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ヒトゲノム・再生医療等研究事業

体外衝撃波を用いた急性心筋梗塞に対する

非侵襲性治療法の開発に関する研究

平成17年度 総括研究報告書

主任研究者 下川 宏明

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厚生労働科学研究費補助金（ヒトゲノム・再生医療等研究事業）
総括研究報告書

体外衝撃波を用いた急性心筋梗塞に対する非侵襲性治療法の開発に関する研究
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主任研究者 下川宏明 東北大学大学院医学系研究科循環器病態学・教授

研究要旨

本研究は、低出力の体外衝撃波を用いて急性心筋梗塞後の心室リモデリング・心不全に対する非侵襲性の新たな治療法を開発するものである。低出力体外衝撃波を用いた血管新生療法は、重症の狭心症患者に対して既に九州大学病院で第一次の臨床試験を実施し、東北大学病院で第二次の臨床試験を実施中であり、その有効性・安全性を確認しつつある。したがって、急性心筋梗塞に対しても十分にその効果が期待できる。

本研究では、初年度の研究として、ブタを用いた急性心筋梗塞モデルにおいて、心筋梗塞作成3日後および28日後に体外衝撃波治療を行い、その4週間後に心機能等を評価した。その結果、本治療法を心筋梗塞発症3日後から開始すると、慢性期の左室リモデリングや心機能低下が有意に抑制されたのに対し、28日後から開始した場合は、有意の抑制効果が認められなかった。また、副作用は認めなかった。これらの結果から、低出力体外衝撃波治療が急性心筋梗塞に対して、有効かつ安全性の高い治療法となりうる可能性が示唆された。平成18年度では、さらに基礎的検討を継続するとともに、臨床応用を目指した臨床研究を計画している。

本治療法の利点は、麻酔や外科的侵襲なしに体外から任意の虚血部位に効率的に血管新生を惹起できること、患者には痛みなどの苦痛を全く与えないこと、副作用がないこと、必要ならば（外来でも）繰り返し実施できること、医療費が大幅に節減できること、などである。

本治療法は、未曾有の高齢化社会を迎えようとしている我が国で増加中の急性心筋梗塞に、非侵襲的で安全かつ効果的な血管新生療法を提供することになり、国民の保健医療の向上や医療費の大幅な削減により国家の保健財政にも大きく貢献することが期待される。

分担研究者氏名・所属施設名および所属施設における職名

伊藤健太 東北大学大学院医学系研究科循環器病態学・助手

A. 研究目的

我が国では、人口の高齢化や生活の欧米化などにより、虚血性心臓病・脳卒中・閉塞性動脈硬化症などの動脈硬化性疾患が増加している。これらの動脈硬化性疾患は、国民の生命予後を悪化させ、日常生活のQOLを著しく低下させ、活力のある社会の実現に大きな障害となっている。これらの動脈硬化性疾患に対しては、これまで、薬物療法・カテーテル療法・バイパス手術などが行われてきたが、最近では、これらの治療法だけでは十分な治療効果が得られない重症の虚血性心臓病や下肢の閉塞性動脈硬化症の症例が増えてきている。これに対して、自家細胞移植や遺伝子導入を用いた血管再生療法が開発され、その一部は試験的に臨床応用され始めているが、これらの先端的血管新生療法は、その安全性が十分には確認されておらず、また侵襲的であり何回も繰り返し実施することは実際上不可能である。

我々は、低出力の衝撃波が血管内皮細胞から各種の血管新生因子の産生を促進するという研究結果に着目し、体外から心臓に衝撃波を照射することにより、動物（ブタ）モデルにおいて高度の心筋虚血を完全に回復させるほどの著明な血管新生を誘導することに成功した（Nishida, Shimokawa, et al. *Circulation* 110: 3055-3061, 2004.）。副作用も全く認められなかった。この実験結果を基に、平成14年12月の九州大学医学部倫理委員会での承認を受けて、重症狭心症患者に対する非侵襲性の体外式心臓血管新生療法を開始し、虚血心筋の血流が改善し自覚症状が著明に改善するなどの良好な結果を得ている。この画期的な体外式血管新生療法は、麻酔や手術操作が一切不要なこと、必要ならば繰り返し実施可能であること、副作用がないこと、医療費が大幅に削減できること、など多くの特徴がある。

本研究では、急性心筋梗塞に対する非侵襲性の体

外式血管新生療法の開発を目指す。

B. 研究方法

本研究では、急性心筋梗塞に対する非侵襲性の体外式血管新生療法の臨床応用を目指し、以下の基礎研究・臨床研究を行う。

(1) ブタ急性心筋梗塞モデルにおける検討

1) 心筋梗塞モデルの作成

ブタの左冠動脈回旋枝近位部を結紮し、急性心筋梗塞を作成する。

2) 体外衝撃波治療

急性心筋梗塞作成後、3日目および28日目から、体外衝撃波治療を開始する。治療条件は、低出力体外衝撃波（0.09 mJ/mm²、レベル3）を、梗塞領域周辺部に200発/spot x 10~20spots照射する。対照群は、衝撃波照射以外の操作を同様に進行。

3) 治療効果の評価

各衝撃波治療の4週間後に、冠動脈造影・左室造影・心エコーによる検討を行い、心機能や心室リモデリングを評価する。さらに、血管新生因子（VEGF, FGF, HGH, 等）の発現をmRNAレベル（RT-PCR）、蛋白レベル（免疫染色・Western blotting）で検討する。

(2) 臨床研究による検討

動物実験の結果を受けて、東北大学病院倫理委員会の承認を得た後に、臨床研究を開始する。対象は、急性心筋梗塞患者とし、発症後3日目から治療を開始する。重症狭心症患者の治療条件と同様に、レベル3（0.09 mJ/mm²）の低出力衝撃波を30~50spots、1spot当たり200発照射する。照射域は、治療機器に内蔵したエコーにより得られる画像により、容易に設定できる。これまでの検討の結果、最も衝撃波の効果を受ける範囲は2×2mmの領域、治療効果が得られる領域は1×1cmであること、また、肋骨

を避ければ痛みも全く生じないことも確認している。この衝撃波治療を隔日に計3回行う。治療後、1ヶ月・3ヶ月・6ヶ月・12ヶ月目に心筋血流の程度を、心筋シンチ法・心エコー法で評価する。

(倫理面での配慮)

動物実験では、動物愛護に十分配慮し、東北大学動物実験審査委員会の審査を受けて、受理された。今後の臨床研究においては、東北大学病院倫理委員会の審査を受けて、承認後、実施する予定である。

C. 研究結果

1) ブタ急性心筋梗塞モデルにおける検討

急性心筋梗塞作成後3日目から体外衝撃波治療を行った群では、4週間後の左室リモデリングの発生が有意に抑制され、結果的に、心機能の低下が予防された。これに対し、急性心筋梗塞作成後28日目から体外衝撃波治療を行った群では有意な治療効果が認められなかった。心筋壁厚の増加率は、衝撃波を照射した梗塞周辺部に認められた。現在、血管新生因子発現に関する詳細な検討を行っている。

3) 臨床研究による検討

上記した臨床試験を開始するために、現在、東北大学病院倫理委員会へ申請する準備中である。

D. 考察

急性心筋梗塞発症後3日目から体外衝撃波治療を行うと、慢性期の左室リモデリングや心機能の低下が有意に抑制されることが初めて示された。発症28日目から治療を開始した場合には治療効果が認められなかったことから、早期の治療の有効性が示唆された。今後、さらに超急性期に治療を行った場合に更なる治療効果が得られるか、検討する予定である。

E. 結論

体外衝撃波治療は急性心筋梗塞後の心室リモデリングに対する有効かつ安全性の高い治療法となる可

能性がある。

F. 健康危険情報

特になし。

G. 研究発表

(1) 国内

口頭発表	10件
原著論文による発表	0件
それ以外(総説等)の発表	3件
そのうち主なもの	

(国内論文発表)

- ①重症虚血性心臓病に対する体外衝撃波を用いた非侵襲性治療. 下川宏明(福岡県臨床外科医学会会誌 29:22-23, 2005.)
- ②体外衝撃波治療による血管新生. 上徳豊和、下川宏明(治療学 39:747-748, 2005.)
- ③先駆的医工学と循環器. 「衝撃波」上徳豊和、下川宏明(循環器科. 58:594-599, 2005.)

(国内学会発表)

- ①虚血性心臓病に対する体外衝撃波治療の開発. 上徳豊和、他(第9回九州・山口心血管研究会、平成17年1月8日、福岡)
- ②体外衝撃波を用いた重症虚血性心臓病に対する非侵襲性先端医療の開発. 下川宏明(第20回高度先進医療研究会、平成17年2月22日、東京)
- ③ Extracorporeal cardiac shock wave therapy improves blood flow in a rabbit model of mild hindlimb ischemia. 大井啓司(第69回日本循環器学会総会、平成17年3月19日、横浜)
- ④ Extracorporeal cardiac shock wave therapy ameliorates severe hindlimb ischemia in rabbits. 大井啓司(第69回日本循環器学会総会)

平成17年3月19日、横浜)

- ⑤ Extracorporeal cardiac shock wave therapy suppresses left ventricular remodeling after myocardial infarction in pigs. 上徳豊和 (第69回日本循環器学会総会 平成17年3月20日、横浜)
- ⑥ Extracorporeal cardiac shock wave therapy prevents left ventricular remodeling after myocardial infarction in a swine model. 上徳豊和. (第68回日本循環器学会総会 平成16年3月27日、東京)
- ⑦ 重症虚血性心臓病に対する低出力体外衝撃波を用いた非侵襲性治療の開発. 上徳豊和 (第2回西日本心臓血管研究会、平成17年4月16日、福岡)
- ⑧ ウサギ慢性虚血モデルにおける低出力体外衝撃波を用いた非侵襲性血管新生療法に関する研究. 福永亮大 (第105回日本外科学会定期学術集会、平成17年5月11—13日、名古屋)
- ⑨ 低出力体外衝撃波治療は急性心筋梗塞後の心室リモデリングの形成を抑制する. 上徳豊和 (第5回心血管再生先端治療フォーラム (平成17年7月9日、東京))
- ⑩ Extracorporeal cardiac shock wave therapy suppresses left ventricular remodeling after myocardial infarction in pigs. 上徳豊和 (第9回日本心不全学会学術集会 (平成17年10月20-22日、下関))

(2) 海外

口頭発表 3件
原著論文による発表 2件 (うち1件はreview中)
それ以外 (レビュー等) の発表 0件
そのうち主なもの

(海外論文発表)

- ① Extracorporeal cardiac shock wave therapy ameliorates myocardial ischemia in patients with severe coronary artery disease. Fukumoto Y, et al. (*Coronary Art Dis.* 17:63-70,2006.)
- ② Extracorporeal cardiac shock wave therapy ameliorates myocardial remodeling after acute myocardial infarction in pigs in vivo. Uwatoku et al. (投稿中)

(海外学会発表)

- ① CSWT ameliorates myocardial dysfunction in pigs and results of the animal trial for acute myocardial infarction. Shimokawa H. (CSWT Consensus Meeting 2005, January 28-29, 2005, Zurich, Switzerland)
- ② CSWT results of the Japanese clinical study. Shimokawa H. (CSWT Consensus Meeting 2005, January 28-29, 2005, Zurich, Switzerland)
- ③ Extracorporeal cardiac shock wave therapy ameliorates myocardial ischemia in patients with severe coronary artery disease. Shimokawa H. (European Society of Cardiology Congress, September 3-7, 2005, Stockholm, Sweden)

H. 知的所有権の出願・取得状況 (予定を含む)
なし

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル	編集者名	書籍名	出版社名	出版地	出版年	ページ

雑誌

発表者名	論文タイトル	発表誌名	巻号	ページ	出版年
Fukumoto Y, Shimokawa H, et al.	Extracorporeal cardiac shock wave therapy ameliorates myocardial ischemia in patients with severe coronary artery disease. Fukumoto Y, et al. (<i>Coronary Art Dis.</i> 17:63-70, 2006.)	Coronary Artery Disease	17	63-70	2006
Uwatoku T, Shimokawa H, et al.	Extracorporeal cardiac shock wave therapy ameliorates myocardial remodeling after acute myocardial infarction in pigs in vivo.	(投稿中)			
下川宏明	重症虚血性心臓病に対する体外衝撃波を用いた非侵襲性治療.	福岡県臨床外科医学会会誌	29	22-23	2005
上徳豊和、 下川宏明	体外衝撃波治療による血管新生.	治療学	39	747-748	2005
上徳豊和、 下川宏明	先駆的医工学と循環器。「衝撃波」	循環器科	58	594-599	2005

Extracorporeal cardiac shock wave therapy ameliorates myocardial ischemia in patients with severe coronary artery disease

Yoshihiro Fukumoto, Akira Ito, Toyokazu Uwatoku, Tetsuya Matoba, Takuya Kishi, Haruki Tanaka, Akira Takeshita, Kenji Sunagawa and Hiroaki Shimokawa

Objective Prognosis of severe coronary artery disease with no indication of percutaneous coronary intervention or coronary artery bypass grafting remains poor. We have recently demonstrated that shock wave therapy effectively induces neovascularization and improves myocardial ischemia in a porcine model *in vivo*.

Methods With permission from the Ethical Committee of our Institute, we treated nine patients with end-stage coronary artery disease with no indication of percutaneous coronary intervention or coronary artery bypass grafting (55–82 years old, five men and four women) with our cardiac shock wave therapy (200 shots/spot at 0.09 mJ/mm² for 20–40 spots, 3 times a week/series). We followed-up the patients at 1, 3, 6, and 12 months after the therapy to examine the amelioration of myocardial ischemia. When needed, shock wave therapy was performed up to three series at 0, and 1, 3 or 6 months.

Results The cardiac shock wave therapy improved symptoms (Canadian Cardiovascular Society functional class score, from 2.7 ± 0.2 to 1.8 ± 0.2, $P < 0.01$) and reduced nitroglycerin use (from 5.4 ± 2.5 to 0.3 ± 0.3/week, $P < 0.05$). The treatment also improved myocardial perfusion as assessed by dipyridamole stress thallium scintigraphy (severity score, 25.2 ± 7.2% improvement, $P < 0.05$; extent score, 23.3 ± 9.0% improvement, $P = 0.10$; washout rate, 20 ± 3 to 34 ± 3, $P < 0.05$). Myocardial

perfusion was improved only in the ischemic area treated with the therapy. These beneficial effects persisted for 12 months. No procedural complications or adverse effects were noted.

Conclusion These results indicate that our extracorporeal cardiac shock wave therapy is an effective and non-invasive treatment for end-stage coronary artery disease, although further careful evaluation is needed. *Coron Artery Dis* 17:63–70 © 2006 Lippincott Williams & Wilkins.

Coronary Artery Disease 2006, 17:63–70

Keywords: angina, angiogenesis, ischemia, non-invasive therapy, shock wave

Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan

Correspondence and requests for reprints to Hiroaki Shimokawa, MD, PhD, Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai 980-8574, Japan
Tel: +81 22 717 7151; fax: +81 22 717 7156;
e-mail: shimo@cardio.med.tohoku.ac.jp

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Introduction

The current management of coronary artery disease (CAD) has three major therapeutic options including medical treatment, percutaneous coronary intervention (PCI), and coronary artery bypass grafting (CABG) [1]. Prognosis of severe CAD without an indication of PCI or CABG, however, still remains poor because medication is the only therapy to treat the disorder. Angiogenesis by gene or cell therapy may be effective but invasive in nature and is still at a preclinical stage [2–5].

Shock wave (SW) therapy has been widely used in the lithotripsy or the treatment of certain orthopedic conditions including bone fracture or calcifying tendonitis [6,7]. Recently, it has been demonstrated that a low level of SW

could upregulate angiogenic factors in cultured endothelial cells *in vitro* [8]. On the basis of this report, we first confirmed that a low level of SW enhances the expression of vascular endothelial growth factor (VEGF) and its receptor, Flt-1, in cultured human endothelial cells *in vitro* [9]. The maximum angiogenic effect of SW was noted at 0.09 mJ/mm², which is approximately 10% of that used for lithotripsy treatment [9]. We then performed animal experiments, in which we were able to demonstrate that extracorporeal cardiac SW therapy effectively induces angiogenesis and markedly ameliorates myocardial ischemia and dysfunction in a porcine model *in vivo* without any adverse effects [9]. In the present study, we thus tested our notion that cardiac SW therapy improves myocardial ischemia in patients with severe CAD.

Methods

The Ethical Committees of Kyushu University Hospital approved the study protocol on 17 January 2003, and all patients provided informed consent.

Patient selection

First, we evaluated the indication of coronary revascularization by symptoms, exercise tolerance, dipyridamole stress thallium scintigraphy, and coronary angiography. Cardiac SW therapy was indicated when patients with severe CAD suffered from stable effort angina with evidence of myocardial ischemia even after adequate medications and when PCI or CABG was not indicated because of the diffuse distal coronary artery narrowing. Exclusion criteria were absence of the inclusion criteria, Q-wave or non-Q-wave myocardial infarction within 3 months, unstable angina, cardiac transplantation, breast plastic surgery with silicon, pregnancy, cardiac shock or uncontrolled heart failure, left ventricular thrombus, poorly controlled diabetic retinopathy, malignant tumor (including operation for the tumor within 5 years), PCI and/or CABG within 3 months, and participation in other clinical trial. The final decision to perform the cardiac SW therapy was based on the agreement of cardiologists and cardiac surgeons in our hospital. In the last 2 years, nine patients with severe CAD were finally enrolled for the cardiac SW therapy and were followed-up for 1 year. Five of them had previously undergone PCI and/or CABG, when indicated. The characteristics of the patients are shown in Table 1. Hypercholesterolemia was defined as total cholesterol ≥ 220 mg/dl or the use of lipid-lowering drug(s). Hypertension was defined as systolic blood pressure > 140 mmHg and/or diastolic blood pressure > 90 mmHg or the use of antihypertensive drug(s). Diabetes was defined as fasting blood sugar ≥ 140 mg/dl, blood sugar during a 75-g oral glucose tolerance test ≥ 200 mg/dl, or the use of antidiabetic drug(s). All patients continued their oral medications including nitrates, β -blockers, and calcium channel blockers (Table 1).

Treatment protocol

We treated the nine patients with our SW therapy (3 times a week/series, 200 shoots/spot at 0.09 mJ/mm² for 20–40 spots each time, Modulith SLC, with electromagnetic SW source; Storz Medical, Kreuzlingen, Switzerland), as we did in the experimental study (Fig. 1) [9]. Continuous electrocardiography monitoring was performed during and for 24h after the SW therapy each time. Blood chemistry (including myocardial creatinine phosphokinase) and hematological analyses were performed the day after the therapy each time. On the basis of the recommendation by the Ethical Committee, from an ethical point of view, this study had no placebo-controlled group. In addition, the placebo procedure was considered to be difficult to perform because patients were able to feel, more or less, the compression (but not pain) on the chest with the SW therapy.

We were able to follow-up all patients at 1, 3, 6, and 12 months after the SW therapy to evaluate the time course of amelioration of myocardial ischemia, including symptoms, exercise tolerance, dipyridamole stress thallium scintigraphy, and, when applicable, coronary angiography. The patients continued their oral medications throughout the study period (Table 1). When we considered that additional improvement could be expected with an additional SW therapy on the basis of the results at 1 and 3 months, the SW therapy was repeated up to three series at 0, and 1, 3, or 6 months, depending on the results of our evaluation (Table 2).

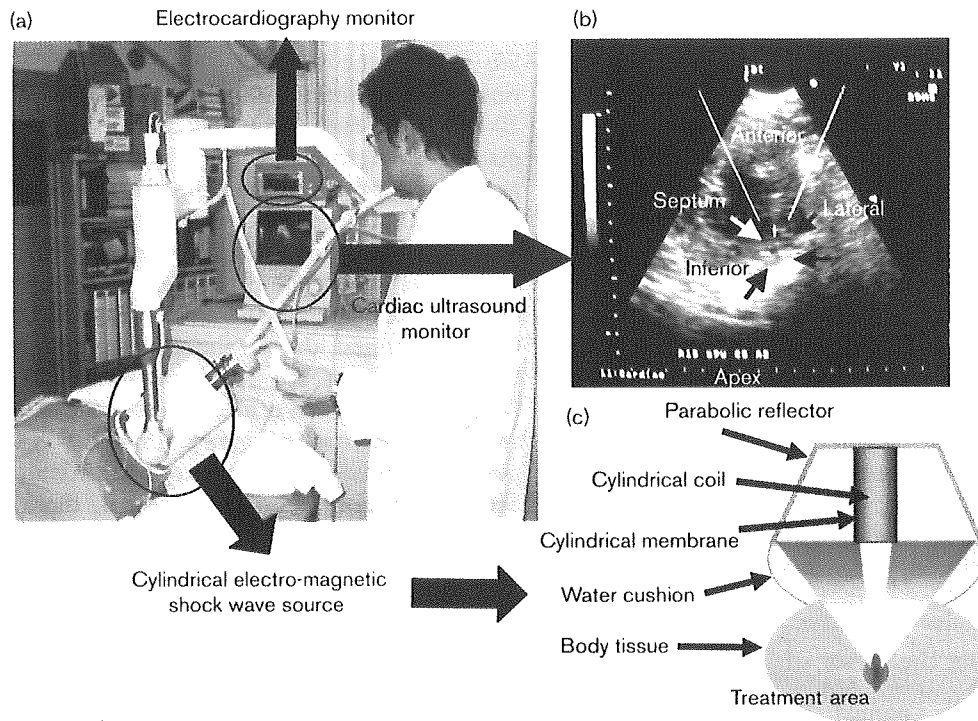
We evaluated the time course of symptoms with Canadian Cardiovascular Society (CCS) class scores (1, ordinary physical activity; 2, slight limitation of ordinary activity; 3, marked limitation of ordinary activity; 4, inability to carry on any physical activity without discomfort) and the use of nitroglycerin per week before and after the SW therapy.

Table 1 Characteristics of the patients

Patient	Sex	Age (years)	CAD	Previous treatment	DM	HT	HL	Current smoking	ASO	HD	OMI	Medications
1	M	82	3 VD	CABG	-	+	-	-	+	-	+	AP, ARB, BB, CCB, N, NCD
2	M	66	3 VD	None	+	+	-	-	-	-	-	ASP, BB, CCB, S
3	F	64	3 VD	CABG, PCI	+	+	+	-	-	-	+	ASP, BB, N, CCB, S
4	F	56	3 VD	CABG, PCI	+	-	+	-	-	+	+	ASP, BB, CCB, NCD, S
5	M	70	3 VD	CABG	+	-	-	-	-	-	+	ACEI, ASP, BB, N, CCB, NCD
6	M	76	1 VD	None	-	+	-	-	+	-	+	ASP, BB, N, CCB, NCD
7	M	62	3 VD	None	-	-	+	-	-	+	+	ACEI, ASP, BB, N
8	F	70	3 VD	PCI	+	+	-	-	+	-	+	ACEI, ASP, BB, CCB
9	F	55	3 VD	None	+	-	+	-	-	-	-	ACEI, AP, N, CCB, NCD, S

M, male; F, female; CAD, coronary artery disease; VD, vessel disease; SW, shock wave; DM, diabetes mellitus; HT, hypertension; HL, hyperlipidemia; ASO, arteriosclerosis obliterans; HD, hemodialysis; OMI, old myocardial infarction; ACEI, angiotensin-converting enzyme inhibitors; AP, anti-platelet; ARB, angiotensin receptor blockers; ASP, aspirin; BB, β -blockers; CCB, calcium channel blockers; N, nitrates; NCD, nicorandil; S, statins; CABG, coronary artery bypass graft surgery; PCI, percutaneous coronary intervention.

Fig. 1



Extracorporeal cardiac shock wave (SW) therapy in action in a patient. (a) The machine is equipped with a SW generator and in-line echocardiography. The SW generator is attached to the chest wall when used. (b) The cardiac ultrasound monitor. The SW pulse is easily focused on the ischemic myocardium under the guidance of echocardiography (black and white arrows). (c) The SW generator is equipped with parabolic reflector, cylindrical coil, and cylindrical membrane with water cushion.

Table 2 Clinical features and outcome of the shock wave therapy

Patient	Target area	SW treatment				CCS class	
		0 month	1 month	3 months	6 months	0 month	12 months
1	Inf, Lat, Pos	+	-	-	-	3	2
2	Ant, Sep, Inf, Lat, Pos	+	+	+	-	3	2
3	Ant, Sep, Lat	+	+	-	+	3	2
4	Ant, Lat	+	-	+	-	2	2
5	Ant, Lat, Inf, Pos	+	+	+	-	2	2
6	Inf, Pos	+	+	-	-	3	1
7	Inf, Lat	+	+	+	-	3	2
8	Ant	+	+	-	-	3	2
9	Inf, Lat, Pos	+	+	+	-	2	2

SW, shock wave; CCS, Canadian Cardiovascular Society; Inf, inferior; Lat, lateral; Pos, posterior; Ant, anterior; Sep, septal. The '+' and '-' refer to shock wave therapy performed and not performed, respectively.

Exercise test

Before and after the cardiac SW therapy, patients underwent a symptom-limited exercise test using a 6-min walk and exercise treadmill test using modified Bruce or Unit Mets protocol. All tests were performed with 12-lead electrocardiography monitoring by well trained cardiologists. Arterial blood pressure was measured with a mercury sphygmomanometer every minute until recovery.

Dipyridamole stress thallium scintigraphy

Dipyridamole (0.6 mg/kg body weight) was intravenously infused over a 4-min period, followed by a bolus of 3 mCi thallium-201. Continuous electrocardiography monitoring and blood pressure measurement were carried out throughout the test. Single photon emission computed tomography imaging was obtained with the same camera (Toshiba, Tokyo, Japan) in the present study. Redistribution images were obtained 4 h after dipyridamole infusion.

The results of the stress thallium scintigraphy were evaluated with a 48-segment model of the left ventricle in a blind manner. The severity of myocardial ischemia was evaluated as the ratio of percentage thallium uptake in the stress image of the most severe ischemic area in comparison with the normal wall [10]. The extent of myocardial ischemia was determined by the number of ischemic segments that indicate less than 80% uptake in the anterior and lateral walls and less than 70% uptake in the inferior, posterior, and septal walls [10]. We determined the assessment areas just after the first SW treatment and evaluated these two indices (percentage improvement of severity score and extent score) in the local segments treated with the SW therapy in all patients. We also evaluated the change in absolute value of washout rate in six patients who showed a washout rate of less than 30% before the SW therapy in a blinded manner.

Coronary angiography

Coronary angiograms [11] were obtained in multiple projections and evaluated by more than five experienced cardiologists in a blind manner. The degree of coronary artery stenosis was visually estimated as a percentage of the angiographically normal segment preceding the stenosis. We performed diagnostic coronary angiography in all patients before the SW therapy; however, we did not perform follow-up coronary angiography in four out of the nine patients because of renal dysfunction.

Statistical analysis

Continuous variables were expressed as mean \pm SEM. Comparisons during the time course after the SW therapy were made by one-way ANOVA followed by a post-hoc test. All statistical analyses were performed using Stat View (SAS Institute, Cary, North Carolina, USA), and *P* values of less than 0.05 were considered to be statistically significant.

Results

Patient selection

All nine patients had at least more than 12 months of history of stable effort angina. They had ischemic myocardium as documented by dipyridamole stress thallium scintigraphy but were diagnosed by coronary angiography to have no indication of PCI or CABG (Table 1). Among them, eight had a three-vessel disease and the remaining one patient had a one-vessel disease, and seven of them had old myocardial infarction (Table 1). Two patients had previously undergone CABG alone, one PCI alone, and two both CABG and PCI. All of them had more than two risk factors of atherosclerosis (Table 1).

Cardiac shock wave therapy

We performed our cardiac SW therapy to the ischemic area that was confirmed by dipyridamole stress thallium scintigraphy (Fig. 1, Table 2). Depending on the time

course of the improvement of myocardial ischemia, we performed up to three series of the therapy at 0, and 1, 3 or 6, months for seven patients, two series at 0 and 1 month for one patient, and only one series for one patient (Table 2). No procedural complications or adverse effects were noted in any patient.

Effects of the cardiac shock wave therapy

The cardiac SW therapy significantly improved symptoms, as evaluated by the CCS class score (Fig. 2a, Table 2) and the use of nitroglycerin (Fig. 2b), and tended to do so for a 6-min walk and treadmill test (Fig. 3a, b). Importantly, the SW therapy improved myocardial perfusion as evaluated by dipyridamole stress thallium scintigraphy only in the ischemic myocardium where SW was applied (Fig. 4). Indeed, when we treated the anteroseptal area with the SW therapy, myocardial perfusion was improved only in the treated area, and when we subsequently treated the lateral area, myocardial perfusion was improved only in the treated area (Fig. 4). The severity score, extent score, and washout rate obtained from the scintigraphy showed that myocardial ischemia was improved in the treated area, while it tended to be worsened in the untreated area (Fig. 5). The SW therapy significantly improved severity score (Fig. 6a) and tended to do so for the extent score (Fig. 6b). Especially, in the six patients with an initial low washout rate (< 30%), the SW therapy also significantly ameliorated the washout rate in the ischemic myocardium (Fig. 6c). Importantly, these anti-ischemic effects of the SW therapy were noted as early as 3 months after the therapy and persisted for 12 months (Figs 2 and 6). By contrast, coronary angiography showed no significant increase in the number of visible coronary arteries.

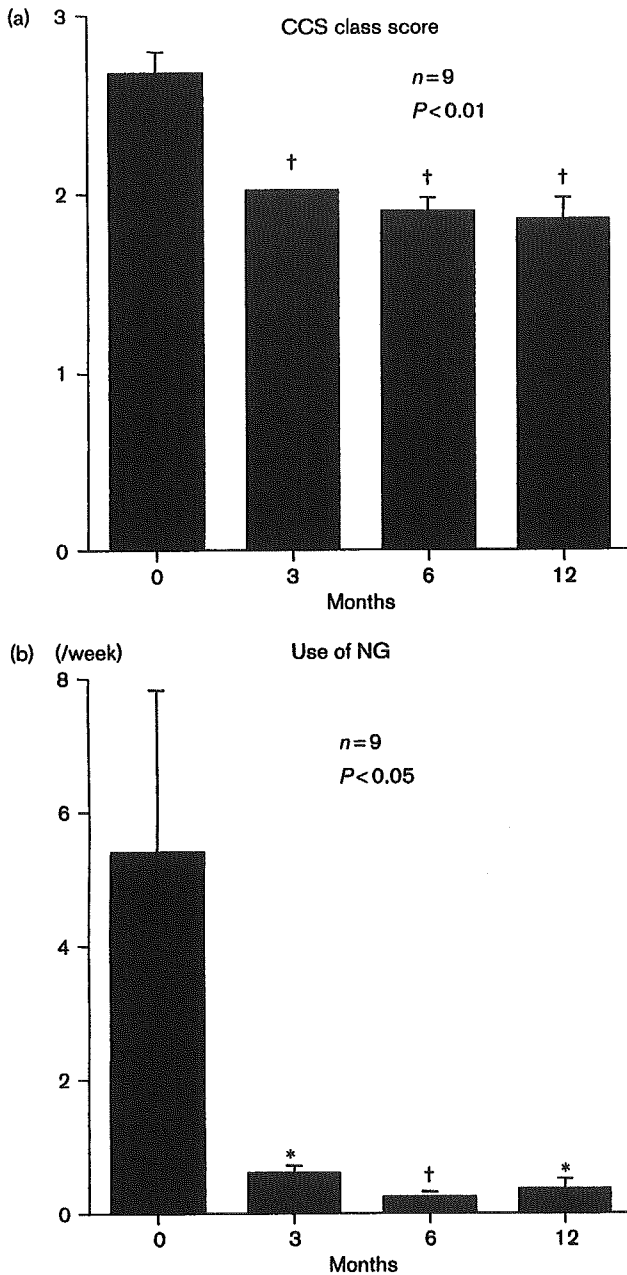
Discussion

The present study demonstrates that our non-invasive extracorporeal cardiac SW therapy ameliorates myocardial ischemia in patients with severe CAD without procedural complications or adverse effects, confirming our findings in a porcine model of chronic myocardial ischemia [9]. The subjective and objective improvements of myocardial ischemia were noted as early as 3 months after the SW therapy in the patients who had at least more than 12 months of history of stable effort angina pectoris. The results with myocardial thallium scintigraphy and coronary angiography suggest that angiogenesis was effectively induced locally at coronary microvascular levels in the ischemic myocardium. The therapeutic effects of the SW therapy persisted during the follow-up period for 1 year. Importantly, no procedural complications or adverse effects were noted in the present study.

Mechanism of the cardiac shock wave therapy

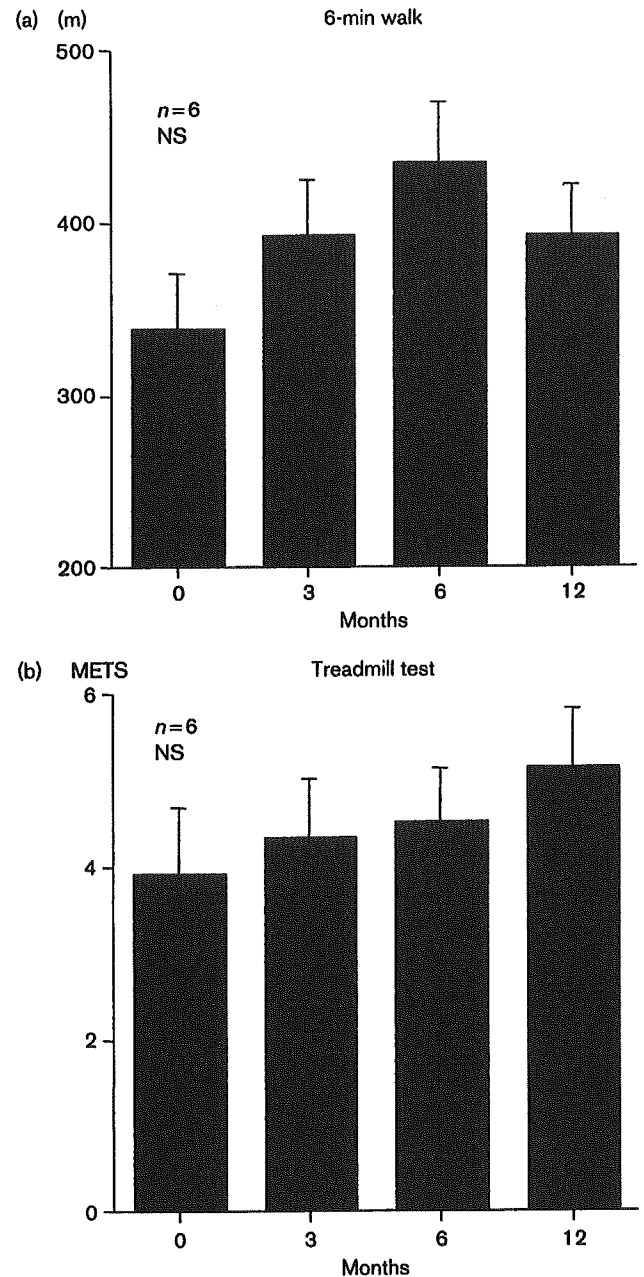
The precise mechanism of SW to induce angiogenesis remains to be fully elucidated. SW is a longitudinal

Fig. 2



Extracorporeal cardiac shock wave therapy significantly improved Canadian Cardiovascular Society (CCS) scores (a) and the use of nitroglycerin (NG) (b). Results are expressed as mean \pm SEM. * $P < 0.05$ and † $P < 0.01$ vs. 0 month (statistically analyzed by post-hoc test after one-way ANOVA).

Fig. 3

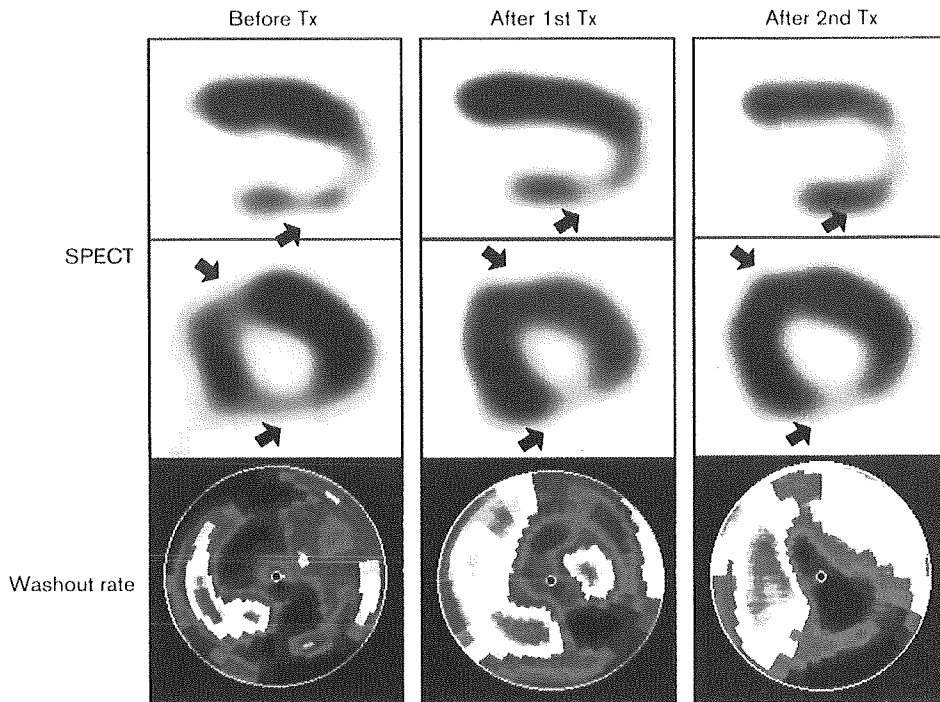


Extracorporeal cardiac shock wave therapy tended to improve exercise tolerance; 6-min walk (a) and treadmill test (b). Results are expressed as mean \pm SEM.

acoustic wave, traveling with the speed in water of ultrasound through body tissue, and could be focused on an area of several millimeters in diameter [12]. SW is known to exert the 'cavitation effect' (a micrometer-sized violent collapse of bubbles inside and outside the cells) [12] and recently has been demonstrated to induce

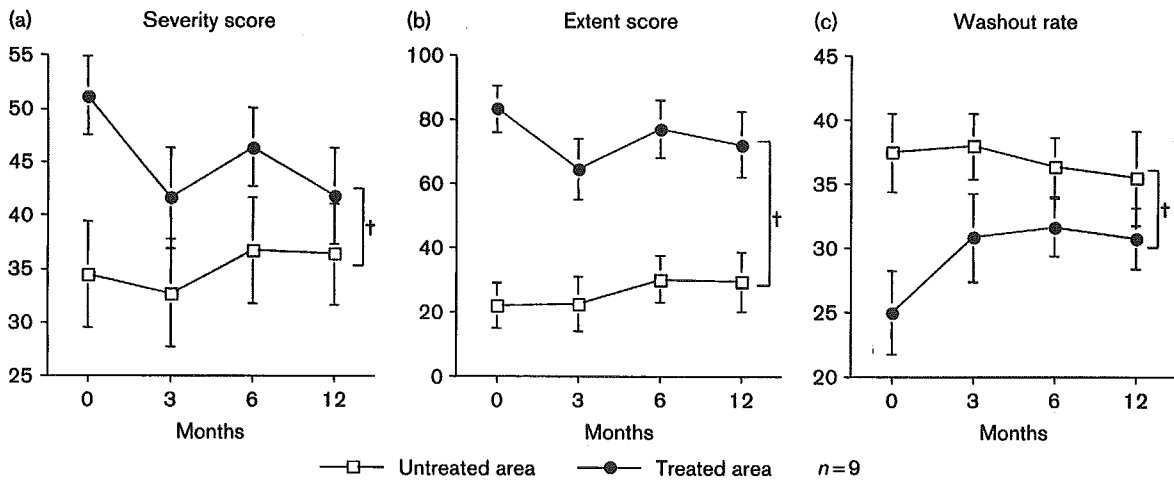
localized stress on cell membranes that resembles shear stress [13]. SW has also been demonstrated to cause non-enzymatic nitric oxide synthesis from L-arginine and hydrogen peroxide [14], which may be involved, at least in part, in the biochemical effects of SW. Furthermore, recent orthopedic studies have demonstrated that SW

Fig. 4



Dipyridamole stress thallium-201 single photon emission computed tomography (SPECT) imaging and polar map demonstrated that the shock wave (SW) treatment ameliorated myocardial perfusion only where SW was applied; in the anteroseptal wall after the first treatment (Tx) and in the lateral wall after the second treatment (arrows).

Fig. 5

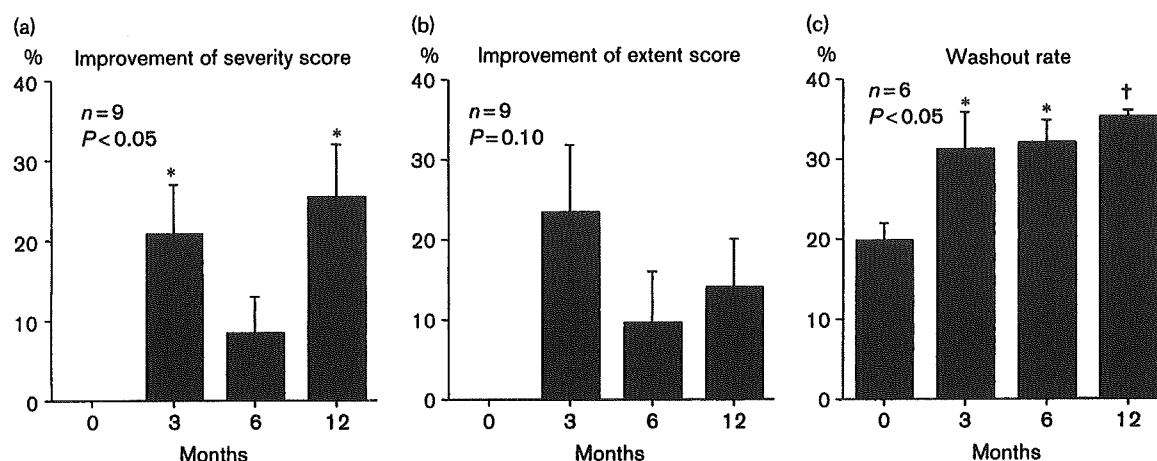


The severity score (a), extent score (b), and washout rate (c) obtained from dipyridamole stress thallium-201 single photon emission computed tomography imaging. The results showed that myocardial ischemia was improved in the treated area, while in the untreated area, it tended to be worsened. Results are expressed as mean \pm SEM. † $P < 0.01$ untreated vs. treated area (statistically analyzed by one-way ANOVA).

therapy induces neovascularization at tendon [7,15], via upregulation of endothelial nitric oxide synthesis, VEGF, and proliferating cell antigen [15]. We have previously confirmed that SW upregulates VEGF and its receptor, Flt-1, in endothelial cells *in vitro* and VEGF in the

ischemic myocardium *in vivo* [9]. As the VEGF-Flt system is essential in initiating vasculogenesis and/or angiogenesis, this effect of SW could explain, at least in part, the underlying mechanisms for SW-induced angiogenesis. In this study, myocardial perfusion in the

Fig. 6



The shock wave therapy significantly improved severity score (a), tended to improve extent score (b), and significantly improved local washout rate in patients with initial low washout rate (<30%) (c) in the dipyridamole stress thallium scintigraphy. Results are expressed as mean \pm SEM. * P <0.05 and † P <0.01 vs. 0 month (statistically analyzed by post-hoc test after one-way ANOVA).

ischemic myocardium was improved only where the SW was applied (Fig. 3), excluding the placebo effect of the therapy.

Advantage of the cardiac shock wave therapy

Transmyocardial laser revascularization is an accepted therapy for an end-stage CAD and is reported to reduce the frequency and the severity of anginal symptoms and to improve exercise tolerance and quality of life, which, however, is an invasive surgical therapy [16]. A major advantage of our extracorporeal cardiac SW therapy over PCI, CABG, and transmyocardial laser revascularization is shown by the fact that it is quite non-invasive and safe, without any procedural complications or adverse effects. If necessary, we could repeatedly treat patients (even outpatients) with the SW therapy because no surgery, anesthesia, or even catheter intervention is required for the treatment. This is an important factor in determining the clinical usefulness of angiogenic therapies in elderly patients with severe CAD. Thus, the extracorporeal cardiac SW therapy appears to be an applicable and non-invasive treatment for ischemic heart disease, although future randomized, controlled studies are required to validate the present encouraging results.

Limitations of the study

Several limitations should be mentioned for the present study. First, from an ethical point of view as discussed by the Ethical Committee, the present study is not a placebo-controlled study, as is always the case in the initial stage of a novel angiogenic therapy. As we discussed above, we noted the improvement of myocardial perfusion only in the ischemic region where we treated with the SW therapy in all patients. In addition, the objective improvement of myocardial

ischemia was noted as early as 3 months after the SW therapy in patients with more than 12 months of history of stable effort angina. We consider that these findings could rule out the placebo effect. Furthermore, as patients are able to feel more or less some compression (but not pain) on the chest by the low level of SW, an effective placebo manipulation is practically difficult. To further confirm the effectiveness of the cardiac SW therapy, however, placebo-controlled, randomized studies are needed in the future. Second, the number of patients ($n = 9$) is small in the present study although it took 2 years for us at one institute to carefully enroll the nine patients for the SW therapy and to follow-up the patients for 1 year. Moreover, the present data are not enough to evaluate the dose dependency of the SW therapy because of the small number of patients. Thus, the present findings including the evaluation of dose-dependent effect of the therapy should be confirmed in a multicenter study with a large number of patients. Third, we need to compare the effectiveness of our cardiac SW therapy with that of gene or cell therapies that are currently being developed with an initial success [2–5].

Conclusion

In the present study, on the basis of the strict inclusion and exclusion criteria approved by the Ethical Committee, we enrolled nine patients with severe CAD without indication of PCI or CABG. We were able to demonstrate that our cardiac SW therapy is effective to treat those patients with severe CAD. Thus, if our present findings are confirmed in future studies with a large number of patients, the use of the cardiac SW therapy may be broadened for the treatment of patients with CAD as a non-invasive therapy.

References

- 1 Gibbons RJ, Chatterjee K, Daley J, Douglas JS, Fihn SD, Gardin JM, et al. ACC/AHA/ACP-ASIM guidelines for the management of patients with chronic stable angina: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Management of Patients With Chronic Stable Angina). *J Am Coll Cardiol* 1999; 33:2092-2197.
- 2 Leschke M, Schoebel FC, Mecklenbeck W, Stein D, Jax TW, Muller-Gartner HW, et al. Long-term intermittent urokinase therapy in patients with end-stage coronary artery disease and refractory angina pectoris: a randomized dose-response trial. *J Am Coll Cardiol* 1996; 27:575-584.
- 3 Isner JM, Pieczek A, Schainfeld R, Blair R, Haley L, Asahara T, et al. Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. *Lancet* 1996; 348:370-374.
- 4 Losordo DW, Vale PR, Symes JF, Dunnington CH, Esakof DD, Maysky M, et al. Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. *Circulation* 1998; 98:2800-2804.
- 5 Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, et al. Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet* 2002; 360:427-435.
- 6 Haupt G, Haupt A, Ekkernkamp A, Gerety B, Chvapil M. Influence of shock waves on fracture healing. *Urology* 1992; 39:529-532.
- 7 Rompe JD, Rumler F, Hopf C, Nafe B, Heine J. Extracorporeal shock wave therapy for calcifying tendinitis of the shoulder. *Clin Orthop Relat Res* 1995; 321:196-201.
- 8 Gutersohn A, Gaspari G. Shock waves upregulate vascular endothelial growth factor m-RNA in human umbilical vascular endothelial cells. *Circulation* 2000; 102 (Suppl I):I-18.
- 9 Nishida T, Shimokawa H, Oi K, Tatewaki H, Uwatoku T, Abe K, et al. Extracorporeal cardiac shock wave therapy markedly ameliorates ischemia-induced myocardial dysfunction in pigs *in vivo*. *Circulation* 2004; 110:3055-3061.
- 10 Aoki M, Sakai K, Koyanagi S, Takeshita A, Nakamura M. Effect of nitroglycerin on coronary collateral function during exercise evaluated by quantitative analysis of thallium-201 single photon emission computed tomography. *Am Heart J* 1991; 121:1361-1366.
- 11 Mohri M, Koyanagi M, Egashira K, Tagawa H, Ichiki T, Shimokawa H, et al. Angina pectoris caused by coronary microvascular spasm. *Lancet* 1998; 351:1165-1169.
- 12 Apfel RE. Acoustic cavitation: a possible consequence of biomedical uses of ultrasound. *Br J Cancer Suppl* 1982; 45:140-146.
- 13 Maisonhaute E, Prado C, White PC, Compton RG. Surface acoustic cavitation understood via nanosecond electrochemistry. Part III: shear stress in ultrasonic cleaning. *Ultrason Sonochem* 2002; 9:297-303.
- 14 Gotte G, Amelio E, Russo S, Marlinghaus E, Musci G, Suzuki H. Short-time non-enzymatic nitric oxide synthesis from L-arginine and hydrogen peroxide induced by shock waves treatment. *FEBS Lett* 2002; 520:153-155.
- 15 Wang CJ, Wang FS, Yang KD, Weng LH, Hsu CC, Huang CS, et al. Shock wave therapy induces neovascularization at the tendon-bone junction. A study in rabbits. *J Orthop Res* 2003; 21:984-989.
- 16 Szatkowski A, Ndubuka-Irobunda C, Oesterle SN, Burkhoff D. Transmyocardial laser revascularization: a review of basic and clinical aspects. *Am J Cardiovasc Drugs* 2002; 2:255-266.

衝撃波*

上 徳 豊 和**
下 川 宏 明***

Key Words : shock wave, angiogenesis, VEGF, Flt-1

はじめに

医療技術の進歩に伴い、循環器領域においても、経皮経管的冠動脈形成術、冠動脈バイパス手術、心臓移植などの先進医療の発達はめざましいものがある。しかし一方で、日常診療において、いわゆる高齢者の占める割合は急速に増加しつつあり、上記のような侵襲度の高い先進医療の恩恵に与れない症例は、今後もますます増加の一途をたどると考えられる。虚血性心疾患に対する治療戦略は、薬物治療・インターベンション・冠動脈バイパス手術の3つが大きな柱であるが、冠動脈病変の重症化や高齢者の増加などにより、これらの従来の治療法では十分な治療効果を得ることが困難な症例が増加しつつある。そこでわれわれは、低出力衝撃波が複数の血管新生因子の発現を増加させるという*in vitro*の実験報告に着目した¹⁾。血管新生を惹起する衝撃波の出力は、結石破碎術の約10%と非常に低く、生体に対しても低侵襲であり、種々の循環器疾患における適応が想定される。また、循環器領域以外でも、とくに整形領域において、低出力の衝撃波による血管新生および局所傷害治癒過程の促進といった報告がなされており²⁾³⁾、

本研究においては、HUVECを用いた*in vitro*での血管新生因子の誘導・またブタ慢性心筋虚血モデルにおける血管新生・またウサギ下肢虚血モデルにおける血管新生・さらにはブタ心筋梗塞モデルにおけるリモデリング抑制効果(機序については現在検討中)を確認した。また、慢性虚血モデルの結果を踏まえ、倫理委員会の承認を得て、従来の治療法の適用が困難な重症冠動脈疾患患者に対して、同治療法の臨床試験を施行した。本稿では、基礎研究から臨床治験に至る本研究室での成果を報告したい。

ヒト培養血管内皮細胞における検討

ヒト培養内皮細胞への衝撃波の照射により血管新生因子が誘導されることは2000年に報告されており¹⁾、今回われわれの研究室でも、HUVEC細胞を用いて衝撃波治療による血管新生因子の誘導の有無を追試した。また、血管新生因子として、*vasculogenesis*および*angiogenesis*に対してもっとも重要であるVEGF/Flt-1をマーカーとし検討を行った⁴⁾。結果、レベル3の出力(結石破碎治療の約10%)で最大の血管新生因子発現亢進作用が認められ、後述する一連の検討においても、同様のエネルギーレベルを用いることとした(図1)。

* Extracorporeal shock wave is a new therapeutic strategy for coronary artery disease, myocardial infarction and arteriosclerosis obliterans.

** Toyokazu UWATOKU, M.D.: 九州大学医学部循環器内科(☎812-8582 福岡市東区馬出3-1-1); Department of Cardiovascular Medicine, Kyushu University School of Medical Sciences, Fukuoka 812-8582, JAPAN

*** Hiroaki SHIMOKAWA, M.D.: 東北大学医学部循環器内科

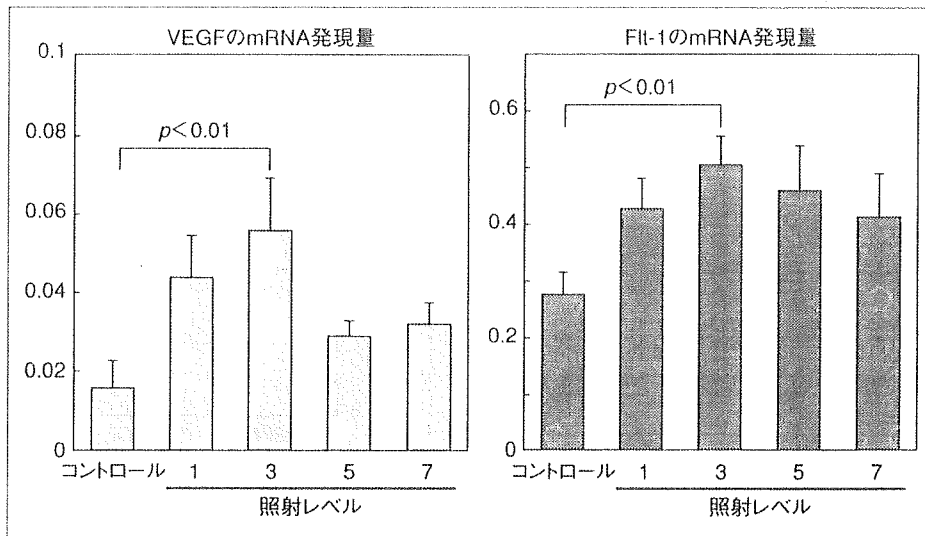


図1 衝撃波刺激による培養内皮細胞における血管新生因子・受容体の発現増加

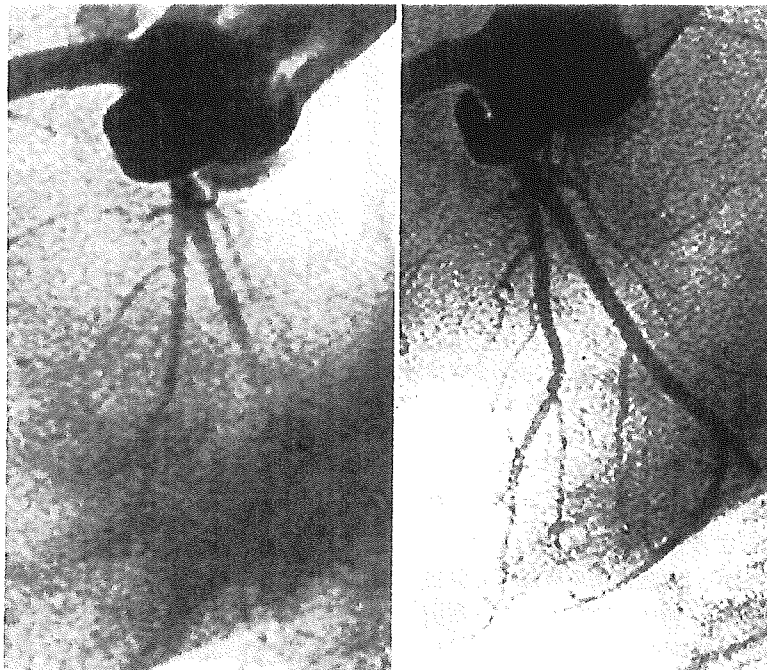


図2 体外衝撃波治療による冠動脈再生
左：治療前，右：治療後

ブタ慢性心筋虚血モデルにおける効果⁵⁾

上記の結果を踏まえ、われわれは過去の報告に準じ⁶⁾ブタの左冠動脈回旋枝基部にアミロイドコンストラクターを植え込み、4週間かけて徐々に閉塞させ心筋虚血領域を作成した。その後、

同部に上記したレベル3の低出力の衝撃波を照射した(200発/1か所×9か所・隔日で3回照射)。4週間後、冠動脈造影による血管新生(図2)、心エコーによる左室の壁運動(図3・左室駆出率49→63%, $p < 0.01$)・マイクロスフィアにより算出される心筋血流量(図4・0.9→1.8ml/min/g, $p <$

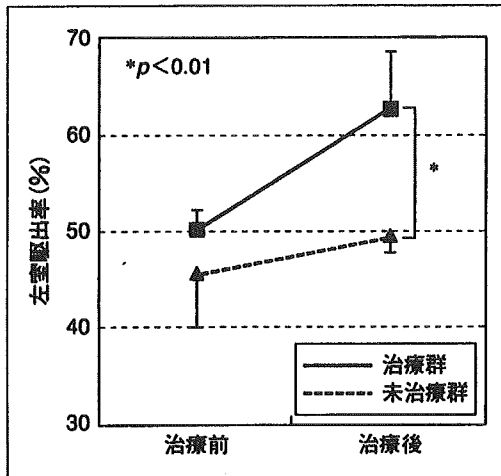


図3 体外衝撃波治療による左室壁運動の改善

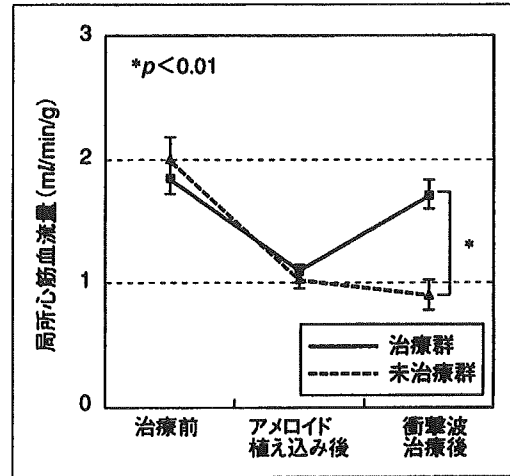


図4 体外衝撃波治療による心筋血流の正常化

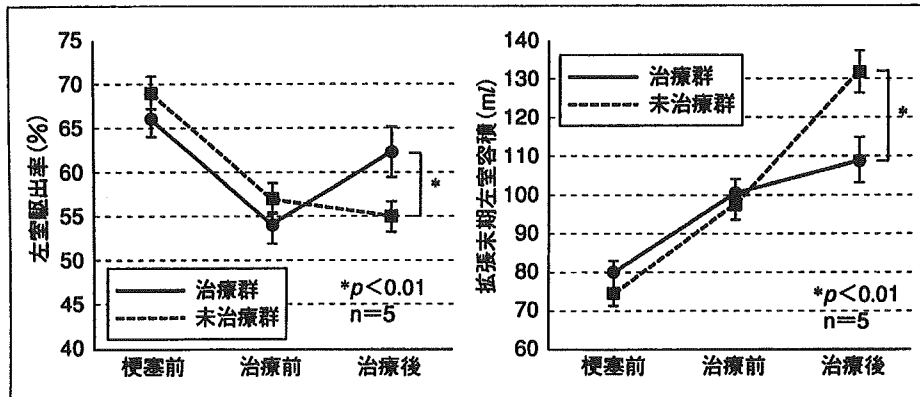


図5 体外衝撃波治療による心機能の改善
左：左室駆出率，右：拡張末期左室容積

0.01)の著明な改善が、衝撃波治療群にのみ認められた。なお衝撃波照射中・慢性期を含め、衝撃波照射自体に由来すると考えられるインシデントの発生は認めなかった(致死的不整脈・心筋出血・肺損傷含)。また、機序の検討として、照射局所での毛細血管数の有意な増加、照射心筋局所でのVEGF/Flt-1を含む血管新生因子の増加を認めており、生体心筋においても本治療が有効な血管新生療法の一選択肢となりうる事が示唆された。

ブタ急性心筋梗塞モデルでの検討

ブタの左冠動脈回旋枝結紮により急性心筋梗塞を作成した。本検討では2つのモデルを作成し、1つは心筋梗塞後3日目から照射を開始する

急性期治療群、および4週間後から照射を開始する慢性期治療群を作成した。梗塞部周辺に前述のとおり衝撃波を照射した(200発/1か所×9か所・隔日で3回照射)。さらに照射終了後4週間目の時点で左室造影、冠動脈造影を行った結果、急性期治療群においてのみ左室拡張末期容量の減少を認め、心筋梗塞急性期における衝撃波治療による心室リモデリングの予防効果が示された(図5・左室駆出率55→62%, $p < 0.01$; 拡張末期左室容積132→109ml, $p < 0.01$)。また、照射部位局所での毛細血管数増加も確認され(図6・916→1,310/sq.mm, $p < 0.01$)、心筋保護の機序の一つと考えられた。心筋局所の血管新生因子誘導を含め現在検討中である。

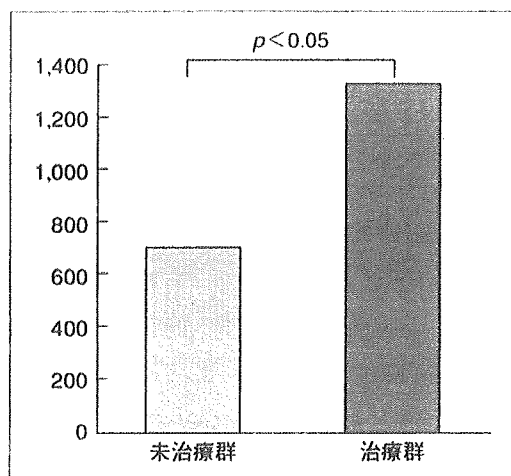


図6 体外衝撃波治療による毛細血管数の増加
左：未治療群，右：治療群

閉塞性動脈硬化症モデルにおける検討

上記における慢性虚血モデル・および心筋梗塞モデルと平行し、われわれは衝撃波治療が血管新生因子の誘導をもたらすことに着目し、閉塞性下肢動脈硬化症に対する衝撃波治療の適応の可能性および安全性を検討した。モデルとしてウサギ下肢動脈結紮モデルを作成し、結紮後3日目から、虚血に陥った下肢筋に対し衝撃波照射を行った(200発/1か所×27か所・週3回×4週間)。治療終了時点においてレーザードップラーにより下肢血流の評価および患側/健側総腸骨動

脈血流比の測定を行った。結果、治療側での有意な下肢血流の増加(図7)および総腸骨動脈血流比の改善を認めた。大動物を含めたさらなる検討を要するが、本結果は、衝撃波治療が循環器領域疾患にとどまらず、全身の虚血に起因する種々の血管疾患に対し有効となる可能性を示唆するものである。

重症冠動脈疾患患者に対する 低出力体外式衝撃波治療の検討⁷⁾

これらの実験結果をもとに、倫理委員会での承認を受けて、2003年1月から重症狭心症患者に対する非侵襲性の体外式心臓血管新生療法を開始し、慎重な症例選択とinformed consent取得ののち、0, 1, 3, 6か月の時点で衝撃波治療を行い、各治療時点で冠動脈造影、左室造影、心筋シンチグラム、心エコーを施行している。その結果、6か月後の時点で、治療を行ったほぼすべての患者(n=9)において、虚血心筋局所のwashout ratio(19.8→33.7%)、severity score(0→+20% change)、extent score(0→-24% change)の改善およびCCS class(2.7→1.9)、3 min walk(337→465m)の改善を認め、顕著な症例においては複数回の照射により、前壁中隔・側壁領域の広範な虚血が改善し、AHA classもⅢ→Ⅰにいたる著明な改善を得た(図8)。また、これまでのところ、衝撃波治療に伴う不整脈・心筋傷害・気胸などの副作用は認めていない。本検討は今後、

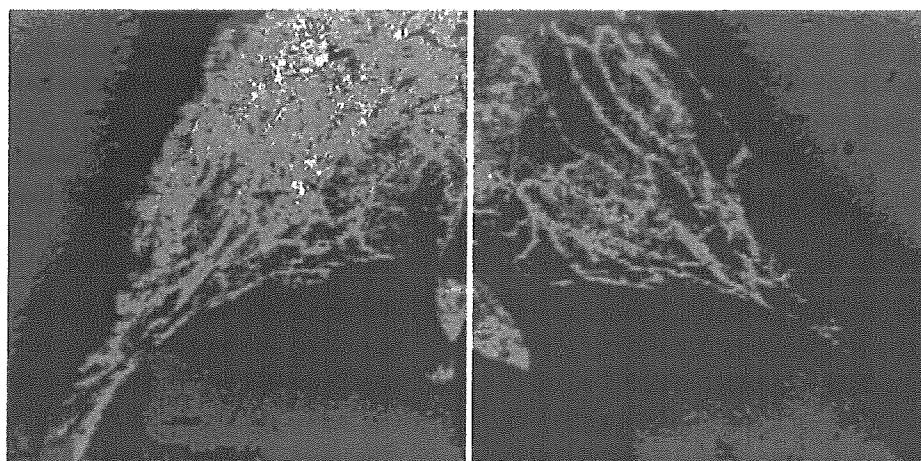


図7 衝撃波治療による下肢血流の著明な改善(レーザードップラー法)
左：右後肢(衝撃波照射側)，右：左後肢(衝撃非波照射側)

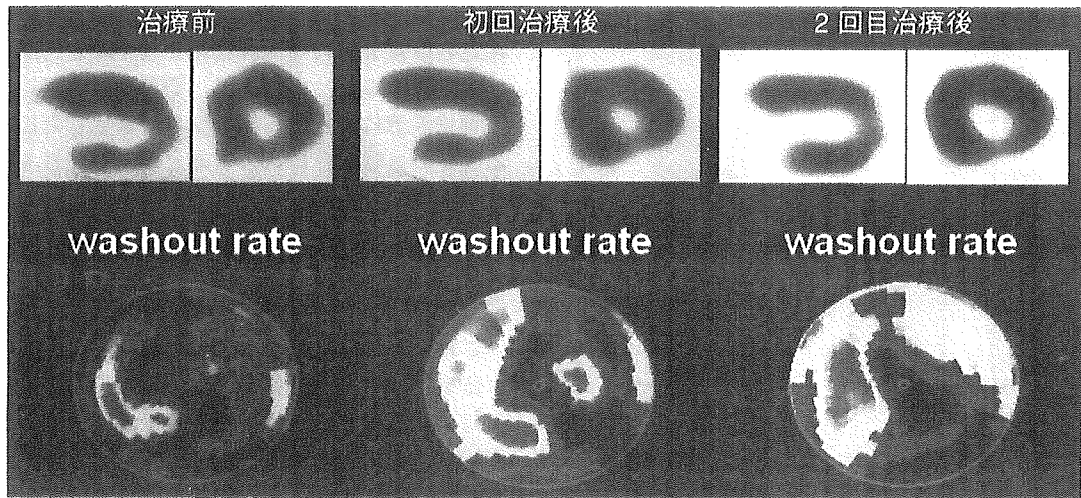


図8 衝撃波治療による心筋血流の著明な改善
 上段：タリウム心筋イメージング，下段：washout ratio

東北大学循環器内科において継続して行う予定であり、今後、より幅広い適応を含めた衝撃波治療の展望を模索中である。

おわりに

以上、本研究室での研究成果を述べたが、低出力体外式衝撃波治療により有効なレベルの血管新生因子の誘導がもたらされ、慢性虚血性心疾患・閉塞性動脈硬化症における血行改善・さらには梗塞心筋のリモデリング予防にいたる幅広い効果が認められた。本来、衝撃波が新生血管を誘導する過程においては、今回検討したVEGF, Flk-1のみならず、シェアストレスにより誘導されると考えられる⁹⁾血管内皮由来のNOの関与も報告されており⁹⁾¹⁰⁾、今後のさらなる検討が必要と考えられる。この画期的な体外式血管新生療法は、過去に報告されている^{11)~13)}外科的な処置・特定蛋白の生成・ベクターの使用を伴う血管新生療法と比較しても非常に簡便であり、麻酔や手術操作が一切不要で非侵襲性であること、必要ならば繰り返し実施可能であること、副作用がないこと、医療費が大幅に削減できることなど、多くの特徴を有しており、今後増加の一途を辿ると考えられる重症冠動脈疾患患者に対しての数少ない治療選択の一つになると期待できる。

文 献

- 1) Gutersohn A, Caspari G. Shock waves upregulate vascular endothelial growth factor m-RNA in human umbilical vascular endothelial cells. *Circulation* 2000 ; 102 Suppl : 18.
- 2) Wang CJ, Huang HY, Pai CH. Shock wave-enhanced neovascularization at the tendon-bone junction : an experiment in dogs. *J Foot Ankle Surg* 2002 ; 41 : 16.
- 3) Wang FS, Wang CJ, Huang HJ, et al. Physical shock wave mediates membrane hyperpolarization and Ras activation for osteogenesis in human bone marrow stromal cells. *Biochem Biophys Res Commun* 2001 ; 287 : 648.
- 4) Millauer B, Witzmann-Voos S, Schnurch H, et al. High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. *Cell* 1993 ; 72 : 835.
- 5) Nishida T, Shimokawa H, Oi K, et al. Extracorporeal cardiac shock wave therapy markedly ameliorates ischemia-induced myocardial dysfunction in pigs *in vivo*. *Circulation* 2004 ; 110 : 19.
- 6) O'Konski MS, White FC, Longhurst J, et al. Ameroid constriction of the proximal left circumflex coronary artery in swine : a model of limited coronary collateral circulation. *Am J Cardiovasc Pathol* 1987 ; 1 :