厚生労働科学研究研究費補助金

循環器疾患等総合研究事業

# 心筋微小血管造影装置の開発による糖尿病性心筋微小循環障害の可視化（臨床研究実施チームの整備）

平成16年度　総括研究報告書

[redacted] 西上 和宏

平成17年（2005年）3月

厚生労働科学研究研究費補助金

循環器疾患等総合研究事業

# 心筋微小血管造影装置の開発による糖尿病性心筋微小循環障害の可視化（臨床研究実施チームの整備）

平成16年度　総括研究報告書

分担研究者　西上 和宏

平成17年（2005年）3月

# 目次

厚生労働科学研究費補助金（循環器疾患等総合研究事業）
（総括）研究報告書

# 心筋微小血管造影装置の開発による糖尿病性心筋微小循環障害の可視化に関する研究
（臨床研究実施チームの整備）

分担研究者　西上和宏　国立循環器病センター　心臓血管内科医長

微小血管障害の可視化のため、病院設置型微小血管造影装置の開発を行った。高出力・高感度の装置で、ファントムを用いた検討では50μmの血管まで評価可能であった。吸収線量は1mの20秒照射にて600mSvで臨床上の許容範囲であった。倫理委員会の承認を得、当施設に移設された。血管再生治療前後で微小血管造影法を施行し、再生血管の描出に成功した。

## A. 研究概要

難知性の重症末梢動脈閉塞症に対する血管再生治療が開始され、臨床症状の著明な改善が得られている。しかしながら、血管造影等の一般検査では、有意な変化がみられず、血管再生治療の適切な評価法が確立していない。また、糖尿病性微小血管障害は、病理診断のみで、臨床現場では病態の把握や治療効果についての手段が未だ存在せず、十分な対応がなされていないのが現状である。本研究では、病院設置型微小血管造影装置を開発、臨床応用し、微小血管障害の病態および治療効果の評価に対する有効性と安全性を検討した。

## B. 研究実績

新エネルギー産業技術総合開発機構(NEDO)の支援のもと、浜松ホトニクス(株)を中心に、NHKエンジニアリングサービス、国立循環器病センター研究所、東海大学医学部等が協力して、病院設置型の微小血管造影装置を開発した。装置は、高出力のCT用X線源とハイビジョンの高感度撮像系により構成されている。解像度の検討では、チャートにおいて、一般の血管造影では250μmが限界であったが、病院設置型微小血管造影装置では、50μmまで観察できた。犬冠動脈のファントムでは一般の血管造影では、第3分岐までしか描出できなかったが、病院設置型微小血管造影装置では、第4分岐以下まで明瞭に描出できた。血管再生治療を施行したウサギ虚血肢モデルでは、100μm以下の蛇行した再生血管が描出でき、アデノシンの反応性も評価できた。安全性の検討では、吸収線量が1mの位置で20秒照射が600mSvであり、散乱線も被写体から1mの位置で0.2mSvであった。臨床応用では、血管再生治療前後に、微小血管造影法を施行し、虚血肢下腿の微小血管を評価し、血管再生治療後に再生血管が描出された。

(倫理面への配慮)

倫理委員会の審議・承認を得、本検査の合併症・効能・不利益・利益を説明し、本人及び家族の同意の元に施行した。

## C. 考察

本研究で開発した微小血管造影装置は、50μmと従来の血管造影法より5分の1の血管まで評価可能であった。また、被曝量も許容範囲であった。臨床応用でも、血管再生治療後の再生血管が描出され、今後、症例を増やし、その有効性と安全性を確認する必要がある。

## D. 健康危険情報

特になし。

## E. その他実施した臨床研究の概要および実績

【原著論文】

1. Nagaya N, Fujii T, Iwase T, Ohgushi H, Itoh T, Uematsu M, Yamagishi M, Mori H, Kangawa K, Kitamura S: Intravenous administration of mesenchymal stem cells improves cardiac function in rats with

acute myocardial infarction through angiogenesis and myogenesis. Am J Physiol Heart Circ Physiol. 287: H2670-2676, 2004

2. Fujii T, Nagaya N, Iwase T, Murakami S, Miyahara Y, Nishigami K, Ishibashi-Ueda H, Shirai M, Itoh T, Ishino K, Sano S: Adrenomedullin enhances therapeutic potency of bone marrow transplantation for myocardial infarction in rats. Am J Physiol Heart Circ Physiol, 288: H1444-1450, 2005
3. Itoh T, Nagaya N, Fujii T, Iwase T, Ishibashi-Ueda H, Yutani C, Kimura H, Kangawa K: C-Type natriuretic peptide ameliorates monocrotaline-induced pulmonary hypertension through multiple vasoprotective effects in rats. Am J Respir Crit Care Med, 170: 1204-1211, 2004
4. Murakami S, Nagaya N, Itoh T, Fujii T, Iwase T, Hamada K, Kimura H, Kangawa K: C-type natriuretic peptide attenuates bleomycin-induced pulmonary fibrosis in mice. Am J Physiol Lung Cell Mol Physiol, 287: L1172-1177, 2004
5. Okumura H, Nagaya N, Itoh T, Okano I, Hino J, Mori K, Tsukamoto Y, Ishibashi-Ueda H, Miwa S, Tambara K, Toyokuni S, Yutani C, Kangawa K: Adrenomedullin infusion attenuates myocardial ischemia/reperfusion injury through phosphatidylinositol 3-kinase/Akt-dependent pathway, Circulation, 109:242-8, 2004
6. Nagaya N, Moriya J, Yasumura Y, Uematsu M, Ono F, Shimizu W, Ueno K, Kitakaze M, Miyatake K, Kangawa K: Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting patients with chronic heart failure. Circulation, 110: 3674-3679, 2004;
7. Nagaya N, Yamamoto H, Uematsu M, Itoh T, Nakagawa K, Miyazawa T, Kangawa K, Miyatake K : Green tea reverses endothelial dysfunction in healthy smokers. Heart, 90: 1485-1486, 2004
8. Hosoda H, Doi K, Nagaya N, Okumura H, Nakagawa E, Enomoto M, Ono F, Kangawa K: Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. Clin Chem, 50: 1077-1080, 2004
9. Nagaya N, Kyotani S, Uematsu M, Ueno K, Oya H, Nakanishi N, Shirai M, Mori H, Miyatake K, Kangawa K: Effects of adrenomedullin inhalation on hemodynamics and exercise capacity in patients with idiopathic pulmonary arterial hypertension. Circulation, 109:351-6, 2004
10. Tokunaga N, Nagaya N, Shirai M, Tanaka E, Ishibashi-Ueda H, Harada-Shiba M, Kanda M, Ito T, Shimizu W, Tabata Y, Uematsu M, Nishigami K, Sano S, Kangawa K, Mori H: Adrenomedullin Gene Transfer Induces Therapeutic Angiogenesis in a Rabbit Model of Chronic Hind Limb Ischemia. Benefits of a Novel Nonviral Vector, Gelatin. Circulation, 109: 526-31, 2004
11. Itoh T, Nagaya N, Yoshikawa M, Fukuoka A, Takenaka H, Shimizu Y, Haruta Y, Oya H, Yamagishi M, Hosoda H, Kangawa K, Kimura H: Elevated plasma ghrelin level in underweight patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med, 170: 879-882, 2004
12. Higashikata T, Yamagishi M, Sasaki H, Minatoya K, Ogino H, Ishibashi-Ueda H, Hao H, Nagaya N, Tomoike H, Sakamoto A: Application of real-time RT-PCR to quantifying gene expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human abdominal aortic aneurysm. Atherosclerosis, 177: 353-360, 2004

13. Shimizu W, Noda T, Takaki H, Nagaya N, Satomi K, Kurita T, Suyama K, Aihara N, SunagawaK, Echigo S, Miyamoto Y, Yoshimasa Y, Nakamura K, Ohe T, Towbin J A, Priori S G, Kamakura S: Diagnostic value of epinephrine test for genotyping LQT1, LQT2, and LQT3 forms of congenital long QT syndrome. Heart Rhythm, 3: 276-283, 2004
14. Nagaya N, Kangawa K: Adrenomedullin in the treatment of pulmonary hypertension. Peptides, 2511: 2013-2018, 2004
15. Nagaya N, Mori H, Murakami S, Kangawa K: Kitamura S. Adrenomedullin: angiogenesis and gene therapy. Am J Physiol (Conditional accept)

【著書】

1. K. Noishigami, T. Nakatani, M.Chiku, H. Mori: A Novel Micro-Angiography Detecting Angiogenesis, Application for Autologous Bone Marrow Mononuclear Cells Transplantation in the Patients with Critical Limb Ischemia, H. Mori, H. Matsuda (Eds.) Cardiovascular Regeneration Therapies Using Tissue Engineering Approaches, Springer, 2005
2. Nagaya N, Fukuyama N, Tabata Y, Mori H: Potentiation of Regenerative Therapy by Non-Viral Vector, Gelatin Hydrogel, H. Mori, H. Matsuda (Eds.) Cardiovascular Regeneration Therapies Using Tissue Engineering Approaches, Springer, 2005

【和文著書】

1. 知久正明、西上和宏、内藤博昭、盛　英三、佐藤英一：画像解析-微小血管造影-　再生医療へのブレイクスルー（その革新技術と今後の方向性)、遺伝子医学 MOOK (1) 223-227、2004
3. 知久正明、西上和宏、盛英三、河合敏明、望月亮：病院設置型微小血管造影装置の開発と臨床応用の可能性、血管医学 (5) 17-25、2004
4. 永谷憲歳、片岡雅晴. 遺伝子-細胞ハイブリット治療. 遺伝子医学 ・MOOK1, 273-277, 株式会社メディカルドゥ, 2004
5. 永谷憲歳、寒川賢治: グレリンのトランスレーショナルリサーチ　－循環器疾患-
日本臨床、臨床分子内分泌学（1）Ⅲ. グレリン, 62: 430-434, 株式会社日本臨床社, 2004
6. 清水嘉人、永谷憲歳: 循環器疾患
日本臨床, 臨床分子内分泌学（1）Ⅲ. グレリン, 62: 396-398, 株式会社日本臨床社, 2004
7. 岩瀬俊、永谷憲歳: アドレノメデュリンと細胞治療, 日本臨床　臨床分子内分泌学（1）Ⅱ. アドレノメデュリン, 62: 296-301 株式会社日本臨床社,2004
8. 永谷憲歳、寒川賢治: 第5章, 重症心不全の治療の実際, B.特殊内科的治療, 5.エンドセリン受容体拮抗薬, 重症心不全, 診断・治療・病理の最前線, 169-172, 医学書院, 2003

【シンポジウム・招待講演】

1. Nagaya N：Emerging roles for G protein Coupled Receptor Systems in Cardiovascular Biology: Ghrelin. 第 77 回 American Heart Association, 2004/11
2. Nagaya N, Kangawa K：Translational research of ghrelin for cardiac and pulmonary cachexia. COE International Symposium on Recent Advance in Biological Active Peptides and Signal Transduction, 2004/11
3. Nagaya N, Kangawa K: Adrenomedullin in the treatment of pulmonary hypertension. CGRP AMY CT AM PAMP 4th Symposium, 2004/3

研究成果の刊行に関する一覧表レイアウト（参考）

書籍

| 著者氏名 | 論文タイトル名 | 書籍全体の編集者名／特集 | 書　籍　名 | 出版社名 | 出版地 | 出版年 | ページ |
|---|---|---|---|---|---|---|---|
| K. Noishigami, T. Nakatani, M. Chiku, H. Mori | A Novel Micro-Angiography Detecting Angiogenesis, Application for Autologous Bone Marrow Mononuclear Cells Transplantation in the Patients with Critical Limb Ischemia | H. Mori H. Matsuda | Cardiovascular Regeneration Therapies Using Tissue Engineering Approaches | Springer | 東京 | 2005 | 191-200 |
| Nagaya N, Fukuyama N, Tabata Y, Mori H | Potentiation of Regenerative Therapy by Non-Viral Vector, Gelatin Hydroge | H. Mori H. Matsuda | Cardiovascular Regeneration Therapies Using Tissue Engineering Approaches | Springer | 東京 | 2005 | 17-30 |
| 知久正明 西上和宏 内藤博昭 盛　英三 佐藤英一 | 画像解析-微小血管造影- | 田畑康彦 | 再生医療へのブレイクスルー（その革新技術と今後の方向性)、遺伝子医学MOOK1 | メディカルドゥ | 大阪 | 2004 | 223-227 |
| 知久正明 西上和宏 盛英三 河合敏明 望月亮 | 病院設置型微小血管造影装置の開発と臨床応用の可能性 | 特集：血管の先進映像医学 | 血管医学（5） | メディカルレビュー | 大阪 | 2004 | 17-25 |
| 永谷憲歳 片岡雅晴 | 遺伝子-細胞ハイブリット治療 | 田畑康彦 | 再生医療へのブレイクスルー（その革新技術と今後の方向性)、遺伝子医学MOOK1 | メディカルドゥ | 大阪 | 2004 | 273-277 |
| 永谷憲歳 寒川賢治 | Ⅲ．グレリン、グレリンのトランスレーショナルリサーチ－循環器疾患- | 日本臨床62 | 臨床分子内分泌学（1） | 日本臨床社 | 大阪 | 2004 | 430-434 |
| 清水嘉人 永谷憲歳 | Ⅲ．グレリン、循環器疾患 | 日本臨床62 | 臨床分子内分泌学（1） | 日本臨床社 | 大阪 | 2004 | 396-39 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 岩瀬俊<br>永谷憲歳 | II．アドレノメデュリン，アドレノメデュリンと細胞治療 | 日本臨床62 | 臨床分子内分泌学（1） | 日本臨床社 | 大阪 | 2004 | 296-301 |

雑誌

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
| Nagaya N, Fujii T, Iwase T, Ohgushi H, Itoh T, Uematsu M, Yamagishi M, Mori H, Kangawa K, Kitamura S | Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis | Am J Physiol Heart Circ Physiol | 287 | H2670-2676 | 2004 |
| Fujii T, Nagaya N, Iwase T, Murakami S, Miyahara Y, Nishigami K, Ishibashi-Ueda H, Shirai M, Itoh T, Ishino K, Sano S | Adrenomedullin enhances therapeutic potency of bone marrow transplantation for myocardial infarction in rats | Am J Physiol Heart Circ Physiol | 288 | H1444-1450 | 2005 |
| Itoh T, Nagaya N, Fujii T, Iwase T, Ishibashi-Ueda H, Yutani C, Kimura H, Kangawa K | C-Type natriuretic peptide ameliorates monocrotaline-induced pulmonary hypertension through multiple vasoprotective effects in rats | Am J Respir Crit Care Med | 170 | 1204-1211 | 2004 |
| Murakami S, Nagaya N, Itoh T, Fujii T, Iwase T, Hamada K, Kimura H, Kangawa K | C-type natriuretic peptide attenuates bleomycin-induced pulmonary fibrosis in mice | Am J Physiol Lung Cell Mol Physiol | 287 | L1172-1177 | 2004 |
| Okumura H, Nagaya N, Itoh T, Okano I, Hino J, Mori K, Tsukamoto Y, Ishibashi-Ueda H, Miwa S, Tambara K, Toyokuni S, Yutani C, Kangawa K | Adrenomedullin infusion attenuates myocardial ischemia/reperfusion injury through phosphatidylinositol 3-kinase/Akt-dependent pathway, | Circulation | 109 | 242-8 | 2004 |

| | | | | | |
|---|---|---|---|---|---|
| Nagaya N, Moriya J, Yasumura Y, Uematsu M, Ono F, Shimizu W, Ueno K, Kitakaze M, Miyatake K, Kangawa K | Effects of ghrelin administration on left ventricular function, exercise capacity, and muscle wasting patients with chronic heart failure | Circulation | 110 | 3674-3679 | 2004 |
| Nagaya N, Yamamoto H, Uematsu M, Itoh T, Nakagawa K, Miyazawa T, Kangawa K, Miyatake K | Green tea reverses endothelial dysfunction in healthy smokers | Heart | 90 | 1485-1486 | 2004<br>(記載なし) |
| Hosoda H, Doi K, Nagaya N, Okumura H, Nakagawa E, Enomoto M, Ono F, Kangawa K | Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples | Clin Chem | 50 | 1077-1080 | 2004 |
| Nagaya N, Kyotani S, Uematsu M, Ueno K, Oya H, Nakanishi N, Shirai M, Mori H, Miyatake K, Kangawa K | Effects of adrenomedullin inhalation on hemodynamics and exercise capacity in patients with idiopathic pulmonary arterial hypertension | Circulation | 109 | 351-6 | 2004 |
| Tokunaga N, Nagaya N, Shirai M, Tanaka E, Ishibashi-Ueda H, Harada-Shiba M, Kanda M, Ito T, Shimizu W, Tabata Y, Uematsu M, Nishigami K, Sano S, Kangawa K, Mori H | Adrenomedullin Gene Transfer Induces Therapeutic Angiogenesis in a Rabbit Model of Chronic Hind Limb Ischemia. Benefits of a Novel Nonviral Vector, Gelatin | Circulation | 109 | 526-31 | 2004 |
| Itoh T, Nagaya N, Yoshikawa M, Fukuoka A, Takenaka H, Shimizu Y, Haruta Y, Oya H, Yamagishi M, Hosoda H, Kangawa K, Kimura H | Elevated plasma ghrelin level in underweight patients with chronic obstructive pulmonary disease | Am J Respir Crit Care Med | 170 | 879-882 | 2004 |

| | | | | | |
|---|---|---|---|---|---|
| Higashikata T, Yamagishi M, Sasaki H, Minatoya K, Ogino H, Ishibashi-Ueda H, Hao H, Nagaya N, Tomoike H, Sakamoto A | Application of real-time RT-PCR to quantifying gene expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in human abdominal aortic aneurysm | Atherosclerosis | 177 | 353-360 | 2004 |
| Shimizu W, Noda T, Takaki H, Nagaya N, Satomi K, Kurita T, Suyama K, Aihara N, SunagawaK, Echigo S, Miyamoto Y, Yoshimasa Y, Nakamura K, Ohe T, Towbin J A, Priori S G, Kamakura S | Diagnostic value of epinephrine test for genotyping LQT1, LQT2, and LQT3 forms of congenital long QT syndrome | Heart Rhythm | 3 | 276-283 | 2004 |
| Nagaya N, Kangawa K: Adrenomedullin in the treatment of pulmonary hypertension. Peptides | Adrenomedullin in the treatment of pulmonary hypertension | Peptides | 2511 | 2013-2018 | 2004 |
| Nagaya N, Mori H, Murakami S, Kangawa K. Kitamura S | Adrenomedullin: angiogenesis and gene therapy | Am J Physiol | | | (Conditional accept)<br>(収録なし) |

# A Novel Micro-Angiography Detecting Angiogenesis, Application for Autologous Bone Marrow Mononuclear Cells Transplantation in the Patients with Critical Limb Ischemia

Kazuhiro Nishigami, Takeshi Nakatani, Masaaki Chiku, Hidezo Mori

National Cardiovascular Center, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan

**Summary.** Conventional Anigiographic Findings in Autologous Bone Marrow Mononuclear Cells Transplantation for Critical Limb Ischemia: Bone marrow mononuclear cells have many of the characteristics of stem cells for mesenchymal tissues, and secrete many angiogenic cytokines. We performed autologous transplantation of bone marrow mononuclear cells in six patients with critical limb ischemia due to Buerger disease, who were not candidates for catheter or surgical revascularization. Leg pains at rest and skin ulcers improved after bone marrow transplantation in all patients, although significant collateral developments after the therapy by conventional angiography could not be observed. Autologous transplantation of bone marrow mononuclear cells including stem cells improved critical limb ischemia due to Buerger disease. Neovascularization after therapeutic angiogenesis might be quite small and could not be visualized by conventional angiography.

Novel Micro-angiograpy:We developed in-hospital micro-angiographic equipment which consisted of a high power X-ray source for computed tomography and an avalanche type detector characterized by a high spatial resolution (20μm) and high sensitivity (100 times of CCD camera). We visualized mid-zone collaterals after femoral arterial exfoliation with and without therapeutic angiogenesis in rabbit ischemic limbs and assessed the radio-absorptions in a clinical setting. The micro-angiography clearly demonstrated mid-zone collaterals after the treatment with a diameter of down to 50μm, but the conventional angiography did not. The sum of ra-

dio-absorptions for 10 seconds in clinical settings was 300 mSv. The newly developed in-house micro-angiography could illuminate micro-vessels with a diameter of down to 50μm in clinical settings safely and could be useful in the evaluation of therapeutic angiogenesis.

**Keywords.** Micro-angiography, Angiogenesis, Autologous bone marrow mononuclear cells transplantation, Critical limb ischemia, Buerger disease

## Introduction

Endothelial progenitor cells (EPCs) possess the ability to mature into cells that line the lumen of blood vessels(Asahara T, et al. 1997). Therapeutic angiogenesis could be induced by the transplantation of bone marrow mononuclear cells including EPCs. Several studies demonstrated that therapeutic angiogenesis using autologous bone marrow mononuclear cells transplantation (BMT) was effective for ischemic vascular diseases although conventional angiography could not precisely detect developed collaterals after therapeutic angiogenesis(Iba O, et al. 2002, Inaba S, et al. 2002, Shintani S, et al. 2001,Tateishi-Yuyama E, et al. 2002). We developed an in-hospital micro-angiographic equipment which consisted of a high power X-ray source for computed tomography and an avalanche type detector characterized by a high spatial resolution (20μm) and high sensitivity (100 times of CCD camera).

The purpose of the present study was to evaluate the clinical effects and conventional angiographic findings on BMT for critical limb ischemia, and to validate the usefulness and safety of the novel micro-angiography technique for the evaluation of therapeutic angiogenesis.

## Methods

### *Patients*

Patients qualified for autologous BMT if they had chronic critical limb ischemia including rest pain and/or non-healing ischemic ulcers for a minimum of 4 weeks without evidence of improvement in response to

conventional therapies and were not optimal candidates for surgical or catheter revascularization. Buerger's disease was diagnosed by segmental occlusion of small- and medium-sized arteries, absence of atherosclerosis, and corkscrew collaterals circumventing the occlusion in angiogram and the exclusion of autoimmune diseases such as scleroderma or systemic lupus erythematosus, hypercoagulable states, diabetes, or acute arterial occlusion secondary to embolism. Patients with retinopathy and/or malignancy were excluded. Although 30 patients with atherosclerotic peripheral artery disease were candidates for BMT, they were excluded from the present study due to their systemic atherosclerotic complications. Six patients with Buerger's disease were recruited for the present study. All patients had legs pain at rest and five patients had foot ulcers. Written consent was obtained from all participants of this study. This clinical trial of autologous BMT for the treatment of patients with critical ischemia was approved by the Medical Ethics Committee of the National Cardiovascular Center.

### *Autologous BMT*

Bone marrow fluid (700-800ml) was collected from the iliac bone under general anesthesia. The harvested bone marrow fluid was diluted with RPMI 1640 (Nikken Bio Medical Laboratory, Kyoto, Japan) containing heparin, then stored in a sterile pack from the Bone Marrow Collection Kit (Baxter, IL, USA). The mononuclear cell fraction was prepared with a Fresenius AS104 (AMCO, USA). The injection volume was 0.5ml and injections were spaced 2-3cm apart, using a 1ml syringe and a 27-gauge needle. Leg pains were measured by a visual analog pain scale and foot ulcers were evaluated by area and appearance.

### *Novel micro-angiography*

The in-hospital micro-angiographic equipment consisted of a high power X-ray source for computed tomography and an avalanche type detector characterized by a high spatial resolution (20μm) and high sensitivity (100 times of CCD camera) (Fig.1).

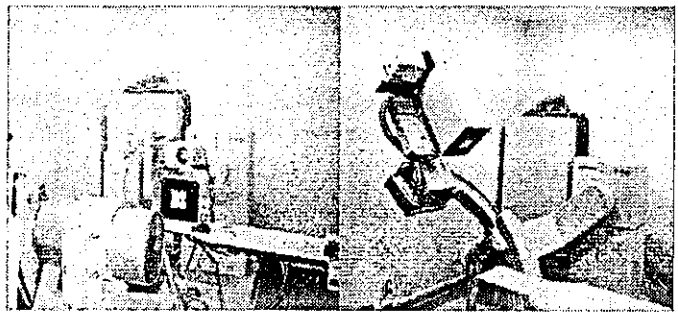


**Fig. 1a, b.**The micro-angiographic equipment that we developed. High-voltage power X-ray source **a** and a detecting system with a high spatial resolution(25μm) and high sensitivity (100 times of CCD camera) **b**.

Limb ischemia models in rabbits were made by ligating the femoral artery and treated by fibroblast growth factor 4 (FGF-4) genes incorporated to gelatin hydro gel (GHG). One month after the treatment, we evaluated collateral micro-vessels by using conventional and micro-angiographic systems. The approach was via the left femoral artery so that the catheter was located in the abdominal aorta. A 5ml bolus of Iodine contrast medium (300mg/ml) was injected at 3ml/sec using an auto-injection system. Imaging was recorded using a digital source in 1000 x 1000 pixels. The sum of radio-absorptions for 10 seconds in clinical settings was studied.

## Results

### *Autologous BMT for Critical Limb Ischemia*

The number of transplanted bone marrow mononuclear cells were one to five multiplied $10^9$. Rest pains decreased or disappeared in one month after BMT (Fig.2) and Skin ulcers improved in one to three months after BMT in all patients (Fig.3).

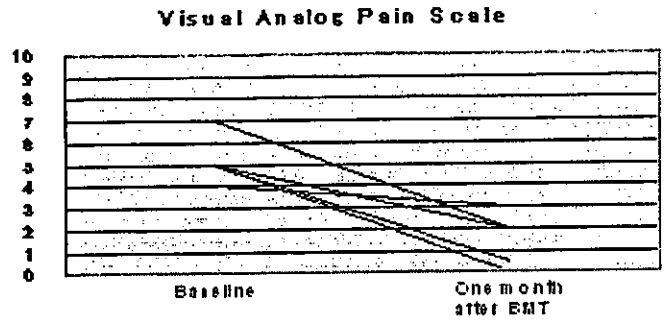



**Fig. 2.** The Visual analog pain scale in all patients.

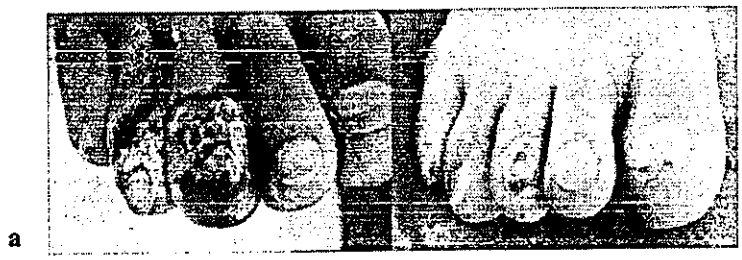
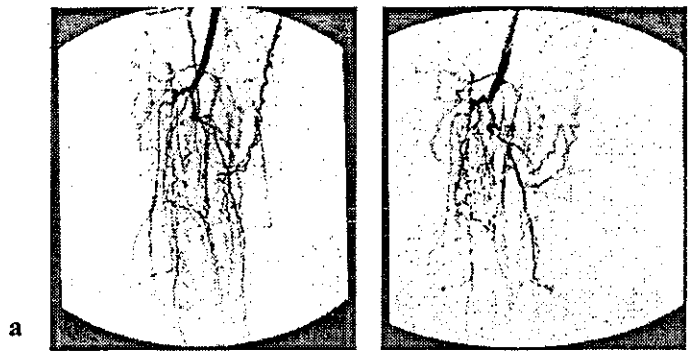



**Fig. 3a, b.** The skin ulcers in a patient before **a** and one month after autologous bone marrow transplantation **b.**

Conventional angiography was performed before and one month after BMT, but there was no significant changes in any of the patients (Fig.4).

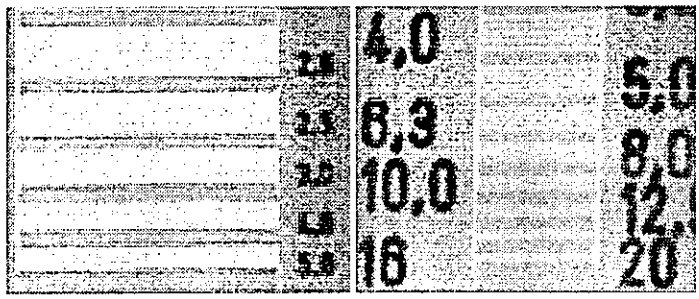


a b

**Fig. 4a, b.** The conventional angiographic findings in the patient before **a** and one month after autologous bone marrow transplantation **b**.

### *Novel micro-angiography*

The novel micro-angiography can detect to within a limit 50 of μm, although a detection limit of a conventional angiography is 250μm (Fig.5).




a b

**Fig. 5a, b.** The detection limits on a conventional angiography **a** and the novel micro-angiography **b** using a line chart

Collateral micro-vessels, which were 100-500μm or less in diameter, were demonstrated more clearly in micro-angiography than conventional angiography (Fig.6).

The sum of radio-absorptions at the point of 1m distance from the X-ray source in clinical settings was 300 mSv. for 10 seconds.

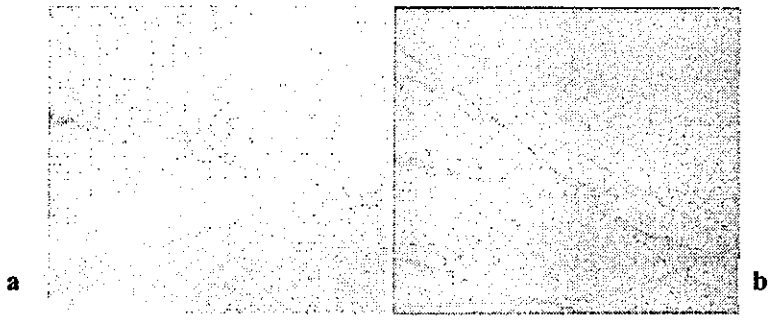



**Fig. 6a, b.** In 2.5x2.5cm view size, Collateral micro-vessels after therapeutic angiogenesis in the rabbit limb ischemia model. Vessel sizes in the range of 100-500μm or less, were demonstrated in the novel micro-angiography **b** more clearly than in a conventional angiography **a**. The diameter of the line in the micro-angiography is 130μm.

## Discussion

Autologous BMT improved chronic severe limb ischemia due to Bue rger's disease. Conventional angiography could not disclose develope d collateral vessels after BMT. A novel micro-angiography technique could illuminate promoted collateral vessels after therapeutic angioge nesis in rabbit models although a conventional angiography did not. The sum of radio-absorptions in the novel angiography could be acc-epted in clinical settings.

### *Autologous BMT and Buerger's disease*

Bone marrow harvests need an amount of more than 500ml bone marrow fluid and general anesthesia in therapeutic angiogenesis using BMT. Such factors have practical limitations to select candidates with peripheral artery disease complicated with systemic atherosclerosis and aging for BMT. Buerger's disease is a segmental vasculitis that affects the distal arteries of the upper and lower extremities. It typically occurs in young people. The majority of patients with Buerger's disease have pain at rest and digital

ulcerations and are hard to treat by revasculaizations, including catheter angioplasty and surgical bypass grafting, because of peripheral artery lesions. Patients with Buerger's disease, however, tend to have less systemic atherosclerotic lesions and normal cardiac function. These suggest that patients with Buerger's disease are the ideal candidates for therapeutic angiogenesis using autologous BMT.

### *Discrepancy between clinical improvements and conventional angiographic findings after BMT*

BMT improved critical limb ischemia clinically. Promoted collateral vessels after the treatment were not, however, visualized well by conventional angiography. These vessels are quite small and the detection limit of small vessels by conventional angiography is about 200μm in diameter.

### *Novel micro-angiography*

Recently, synchrotron radiation system characterized by high brightness, monochromatic and collimated nature bypass, revealed micro-vessels in situ. However the high cost of a synchrotron system strictly limits its clinical application (100 million dollars or more). We developed an in-house micro-angiographic system with a relatively low cost of approximately 1million dollars, which consisted of a high-voltage power X-ray source and a detecting system with a high spatial resolution (25μm) and high sensitivity (100 times of CCD camera). We evaluated collateral micro-vessels one month after therapeutic angiogenesis by using the conventional and micro-angiographic system. The in-house micro-vessel angiographic system could detect the micro-vessels more precisely than conventional angiographic system. We thought that the present micro-angiography should be useful for evaluating efficacy of therapeutic angiogenesis in clinical settings.

## Conclusions

Conventional angiography failed to disclose the promoted collateral vessels after BMT although BMT improved the critical limb ischemia clini-

cally. The in-house micro-angiographic system could detect the micro-vessels more precisely than conventional angiographic system and the sum of the radio-absorption in the equipment could be acceptable in clinical settings. The novel in-house micro-angiographic system can be useful in the evaluation of therapeutic angiogenesis clinically.

## References

Asahara T, Murohara T, Sullivan A, et al.(1997) Isolation of Putative Progenitor Endothelial Cells for Angiogenesis. Science 275:964-966

Iba O, Matsubara H, Nozawa Y, et al.(2002) Angiogenesis by Implantation of Peripheral Blood Mononuclear Cells and Platelets into Ischemic Limbs. Circulation 106:2019-2025

Inaba S, Egashira K, Komori K (2002) Peripheral-blood or bone-marrow mononuclear cells for therapeutic angiogenesis? Lancet 360:2083.

Shintani S, Murohara T, Ikeda H, et al.(2001) Augmentation of Postnatal Neovascularizaion with Autologous Bone Marrow Transplantation. Circulation 103: 897-903

Tateishi-Yuyama E, Matsubara H, Murohara T, et al.(2002) Therapeutic angiogenesis for patients with imb ischemia by autologous transplantation of bone-marrow cells:a pilot study and a randomised controlled trial. Lancet,360:427-35

# Potentiation of Regenerative Therapy by Non-Viral Vector, Gelatin Hydrogel

Noritoshi Nagaya[1], Naoto Fukuyama[2], Yasuhiko Tabata[3], Hidezo Mori[4]

[1]Department of Regenerative Medicine and Tissue Engineering, National Cardiovascular Center Research Institute, Osaka, Japan, [2]Department of Physiology, Tokai University School of Medicine, Kanagawa, Japan, [3]Department of Biomaterials, Field of Tissue Engineering, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan, and [4]Department of Cardiac Physiology, National Cardiovascular Center Research Institute, Osaka, Japan.

**Summary**. Both gene therapy and cell transplantation are promising approaches for therapeutic angiogenesis. However, gene therapy must overcome biohazard of viral vectors, transfection efficiency, and premature tissue-targeting. Conventional cell therapy is insufficient in some cases because of small cell numbers, poor survival, impaired differentiation, etc. Endothelial progenitor cells (EPCs) play an important role in modulating angiogenesis and vasculogenesis. Here, we present a new concept for hybrid cell-gene therapy using a nonviral vector, gelatin. Genetically-modified EPCs may serve, not only as a tissue-engineering tool to reconstruct the vasculature, but also as a vehicle for gene delivery to injured endothelium. Thus, hybrid cell-gene therapy may be a new therapeutic strategy for the treatment of intractable cardiovascular diseases.

**Key words.** Cell therapy, Transplantation, Angiogenesis, Adrenomedullin, Gene therapy