向性のある性質から微小血管造影に適した条件を有している．放射光施設での単色化は，白色放射光をシリコン結晶によってBragg反射させ，その格子面の角度により回折を受け単色X線のエネルギーを決定する（図Ⅲ-74）．前述したが，単色化は放射線量を減衰させてしまうが，シンクロトロン放射光は非常に高い輝度を有しており，単色化しても通常のX線白色光と同等量の単色X線を確保することができる．格子面の角度θを変化させることにより，エネルギーレベル（反射光の波長）の調節が可能となる．

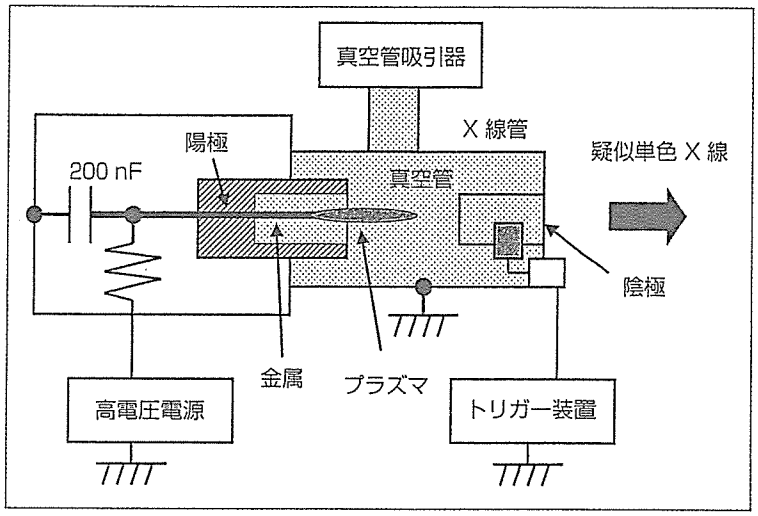
b．普及型微小血管造影装置

放射光はその輝度の高さと平行性では非常に優れているが，多額のコストが掛かり，広大な施設を必要とする．実際に臨床応用へ普及するにはその施設へ行くしかなく，時間的・空間的にも問題がある．そこで新エネルギー・産業技術開発機構（NEDO）の支援により，次世代単色X線診断・治療システムを開発し，開発グループの代表である浜松ホトニクスにより製造され，検出系はNHKエンジニアリングの技術により超高感度ハイビジョンカメラシステムが導入された（図Ⅲ-75）．普及型微小血管造影装置の線源として，高輝度のX線を得るために大容量大出力をもつCT用のX線管を用いた．撮影は冷却性能の改善により，連続20秒照射・8分間休止で駆動することができる．単色化には金属フィルターを用い，高いエネルギーと低いエネルギーをカットし，33.3keVよりに近いエネルギーレベルの疑似単色X線を得る．単色X線の強度は7.74×10^{-4}C/kg/s（＝3R/s）に設定している．また，単色X線にX線コリメータを使用し，ポリキャピラリーを通し平行化し，空間分解能を向上できる．これらにより50 μm以下の空間解像度を確保する．検出系は高解像度・高感度蛍光板（イメージングプレート）で作成した蛍光像を，超高感度・高精細撮像管であるアバランシェ型ハイビジョンモノクロ新Super-HARPカメラ（NHK）で撮影する方式で



図Ⅲ-75　普及型微小血管造影装置の概略

Cアーム上端には蛍光板を有した検出器とHARP管を有するカメラを搭載している（黒矢印）．筒型のX線管は，現在臨床で使用されているCT用の高出力線源である（白矢印）．



高電圧電源を用い50～60kVまで充電し，トリガ装置による印加によりX線管内へ放電される時に，陽極のターゲット金属が気化され，疑似単色X線が発生される．

図Ⅲ-76　プラズマX線装置の概略

ある．この装置の空間分解能は25 μmで，さらに感度は放送用CCDカメラの60～100倍の感度を持つ．現段階では50～100 μmの血管の描出を確認している．本装置は，臨床応用の対象として，末梢動脈閉塞症に対する血管再生療法の効果判定を念頭においている．すなわち，体厚10cm程度の下肢血管造影に応用する．この普及型単色X線装置では，放射光を線源とした場合とは異なり，心血管系など厚い被写体を撮影することはできない．

c．プラズマX線

プラズマX線の性質は高輝度でシャープなK系列特性X線で，SN比が非常に高い特徴がある．プラズマX線発生装置の原理は図に示すように，コンデンサーに50～60kV程度まで充電し，トリガ電圧の印加でX線管に放電する．放電された陽極側の金属は管電流により気化され，弱電離線状プラズマの成長とともに特性X線が発生する（図Ⅲ-76）．ヨードのK吸収端である33.3keV近傍の単色に近いX線を得たい場合には，ターゲットとしてCe（原子番号58）を選択すれば34.566keVにピークを有する特性X線が得られる．こうして得られたX線は，そのターゲット特性の疑似単色X線が得られるので，フォトン量を減少させるような金属フィルターなどの操作を必要としない．このプラズマX線装置は前述の普及型X線装置に比べ高輝度であり，人体のような比較的厚い被写体も通過することができる[3]．

4．検出法

a．高解像度・高感度蛍光体

本微小血管造影法では浜松ホトニクスのファイバオプティクプレート（FOS）（Gd2O2S（Tb）またはCsI（T 1））を用いてX線透亮像を可視光線に変換し，HARPカメラで撮影する．FOSは数ミクロン径のガラスファイバを数千万本束ねた光学デバイスに，X線シンチレータを付加したX線イメージングデバイスである．蛍光体のみを堆

積しているため膜厚を薄くでき，光の拡散を小さくすることができ高解像度（50 μm (20 ラインペア)）となる．また，蛍光体だけを堆積させ不純物を含まない高密度と微細な柱状結晶構造は，光ファイバに似た微細かつ緻密な構造が減光を大幅に削減し高感度を保てる．

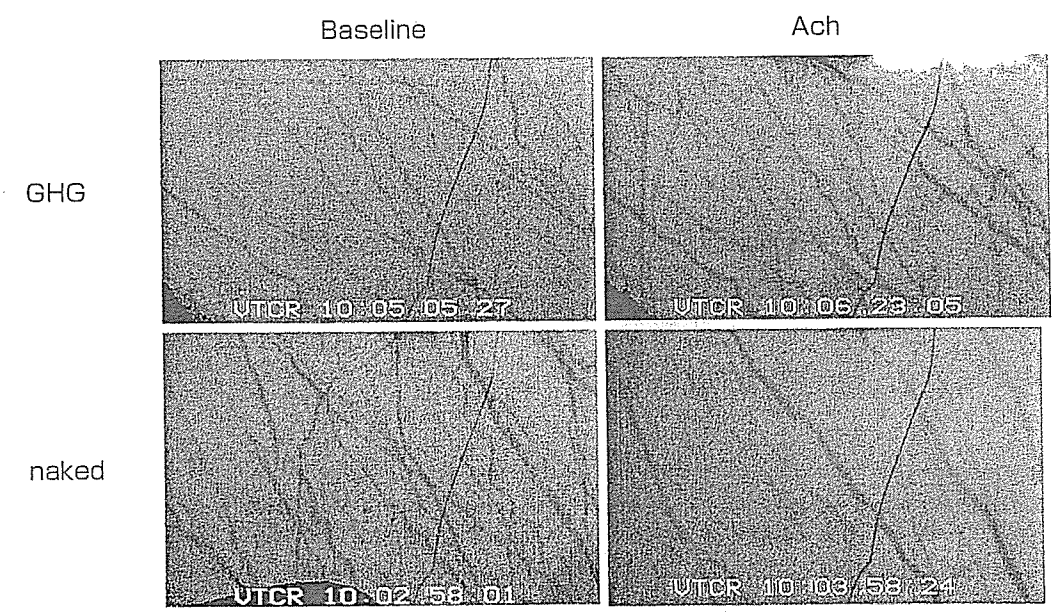
b．HARP 法

CCD を用いたハイビジョンカメラでは，画素あたりの光子数が減少し感度が低下してしまうため，高精細画像として微小血管を描出するには限界がある．アバランシェ型ハイビジョン用撮影管は高解像度で，高感度の撮影が可能であり，その構造は非セレン膜で構成された光伝導電層を有し，高電圧操作下で電子なだれ現象が生じ，実効量子効率が数百倍の光電変換をすることができる[4]．NHK の開発したモノクロ新 super-HARP カメラは，25 μm 非セレン膜の構造を持ち，CCD カメラより 100 倍以上の感度を持ち合せている[5]．

5．症例提示

a．放射光を用いた微小血管造影

ウサギ虚血肢の血管再生モデルを用いた，放射光施設での実験で血管径 100 μm 以下の血管が鮮明に描出されているのがわかる（図Ⅲ-77）．DNA は体内に投与すると，DNA 分解酵素の働きにより可及的速やかに分解されてその効果が失われてしまう．そこで著者らは，ゼラチンハイドロゲル（GHG）と DNA の複合体を形成し再生



図Ⅲ-77　放射光を用いた微小血管の描出と血管機能の評価方法

再生治療により新生された血管径 100 μm 以下の血管が描出されている．GHG・VEGF 複合体投与群ではアセチルコリン（Ach）投与にて Baseline と比べ，血管の拡張と血管数の増加を認めるが（図上），DNA 単独群（naked）では血管の拡張と増加は認められない（図下）．

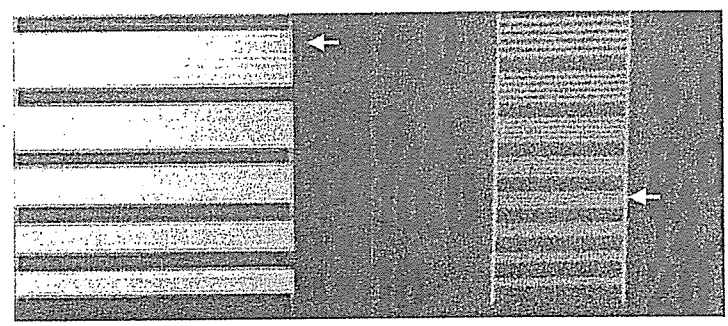
効果を徐放することにより，血管がより成熟度を増すかどうか微小血管造影法を用い検討した．DNAには血管成長因子であるVEGFを使用し，対象としてVEGF単独群，GHG・VEGF複合体群に血管再生治療を施行した．血管成熟度の評価方法はアセチルコリンの血管内投与による血管の反応性を用いた．結果はDNA単独群では血管の反応性は認めなかったが，GHG・VEGF複合体群では血管が拡張し，血管数が増加した（図Ⅲ-77）．

b．普及型単色X線装置を用いた微小血管造影

微小血管撮影を通常の血管造影装置と普及型単色X線装置の比較を提示する．テストチャートとファントムを用い，両装置の空間解像度と微小血管の描出を検討した．テストチャートによる解像度は，通常型の造影装置は250 μm（2ラインペア），普及型単色X線装置は50 μm（10ラインペア）であった（図Ⅲ-78）．単色X線装置の画像では，イヌ冠動脈の中隔枝が末梢まで分岐するたびに血管径が減ずることが観察できるが（図Ⅲ-79右），通常型X線装置の画像では血管端がぼやけて，分岐に伴う血管径の減少を確認できないので（図Ⅲ-79左），微小血管の評価には不向きである．

ポリキャピラリーによるX線平行化の効果を検討した．X線を平行化しない画像では，被写体を検出器から離すと血管端がぼやける．一方，キャピラリーによりX線を平行化すると，被写体を検出器から離した場合でも血管両端の歪みがなく微小血管の描出をさらに向上させる（図Ⅲ-80）．

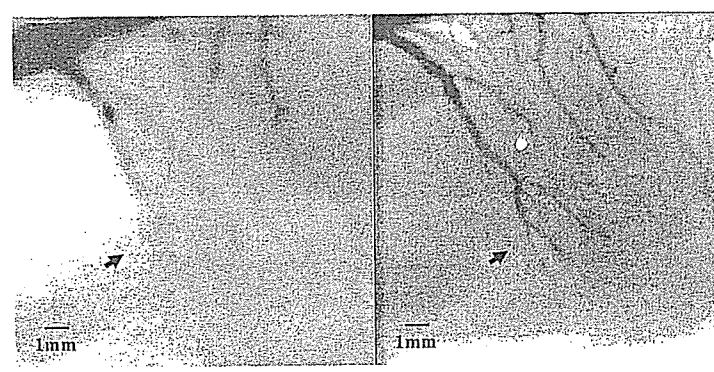
チャートを用いた図左の通常型血管造影装置の解像度は250 μm（2ラインペア），図右の普及型微小血管造影装置の解像度は50 μm（10ラインペア）を示す．



通常型造影装置　　普及型微小血管造影装置

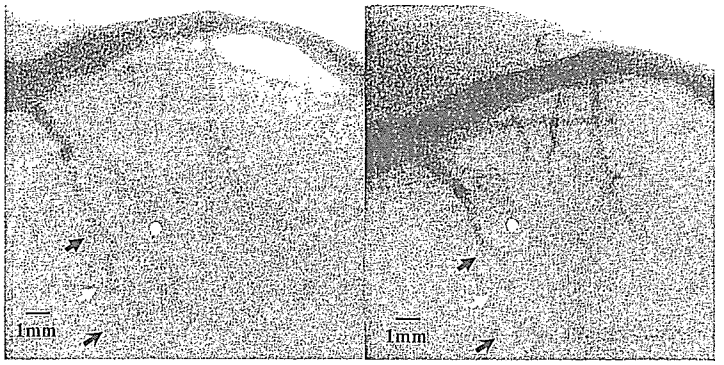
図Ⅲ-78　チャートによる通常型血管造影装置と普及型微小血管造影装置の比較

イヌ冠動脈にヨードマイクロスフィアー（直径25 μm）を詰めて結紮したファントムを用い，撮影した．図左は通常型血管造影装置で撮影したものであり，冠動脈末梢側はぼやけてしまっているが，図右の普及型微小血管造影装置で撮影したファントムは末梢側まで血管を追うことができる．



通常型造影装置　　普及型微小血管造影装置

図Ⅲ-79　イヌ冠動脈ファントムによる通常型血管造影装置と普及型微小血管造影装置の比較



イヌ冠動脈ファントムを検出器から15cm離した状態で撮影した．血管は分岐すると血管径が小さくなるが，平行化していない図左の血管は分岐するごとに血管径が細くならないが，右のキャピラリーで平行化した場合には，分岐ごとに血管径が細くなるのが観察できる．

図Ⅲ-80　キャピラリーによる平行化の効果

6．まとめ

再生医療が注目されているにもかかわらず，臨床において再生血管を可視化して評価する方法は確立されておらず，臨床症状の改善が唯一の治療評価となっているのが現状である．微小血管造影法の可及的速やかな普及化が期待されている．また，微小血管造影法は病理学的診断方法とは異なり，治療前後で血管の変化を検討することができ，臨床応用に有用と考えられる．さらに，動脈硬化・糖尿病などによる微小血管疾患，悪性腫瘍などの早期診断にも発展していく可能性も有する．本稿では，再生血管の評価方法として，微小血管造影法に必要な要素と放射光および普及型微小血管造影装置について概説した．

〔知久正明・西上和宏・佐藤英一・盛　英三〕

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ORIGINAL CONTRIBUTION

Naoto Fukuyama
Etsuro Tanaka
Yasuhiko Tabata
Hisanori Fujikura
Masao Hagihara
Hiromi Sakamoto
Kiyoshi Ando
Hiroe Nakazawa
Hidezo Mori

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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N. Fukuyama (✉) · H. Fujikura · M. Hagihara · K. Ando · H. Nakazawa
Depts. of Physiology, Internal Medicine and Center for Regenerative Medicine
Tokai University School of Medicine
Isehara, 259-1193, Japan
Tel.: +81-463/93-1121
Fax: +81-463/93-6684
E-Mail: fukuyama@is.icc.u-tokai.ac.jp

E. Tanaka
Dept. of Nutritional Sciences
Tokyo University of Agriculture
Tokyo, Japan

Y. Tabata
Research Center for Biomedical Engineering
Kyoto University
Kyoto, Japan

H. Sakamoto
Genetics Division
National Cancer Center Research Institute
Tokyo, Japan

H. Mori
National Cardiovascular Center Research Institute
Suita, Japan

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).

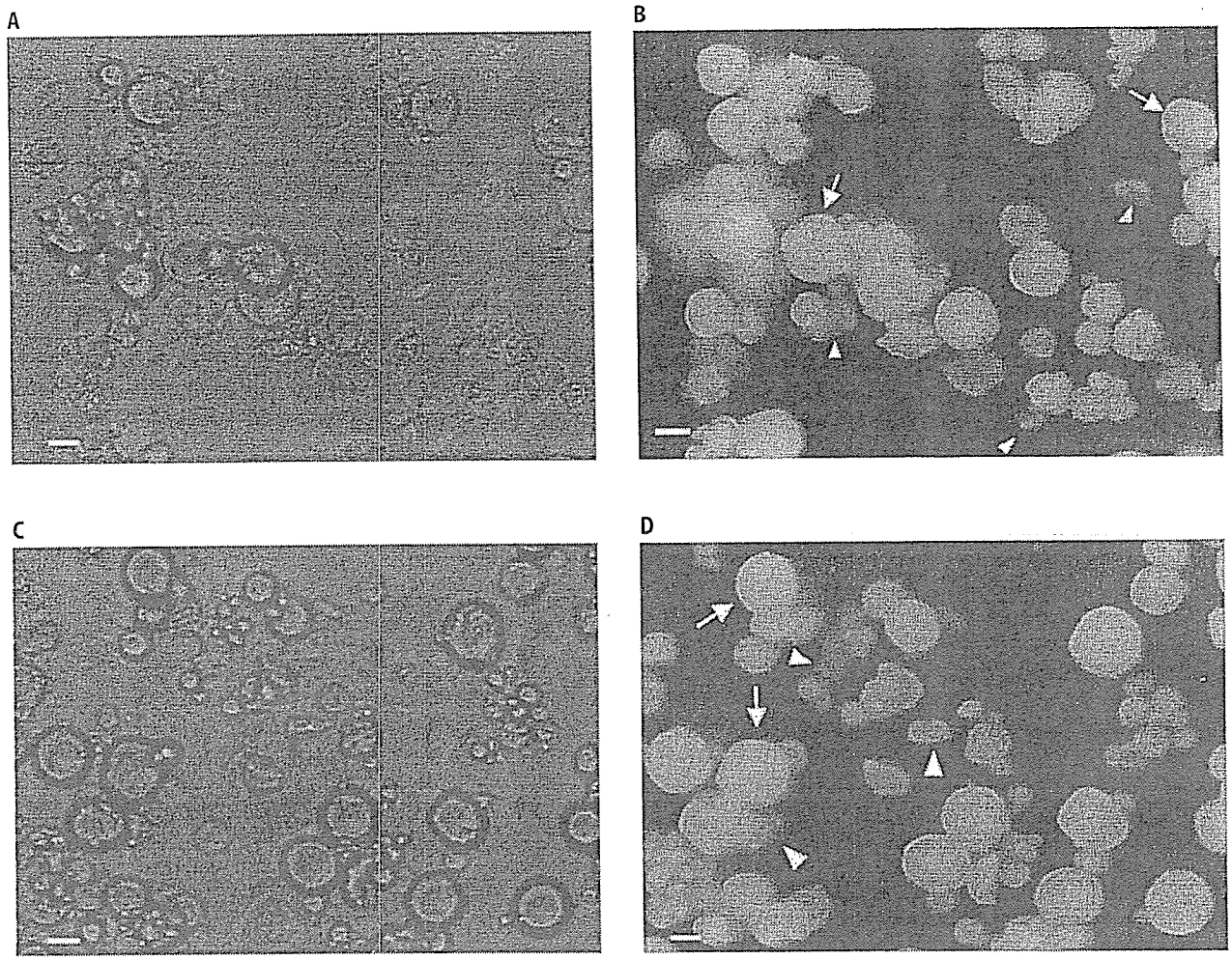

Fig. 1 Fluorescent presentation of ex vivo gene transfection with gelatin-DNA complex in macrophages/monocytes as well as fibroblasts. Rat macrophages (**A** and **B**) and human monocytes (**C** and **D**) were cultured with gelatin-GFP-gene complex for 14 days. Transmittance microscopic images (**A** 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-

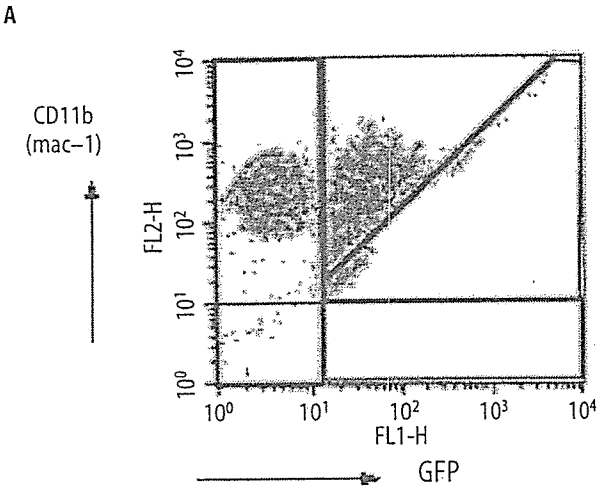

Fig. 2 Quantitative assessment of gene transfection into rat macrophages. (A) Fluorescence-activated cell sorting analysis of transfected macrophages done on day 14 of culture with reference to GFP-positive and Mac1-positive cells. (B) Sequential changes of luciferase activity in cultured macrophages 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-

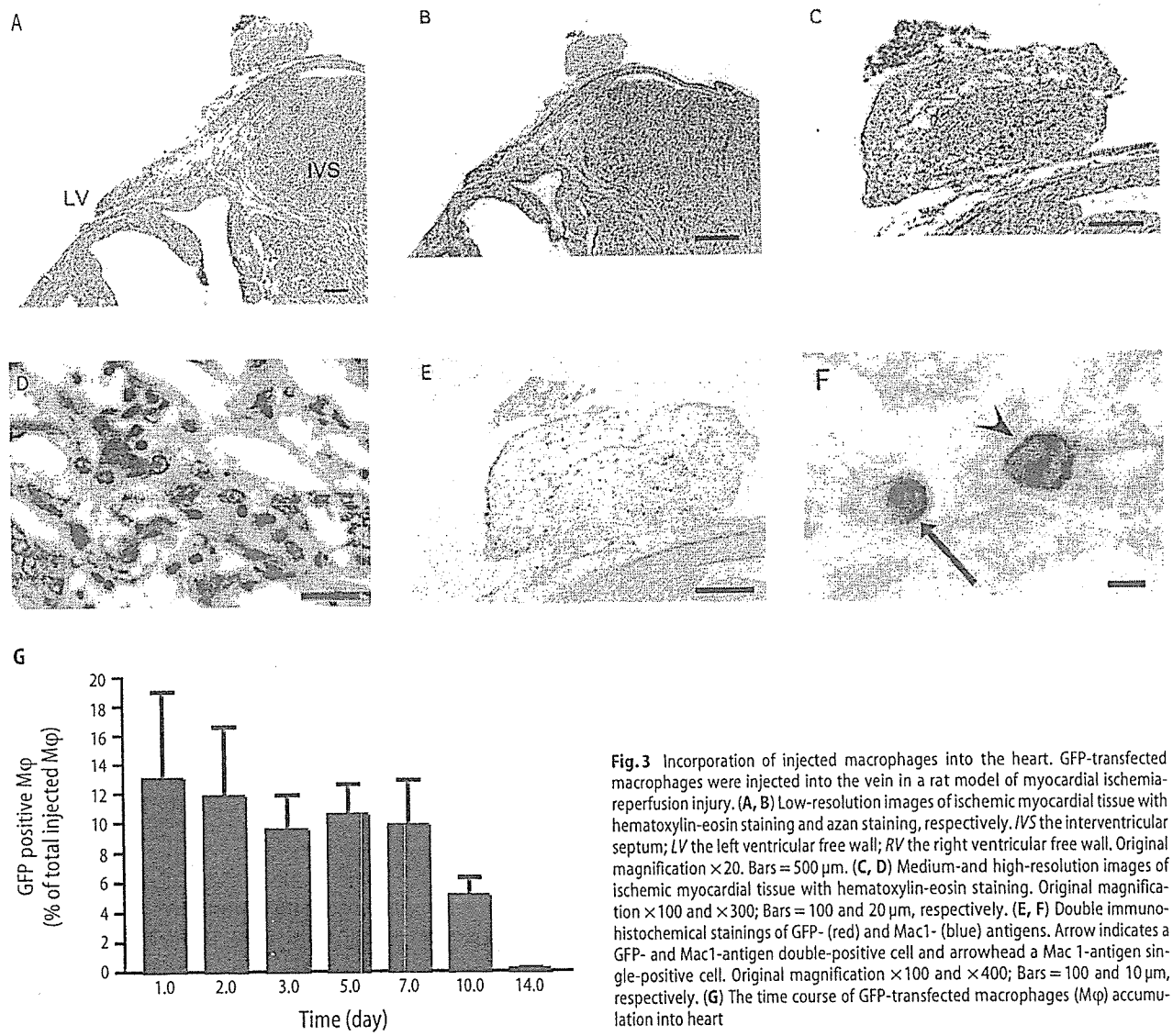

Fig. 3 Incorporation of injected macrophages into the heart. GFP-transfected macrophages were injected into the vein in a rat model of myocardial ischemia-reperfusion injury. (**A, B**) Low-resolution images of ischemic myocardial tissue with hematoxylin-eosin staining and azan staining, respectively. *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

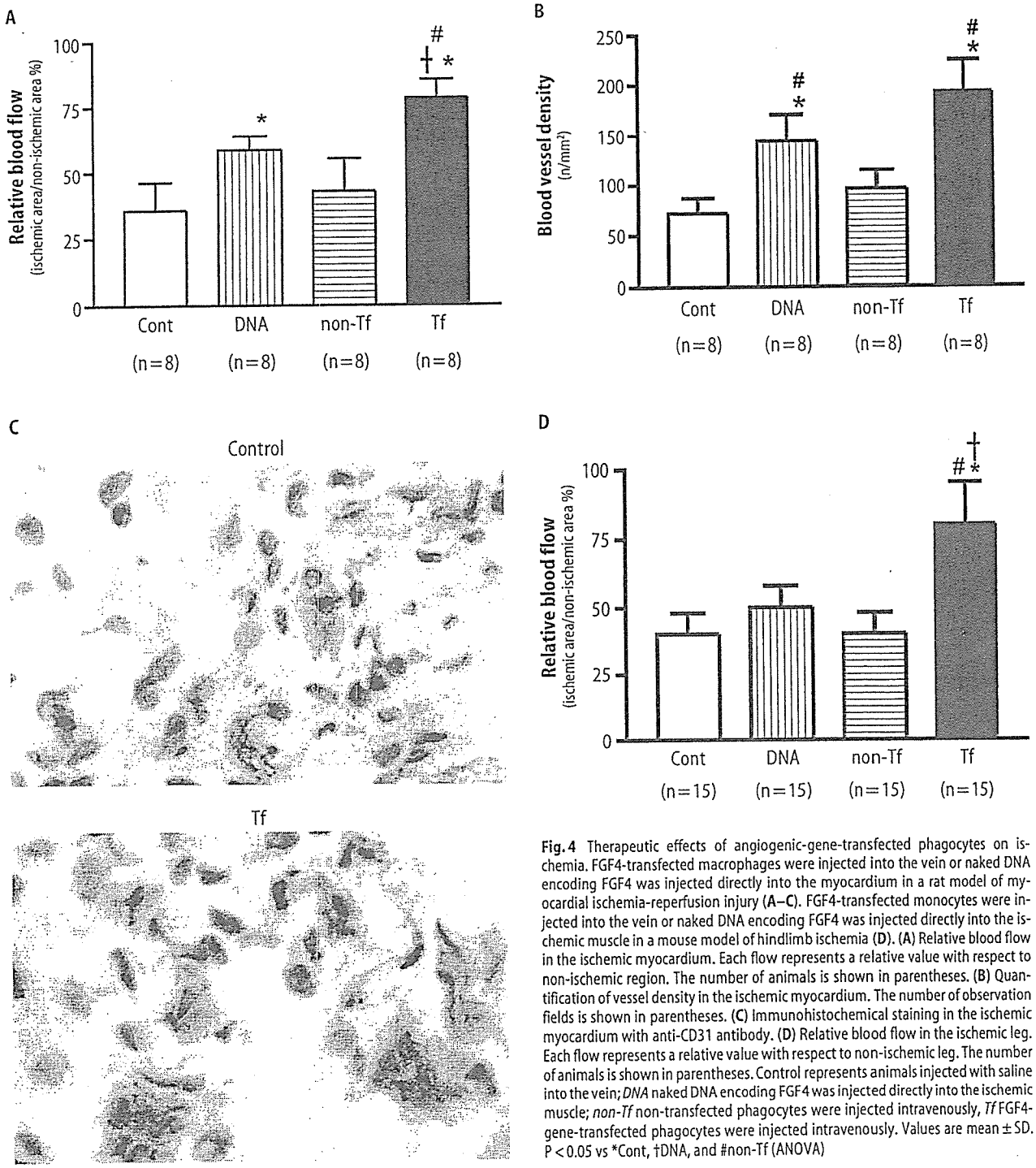

Fig. 4 Therapeutic effects of angiogenic-gene-transfected phagocytes on ischemia. FGF4-transfected macrophages were injected into the vein or naked DNA encoding FGF4 was injected directly into the myocardium in a rat model of myocardial ischemia-reperfusion injury (**A–C**). FGF4-transfected monocytes 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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Poor Implementation of Cardiac Rehabilitation Despite Broad Dissemination of Coronary Interventions for Acute Myocardial Infarction in Japan

— A Nationwide Survey —

Yoichi Goto, MD; Muneyasu Saito, MD; Toshiji Iwasaka, MD; Hiroyuki Daida, MD; Masahiro Kohzuki, MD; Kenji Ueshima, MD; Shigeru Makita, MD; Hitoshi Adachi, MD; Hiroyoshi Yokoi, MD; Kazuto Omiya, MD; Hiroshi Mikouchi, MD; Hiroyuki Yokoyama, MD 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*

Mailing address: Yoichi Goto, MD, Division of Cardiology, National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita 565-8565, Japan. E-mail: ygoto@hsp.ncvc.go.jp

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[3] 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.[14] 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.

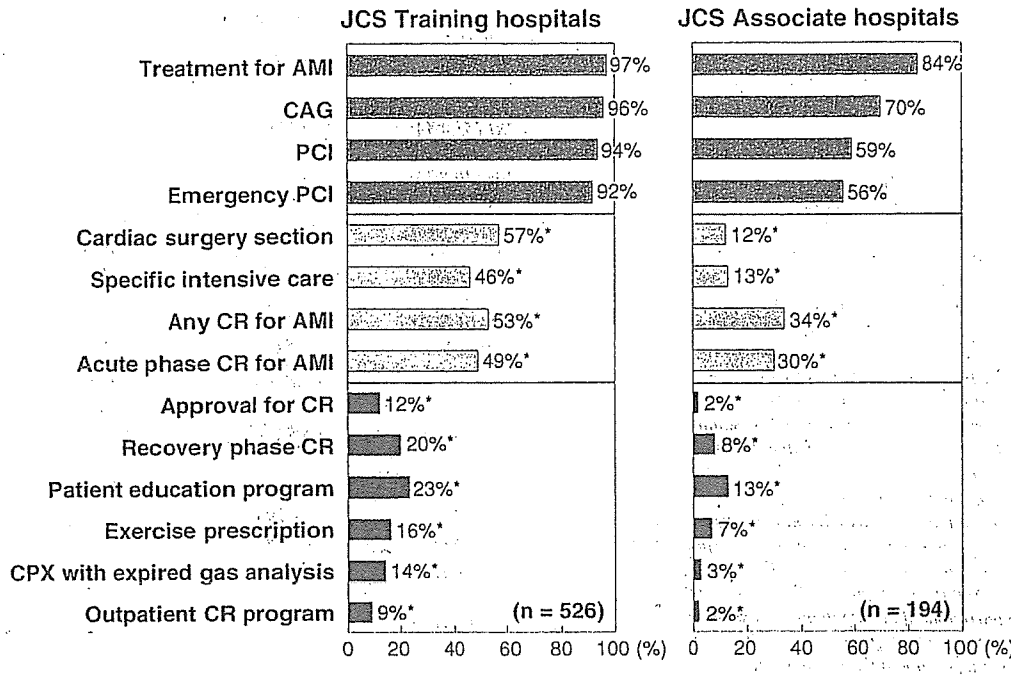

Fig 1. Implementation rates of various types of medical care for AMI in cardiology training hospitals authorized by the JCS. The implementation rates of care related to CR were remarkably low compared with the very high implementation rates of invasive procedures in both training and associate hospitals. 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-