Complications

Spontaneously terminating torsades de pointes was induced by epinephrine infusion in one LQT1 patient, and spontaneous premature ventricular contractions were induced in one LQT1 and two LQT2 patients.

Discussion

The main findings of the present study are as follows: (1) penetrance in the absence of sympathetic stimulation was lower in LQT1 than in LQT2 or LQT3 syndrome and was improved with steady-state epinephrine in LQT1 and LQT2, but not in LQT3 syndromes; and (2) epinephrine infusion was a powerful test to predict the genotype of LQT1, LQT2, and LQT3 syndromes by comparing the Δ corrected Q-Tend at peak and steady-state epinephrine effects.

Penetrance in LQT1, LQT2, and LQT3 syndromes

It has long been expected that all genotype-positive patients could not be diagnosed by using ECG diagnostic criteria.[17,18] Priori et al[19] conducted molecular screening in nine families with sporadic cases of LQTS and suggested that clinical diagnostic criteria had low sensitivity (penetrance; 38%) in identifying mutation carriers. Swan et al[20] reported that the sensitivity and specificity for identifying genotype-positive patients were 53 and 100%, respectively, in a LQT1 family (D188N). Similarly, in the 12 LQT1 families of the prospective study, the sensitivity for identifying LQT1 patients was low under baseline conditions and was substantially improved with the epinephrine test without the expense of specificity. In contrast, the sensitivity for identifying LQT2 and LQT3 patients was relatively high under baseline conditions in the 12 LQT2 and 3 LQT3 families. These findings suggest the need for molecular screening of all family members regardless of clinical diagnosis to confirm genotype-positive patients, especially in LQT1 syndrome.

Epinephrine test for predicting genotype of LQT1, LQT2, and LQT3 syndromes

Recent clinical data on genotype-phenotype correlation and experimental data in LQTS models have demonstrated the genotype-specific response to sympathetic stimulation and the possibility of genotype-specific therapy.[5–8,11–14,21–23] The LQT1, LQT2, and LQT3 syndromes constitute approximately two thirds of genotyped LQTS patients.[24] Therefore, genotyping of the three forms as well as identifying latent genotype-positive patients are of particular importance in the management and treatment of LQTS patients. Because molecular diagnosis still is unavailable to many institutes, is costly, and is time consuming, genotype identification by clinical tests would be useful for stratifying molecular screening by targeting suspected genes for an initial study.[25–28] Moreover, there are still 30% to 40% of patients clinically affected with LQTS in whom no responsible mutations can be identified. Therefore, it is of great importance to diagnose, based on clinical findings, the form of LQTS that patients are affected with.

Our data demonstrate that epinephrine infusion enables us to predict the genotype of LQT1, LQT2, and LQT3 syndromes as well as to improve the clinical diagnosis of genotype-positive patients, especially in LQT1 syndrome. Genotype prediction of the three syndromes by the epinephrine test would facilitate molecular screening by targeting suspected genes. In fact, molecular screening identified the responsible mutations in the first targeted gene suspected by the epinephrine test in all of the 12 LQT1, 12 LQT2, and 3 LQT3 families of the prospective study. On the other hand, the other 15 probands were assigned to a likely genotype by the epinephrine test, but no mutations were found in any LQTS genes. Because the response to the epinephrine test was LQT1 (11 probands and 3 family members) or LQT2 pattern (4 probands and 3 family members), some ion channel or membrane adapter genes, which are sensitive to catecholamines, may be candidates for responsible genes. It is noteworthy that the positive predictive values for LQT1 and LQT2 syndromes still were high (67% for LQT1 and 73% for LQT2), even though the 29 patients without responsible mutations in any LQTS genes were included in the analysis for genotype prediction. The genotype prediction also may help to stratify the management and treatment of LQTS patients, if the patients cannot be genotyped by the molecular screening.

Conclusion

Epinephrine infusion is a powerful test to predict the genotype of LQT1, LQT2, and LQT3 syndromes as well as to improve the clinical diagnosis of genotype-positive patients, especially in LQT1 syndrome.

Acknowledgments

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Adrenomedullin Gene Transfer Induces Therapeutic Angiogenesis in a Rabbit Model of Chronic Hind Limb Ischemia

Benefits of a Novel Nonviral Vector, Gelatin

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Background—Earlier studies have shown that adrenomedullin (AM), a potent vasodilator peptide, has a variety of cardiovascular effects. However, whether AM has angiogenic potential remains unknown. This study investigated whether AM gene transfer induces therapeutic angiogenesis in chronic hind limb ischemia.

Methods and Results—Ischemia was induced in the hind limb of 21 Japanese White rabbits. Positively charged biodegradable gelatin was used to produce ionically linked DNA-gelatin complexes that could delay DNA degradation. Human AM DNA (naked AM group), AM DNA-gelatin complex (AM-gelatin group), or gelatin alone (control group) was injected into the ischemic thigh muscles. Four weeks after gene transfer, significant improvements in collateral formation and hind limb perfusion were observed in the naked AM group and AM-gelatin group compared with the control group (calf blood pressure ratio: 0.60±0.02, 0.72±0.03, 0.42±0.06, respectively). Interestingly, hind limb perfusion and capillary density of ischemic muscles were highest in the AM-gelatin group, which revealed the highest content of AM in the muscles among the three groups. As a result, necrosis of lower hind limb and thigh muscles was minimal in the AM-gelatin group.

Conclusions—AM gene transfer induced therapeutic angiogenesis in a rabbit model of chronic hind limb ischemia. Furthermore, the use of biodegradable gelatin as a nonviral vector augmented AM expression and thereby enhanced the therapeutic effects of AM gene transfer. Thus, gelatin-mediated AM gene transfer may be a new therapeutic strategy for the treatment of peripheral vascular diseases. (*Circulation.* **2004;109:526-531.**)

Key Words: peripheral vascular disease ■ angiogenesis ■ gene therapy ■ ischemia

Adrenomedullin (AM) is a potent vasodilator peptide that was originally isolated from human pheochromocytoma.[1] AM and its receptor are expressed mainly in vascular endothelial cells and vascular smooth muscle cells.[2–4] AM not only induces vasorelaxation but also regulates growth and death of these vascular cells.[5–10] These findings suggest that AM plays an important role in maintaining vascular homeostasis in an autocrine and/or paracrine manner.

A recent study has shown that vascular abnormalities are present in homozygous AM knockout mice, suggesting that AM is indispensable for vascular morphogenesis.[11–13] More recently, AM has been shown to activate the PI3K/Akt-dependent pathway in vascular endothelial cells, which is considered to regulate multiple critical steps in angiogenesis, including endothelial cell survival, proliferation, migration, and capillary-like structure formation.[7,14] These results raise the possibility that AM plays a role in modulating vasculogenesis and angiogenesis. However, whether AM induces therapeutic angiogenesis remains unknown.

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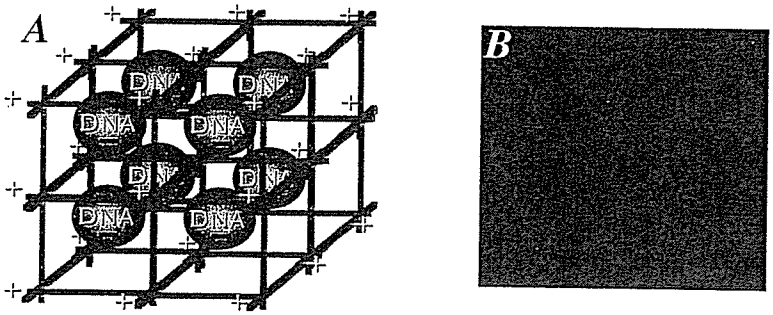

Figure 1. A, Schema of DNA-gelatin complex. Biodegradable gelatin can hold negatively charged plasmid DNA in its positively charged lattice structure. B, RITC-labeled AM DNA particles were incorporated into gelatin.

We prepared biodegradable gelatin that could hold negatively charged protein or plasmid DNA in its positively charged lattice structure.[15,16] Biodegradable gelatin has been widely used as a carrier of protein because of its capacity to delay protein degradation.[15] Similarly, ionically linked DNA-gelatin complexes can delay gene degradation.[16] These findings raise the possibility that gelatin may serve as a nonviral vector for gene therapy.

Thus, the purposes of this study were (1) to investigate whether AM gene transfer induces therapeutic angiogenesis in a rabbit model of chronic hind limb ischemia and (2) to examine whether the use of biodegradable gelatin as a vector augments AM expression and thereby enhances the therapeutic effects of AM gene transfer.

Methods

Animal Model

All protocols were performed in accordance with the guidelines of the Animal Care Ethics Committee of the National Cardiovascular Center Research Institute. Twenty-one male Japanese White rabbits (body weight, 2.9±0.1 kg; Japan Animal Co, Osaka, Japan) were used for physiological and morphological assessment. In addition, 30 rabbits were used for radioimmunoassay, immunohistochemical examination, and Western blot analysis. After anesthetization with pentobarbital sodium (30 to 35 mg/kg), a longitudinal incision was made in the left thigh, extending inferiorly from the inguinal ligament to a point just proximal to the patella. Hind limb ischemia was induced by ligation of the distal left external iliac artery and complete resection of the left femoral artery, as described previously.[17]

Construction of Plasmid DNA

To construct the expression vector for human AM, the *EcoRI/XhoI* fragment of the full-length human AM cDNA was ligated into the EcoRI/XhoI fragment of the pcDNA1.1-CMV expression plasmid (Invitrogen). To verify that the pcDNA1.1-CMV vector encoding AM cDNA produces a biologically active AM protein, the expression vector was transfected into 293 cells, and AM activity in the transfected cells was measured by high-performance liquid chromatography and radioimmunoassay. The pcDNA1.1-CMV vector encoding β-galactosidase (LacZ) cDNA was used as a control DNA.

Preparation of AM DNA-Gelatin Complex

Biodegradable gelatin was prepared from pig skin. The gelatin was characterized by a spheroid shape with a diameter of approximately 30 μm, water content of 95%, and an isoelectric point (pI) of 9 after swelling in water.[15,16] Gelatin can hold negatively charged protein or plasmid DNA in its positively charged lattice structure (Figure 1A). Dried gelatin (4 mg, pI 9) was added to human AM DNA solution (500 μg/100 μL in phosphate-buffered saline, pH 7.4). After mixture of DNA and gelatin, DNA-gelatin complexes were incubated at 37°C for 2 hours.

To visualize incorporation of DNA into gelatin, AM plasmid DNA was labeled with rhodamine B isothiocyanate (RITC), as reported previously.[16] In brief, the coupling reaction of RITC to plasmid DNA was carried out by mixing the two substances in 0.2 mol/L sodium carbonate-buffered solution (pH 9.7), followed by gel filtration with a PD 10 column (Amersham-Pharmacia). RITC-labeled AM DNA was incorporated into positively charged gelatin (Figure 1B).

Study Protocol

Ten days after the induction of hind limb ischemia (day 10), AM DNA (naked AM group, n=7), AM DNA-gelatin complex (AM-gelatin group, n=7), or gelatin alone (control group, n=7) was administered intramuscularly into 3 different sites in the ischemic adductor muscle and 2 different sites in the semimembranous muscle. In addition, Lac Z DNA-gelatin complex served as a control DNA (Lac Z-gelatin group, n=5). The amount of plasmid was 500 μg (1 mL) and that of gelatin was 4 mg. Morphological and angiographic analyses and measurements of calf blood pressure and laser Doppler flow were performed 4 weeks after gene transfer (day 38). After completion of these measurements, the adductor, semimembranous, and gastrocnemius muscles were weighed in each hind limb.[18] The muscle weight ratio was calculated for each muscle as follows: muscle weight ratio=muscle weight in ischemic hind limb/muscle weight in nonischemic hind limb. Specimens of the adductor muscle of the ischemic hind limb were obtained for histological examination.

Measurement of Calf Blood Pressure

Calf blood pressure was measured on days 10 and 38 in both hind limbs with a Doppler flowmeter (Hayashi Denki Co, Ltd) and a 25-mm-wide cuff. The pulse of the posterior tibial artery was identified with the use of a Doppler probe, and the systolic blood pressure in both hind limbs was determined by standard techniques. The calf blood pressure ratio was defined for each rabbit as the ratio of systolic pressure of the ischemic hind limb to that of the normal hind limb.[17]

Laser Doppler Blood Perfusion Analysis

Blood flow of the ischemic hind limb was measured with the use of a laser Doppler blood perfusion image system (moorLDI, Moor Instruments) on day 38.

Angiographic Analysis

Development of collateral arteries was evaluated by angiography on days 0 and 38. A 4F catheter was placed in the left internal iliac artery through the common carotid artery, and 3 mL contrast medium (Iopamiron 300, SCHERING) was injected with an automated angiography injector at a rate of 2.5 mL/s. Quantitative angiographic analysis of collateral vessel development in the ischemic hind limb was performed with the use of a 5-mm^2 grid overlay, as described previously.[17] The angiographic score was calculated for each film as the ratio of grid intersections crossed by opacified arteries divided by the total number of grid intersections in the ischemic medial thigh. The angiographic score was determined by 2 blinded observers.

Morphological and Histological Examination

The degree of lower hind limb necrosis and thigh muscle necrosis was macroscopically evaluated on graded morphological scales (grade 1 to 3) for peripheral tissue damage and muscle necrosis area of the adductor, semimembranous, and medial large muscles. Capillary density of the ischemic hind limb was evaluated by alkaline phosphatase staining, as reported previously.[17] A total of 10 different fields from three different sections were randomly selected, and the number of capillaries was counted under a ×40 objective. Capillary density was expressed as the mean number of capillaries per square millimeter. The number of myofibers in each field was also examined and the capillary/muscle fiber ratio calculated.

Radioimmunoassay for Human AM

Human AM production was examined 1, 2, and 4 weeks after gene transfer in the naked AM group, AM-gelatin group, and control group (n=5 each). The muscles were harvested for radioimmunoassay and immunohistochemical examination. Immunoreactive human AM level in rabbit muscles was determined by immunoradiometric assay with the use of a specific kit (Shionogi Co, Ltd).[19] Tissue content of vascular endothelial growth factor (VEGF) was examined by ELISA kit (R&D systems).

Immunohistochemistry for Human AM, Ki67 Antigen, and Phosphorylated Akt

Immunohistochemical studies were performed on formalin-fixed, paraffin-embedded 4-μm sections of ischemic thigh muscles 7 days after gene transfer. To elucidate AM expression after gene therapy, immunohistochemistry for human AM was performed with the use of a monoclonal antibody recognizing AM-(12–25) (1:100), as reported previously.[20] To evaluate the proliferative potential of AM, tissue sections were stained for Ki67, a marker for cell proliferation, with the use of monoclonal anti-Ki67 antibody (1:100) (DAKO). AM has recently been shown to promote proliferation of vascular endothelial cells at least in part through the PI3k/Akt pathway.[21] Thus, immunohistochemistry for phosphorylated Akt was performed with mouse monoclonal anti-phosphorylated Akt antibody (1:100) (Cell Signaling Technology).

Western Blot Analysis

To identify Akt phosphorylation in ischemic muscles after AM gene transfer, Western blotting was performed with the use of a commercially available kit (PhosphoPlus Akt [Ser473] Antibody Kit, Cell Signaling Technology). Ischemic muscles in the 3 groups were obtained 7 days after AM gene transfer. These samples were homogenized on ice in 0.1% Tween 20 homogenization buffer with a protease inhibitor (Complete, Roche). After centrifugation for 20 minutes at 4°C, the supernatant was used for Western blot analysis. The 50 μg of protein was transferred into sample buffer, loaded on 7.5% SDS-polyacrylamide gel, and blotted onto nitrocellulose membrane through the use of a wet blotting system. After blocking for 60 minutes, the membranes were incubated with primary antibodies (1:500) at 4°C overnight. The membranes were then incubated with secondary antibodies, which were conjugated with horseradish peroxidase (Cell Signaling Technology), at a final dilution of 1:2000. Signals were detected through the use of LumiGLO chemiluminescence reagents (Cell Signaling Technology).

Statistical Analysis

All results are expressed as mean±SEM. Statistical significance was evaluated by 1-way ANOVA followed by Fisher's analysis, Scheffe's F analysis, or Kruskal-Wallis test. A value of $P<0.05$ was considered statistically significant.

Results

Physiological and Morphological Assessment

Complete resection of the left femoral artery resulted in a similar decrease in calf blood pressure ratio among the 3 groups before the initiation of therapy (day 10) (Figure 2A). However, the calf blood pressure ratio on day 38 was highest in the AM-gelatin groups, followed by the naked AM group and subsequently the control group. The laser Doppler flow in hind limb was highest in the AM-gelatin group, followed by the naked AM group and the control group (Figure 2B). The calf blood pressure ratio and laser Doppler flow 4 weeks after gene transfer did not significantly differ between the control group and Lac Z-gelatin group. Lower hind limb necrosis was minimal in the AM-gelatin group, followed by the naked AM group and the control group (Figure 2C). Thigh muscle necrosis was also minimal in the AM-gelatin group. Similarly, the muscle weight ratio (ischemic/normal) on day 38 was highest in the AM-gelatin group (Table). Neither mean arterial pressure nor heart rate significantly differed among the 3 groups.

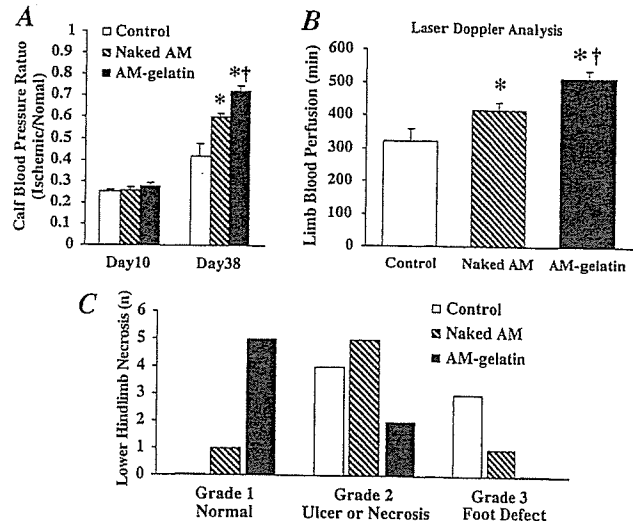

Figure 2. A, Calf blood pressure ratio (ischemic/normal hind limb) before (on day 10) and after (on day 38) gene transfer. B, Measurement of laser Doppler flow on day 38. Data are mean±SEM. *$P<0.05$ vs control group; †$P<0.05$ vs naked AM group. C, Number of cases of each grade of lower hind limb necrosis on day 38. Lower hind limb necrosis was minimal in the AM-gelatin group. Number of necrosis or foot defect is statistically significant among the 3 groups ($P<0.05$ by Kruskal-Wallis test).

Angiographic Analysis

Angiograms 4 weeks after gene transfer (day 38) showed the development of collateral arteries in the naked AM and

Physiological Characteristics

	Control	Naked AM	AM-Gelatin
No. of rabbits	7	7	7
Body weight, kg	2.46±0.06	2.65±0.10	3.16±0.09
MAP, mm Hg	112±3	114±3	116±2
HR, beats/min	269±12	253±5	262±7
Muscle weight ratio	0.71±0.03	0.84±0.02*	0.95±0.02*†

MAP indicates mean arterial pressure; HR, heart rate; and muscle weight ratio, ratio of muscle weight in ischemic hind limb to that in nonischemic hind limb. Data are mean±SEM.

*$P<0.01$ vs control group; †$P<0.05$ vs naked AM group.

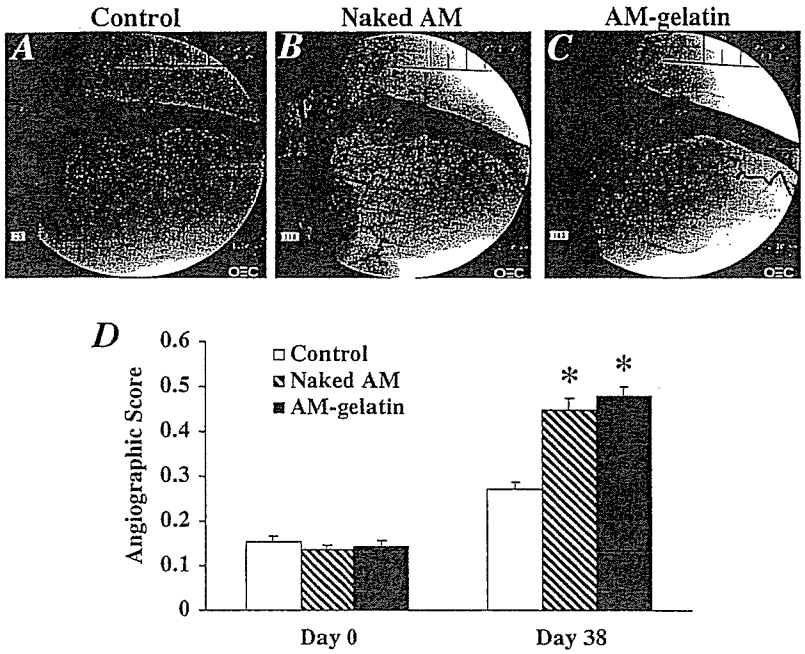

Figure 3. Representative angiograms of control group (A), naked AM group (B), and AM-gelatin group (C) on day 38. Collateral arteries were well developed in the naked AM and AM-gelatin groups. D, Angiographic score on days 0 and 38 in each group. Angiographic score on day 38 was significantly higher in the naked AM and AM-gelatin groups than in the control group. Data are mean±SEM. *$P<0.001$ versus control group.

AM-gelatin groups compared with that in the control group (Figure 3, A through C). Quantitative analysis of collateral vessels demonstrated that the angiographic score in both the naked AM and AM-gelatin groups was significantly higher than that in the control group (Figure 3D). Angiographic score did not significantly differ between the control group and Lac Z-gelatin group.

To examine the development of collateral vessels in an earlier stage, other rabbits (n=4 each) were examined 2 weeks after gene transfer (day 24). Angiograms showed significant collateral development in the naked AM and AM-gelatin groups compared with that in the control group.

Histological Examination

Alkaline phosphatase staining of ischemic hind limb muscle showed marked augmentation of neovascularization in both the naked AM and AM-gelatin groups compared with the control group (Figure 4, A through C). Quantitative analysis demonstrated that capillary density of the ischemic adductor muscle was highest in the AM-gelatin group (Figure 4D). Analysis of the capillary/muscle fiber ratio yielded similar results. Seven days after gene transfer, intense immunostaining for Ki67 was observed in vascular endothelial cells of the naked AM and the AM-gelatin groups (Figure 4, E through G).

AM Expression and Akt Phosphorylation After Gene Transfer

Seven days after gene transfer, modest immunostaining for human AM was observed in the naked AM group, whereas AM immunoreactivity was intense surrounding the gelatin in the AM-gelatin group (Figure 5, A through C). Tissue content of human AM was significantly increased both in the naked AM and the AM-gelatin groups 7 days after gene transfer (Figure 5D). The AM level in the AM-gelatin group was significantly higher that in the naked AM group. Two weeks after gene transfer, AM overexpression was observed only in the AM-gelatin group. The expression of endogenous VEGF and its receptors (Flt-1 and Flk-1) did not differ among the 3 groups (data not shown). Western blot analysis revealed that phosphorylated Akt in ischemic muscles was increased in both the naked AM and AM-gelatin groups 7 days after gene transfer (Figure 5E). Intense immunostaining for phosphory-

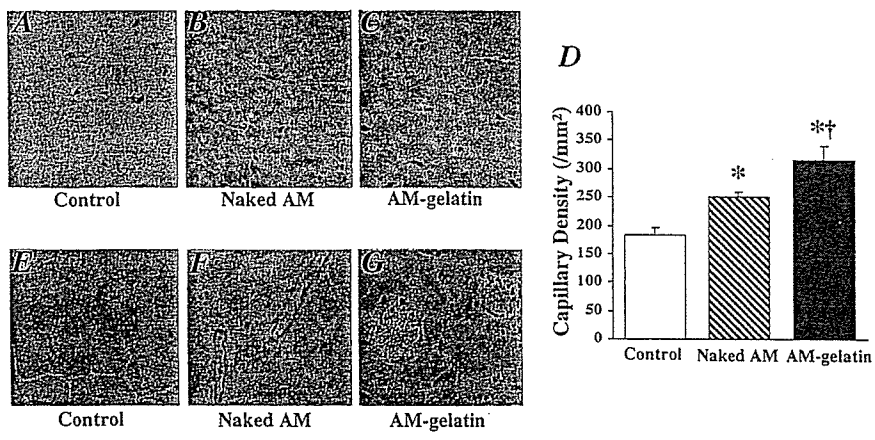

Figure 4. A through C, Representative examples of alkaline phosphatase staining in ischemic hind limb muscles. Magnification ×200. D, Quantitative analysis of capillary density in ischemic hind limb muscles. Data are mean±SEM. *$P<0.05$ vs control group; †$P<0.05$ vs naked AM group. E through G, Immunohistochemical analysis of Ki67 antigen, a marker for cell proliferation. Magnification ×400.

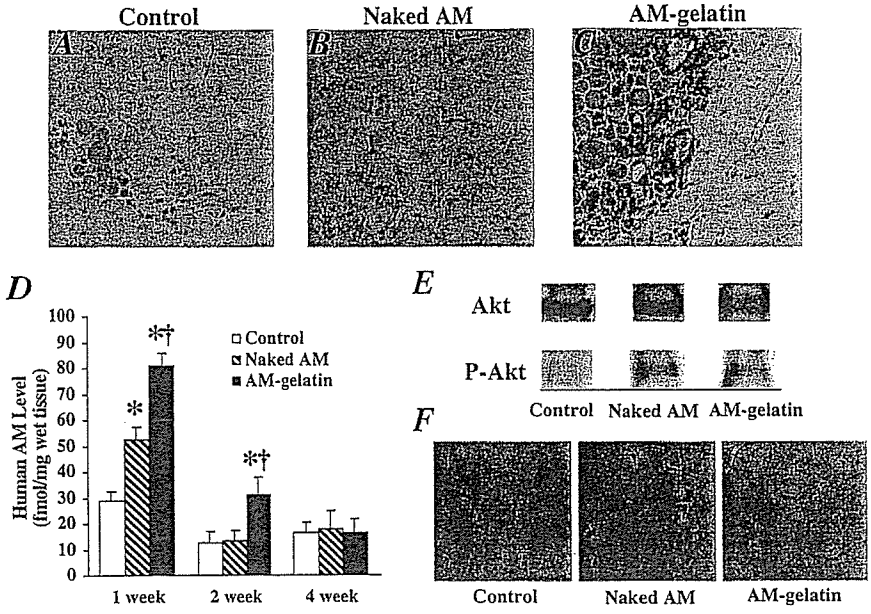

Figure 5. A through C, Immunohistochemistry for human AM 7 days after gene transfer. Intense immunostaining was observed surrounding gelatin in the AM-gelatin group. Magnification ×200. D, Time course of AM production in ischemic muscles after gene transfer. Data are mean±SEM. *$P<0.01$ vs control group; †$P<0.01$ vs naked AM group. E, Western blot analysis for Akt phosphorylation in muscles. F, Immunohistochemical staining for phosphorylated Akt 7 days after gene transfer. Phosphorylated Akt was distributed at least in endothelial cells. Magnification ×400.

lated Akt was observed at least in endothelial cells of the Naked AM and the AM-gelatin groups (Figure 5F).

Discussion

We demonstrated that (1) AM gene transfer induced hemodynamic and angiographic improvements in association with an increase in capillary density in a rabbit model of chronic hind limb ischemia. We also demonstrated that (2) administration of AM DNA-gelatin complexes markedly augmented AM expression and thereby enhanced the therapeutic effects of AM gene transfer.

AM has a variety of effects on the vasculature that include vasodilation,[1,5–7] inhibition of endothelial cell apoptosis,[8,9] and regulation of smooth muscle cell proliferation.[10] However, whether AM has angiogenic potential has remained unknown. In the present study, intramuscular administration of naked AM DNA augmented AM production in skeletal muscles, as indicated by increased tissue content and significant immunostaining of AM. As a result, AM gene transfer increased hind limb perfusion and ameliorated lower hind limb and thigh muscle necrosis in a rabbit model of hind limb ischemia. AM gene transfer may protect the ischemic hind limb partly by improving the blood flow in the ischemic hind limb because AM is originally identified as a potent vasodilating peptide.[1] Nevertheless, angiographic collateral development and high capillary density were observed in ischemic muscles after AM gene transfer. Ki67, a marker for cell proliferation, was detected in endothelial cells of microvessels after AM gene transfer. These results suggest that AM overproduction resulting from gene transfer may induce angiogenesis in a rabbit model of hind limb ischemia. Recent studies using AM gene knockout mice have shown that AM is essential for development of the vasculature during embryogenesis.[11–13] These studies support our results that AM may be an angiogenic factor. VEGF is known to induce angiogenesis and to regulate endothelial cell survival through the phosphatidylinositol 3-kinase (PI3K)/Akt pathway.[22] Thus, the PI3K/Akt pathway is considered to regulate multiple critical steps in angiogenesis, including endothelial cell survival, proliferation, migration, and capillary-like structure formation.[14] A recent study has reported that AM promotes proliferation and migration of human umbilical vein endothelial cells at least in part through the PI3K/Akt pathway.[21] The present study demonstrated that phosphorylated Akt is increased at least in endothelial cells after AM gene transfer. AM gene transfer did not influence endogenous VEGF and its receptors. Taken together, it is interesting to speculate that AM may directly induce angiogenesis through the PI3K/Akt pathway.

In the present study, we used positively charged biodegradable gelatin as a nonviral vector. We have shown that basic fibroblast growth factor (bFGF) is ionically linked with gelatin, which enhances the angiogenic effects of bFGF by delaying protein degradation.[15] Thus, biodegradable gelatin has been used as a carrier of protein. However, little information is available regarding the therapeutic potential of gelatin as a nonviral vector for gene transfer. In the present study, we demonstrated that RITC-labeled AM DNA was incorporated into positively charged gelatin. In addition, intramuscular administration of AM DNA-gelatin complexes strongly enhanced AM production compared with that of naked AM DNA. These results suggest that biodegradable gelatin may serve as a vector for gene transfer. In fact, AM DNA-gelatin complexes induced more potent angiogenic effects in a rabbit model of hind limb ischemia than naked AM DNA, as evidenced by significant increases in histological capillary density, calf blood pressure ratio, laser Doppler flow, and muscle weight ratio and a decrease in necrosis of lower hind limb and thigh muscles. These results suggest that the use of biodegradable gelatin as a nonviral vector augments AM expression and enhances AM-induced angiogenic effects. The angiogenic effects of AM-gelatin complexes were comparable to those of bFGF-gelatin complexes (data not shown). AM DNA-gelatin complexes were distributed mainly in connective tissues. We have recently demonstrated that gelatin-DNA complex is readily phagocytosed by mac-

rophages, monocytes, endothelial progenitor cells, and so on, resulting in gene expression within these phagocytes.[23,24] These findings raise the possibility that AM secreted from these cells acts on muscles in a paracrine fashion. Unlike AM production in the naked AM group, AM overexpression in the AM-gelatin group lasted for longer than 2 weeks. Thus, it is interesting to speculate that delaying gene degradation by gelatin may be responsible for the highly efficient gene transfer.

Currently, a highly efficient and safe gene delivery system is needed for gene therapy in humans. The present study demonstrated that the use of gelatin, which is considered to be less biohazardous than viral vectors, enhanced the angiogenic potential of AM DNA. Thus, gelatin-mediated AM gene transfer may be a new therapeutic strategy for the treatment of severe peripheral vascular diseases. However, the initial success of gelatin-mediated AM gene therapy reported here should be confirmed by long-term experiments, and extensive toxicity studies in animals are needed before clinical trials.

Study Limitation

First, histological capillary density, calf blood pressure ratio, and laser Doppler flow were significantly higher in the AM-gelatin group than in the naked AM group. However, the angiographic score did not significantly differ between the two. This discrepancy raises the possibility that conventional angiography may have insufficient resolution to fully visualize the angiogenic microvessels. Second, human AM level was slightly elevated in the control group. This implies that the anti-human AM antibody used in this radioimmunoassay had some cross-reactivity with endogenous rabbit AM. Nevertheless, human AM level in the muscles was highest in the AM-gelatin group within 2 weeks after gene transfer. These results suggest that AM DNA-gelatin complexes induces potent and long-lasting AM production.

Conclusions

Intramuscular administration of AM DNA induced therapeutic angiogenesis in a rabbit model of chronic hind limb ischemia. Furthermore, the use of biodegradable gelatin as a nonviral vector augmented AM expression and thereby enhanced the therapeutic effects of AM gene transfer. Thus, gelatin-mediated AM gene transfer may be a new therapeutic strategy for the treatment of peripheral vascular diseases.

Acknowledgments

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特　論

アドレノメデュリンと細胞治療

Combined therapy with adrenomedullin and cell transplantation

岩瀬　俊　　永谷憲歳

Key words：adrenomedullin, cell therapy

はじめに

近年，成人個体の骨髄および末梢血中における血管内皮前駆細胞(EPC)の存在が報告されてから，循環器領域における細胞移植治療に関する研究が活発に行われている[1]．実際，臨床においても自己の骨髄単核球(MNC)を用いた血管新生療法が主に閉塞性動脈疾患症例に対して行われている[2]．しかし，現在の方法では細胞採取時の侵襲が大きいことや，時間経過とともに治療効果が減弱することなどの問題点が存在する．したがって，細胞治療の移植効果を上げる機能強化法の開発が必要である．

アドレノメデュリン(adrenomedullin: AM)は，北村，寒川らによってヒト褐色細胞腫より発見された強力な血管拡張ペプチドである[3]．AMは近年その血管拡張作用に加え，血管新生および胎児期の血管形成においても重要な役割を果たしていることが明らかとなっている[4]．AMはこれらの多面的作用を有することより，現在の血管再生を主眼とした細胞移植治療においてその効果を相加的または相乗的に増強する可能性がある．

本稿では，①AMの血管新生作用およびEPC，MNCに対する相互作用，②疾患動物モデル(原発性肺高血圧および末梢動脈閉塞症)に対するAMと細胞移植治療併用の治療効果に関して，今まで著者らが行ってきた検討を文献的考察も含めて報告する．

1. AMの血管新生作用ならびにEPC，MNCに対する相互作用

AMはPI3K-Akt経路を活性化することで血管内皮細胞の生存，遊走，増殖に関与することが知られている．更に近年，AMがPI3K-Akt経路を介して血管新生作用を有することが相次いで報告されている[5,6]．著者らもウサギ下肢虚血モデルにAM遺伝子を導入すると，虚血下肢において著明な血管新生が誘導されることならびに，AMの血管新生作用は代表的な血管新生因子であるVEGFを介さない独自の作用であることを報告してきた[7]．

EPCは生体内で虚血や血管内皮障害が起きたときに，その障害部位へ遊走，付着して血管内皮細胞に分化して血管を形成する能力を有するが[1]，移植に必要な細胞数を確保するのに多量の血液が必要である．著者らはAMがEPCの機能増強作用を有するか否かについて*in vitro*での検討を行った．正常電ゼラチンを用いてAM遺伝子をEPCに導入すると(図1)，EPCのアポトーシスが抑制され，増殖が促進された．更にAM遺伝子導入EPCはEPC単独の10倍以

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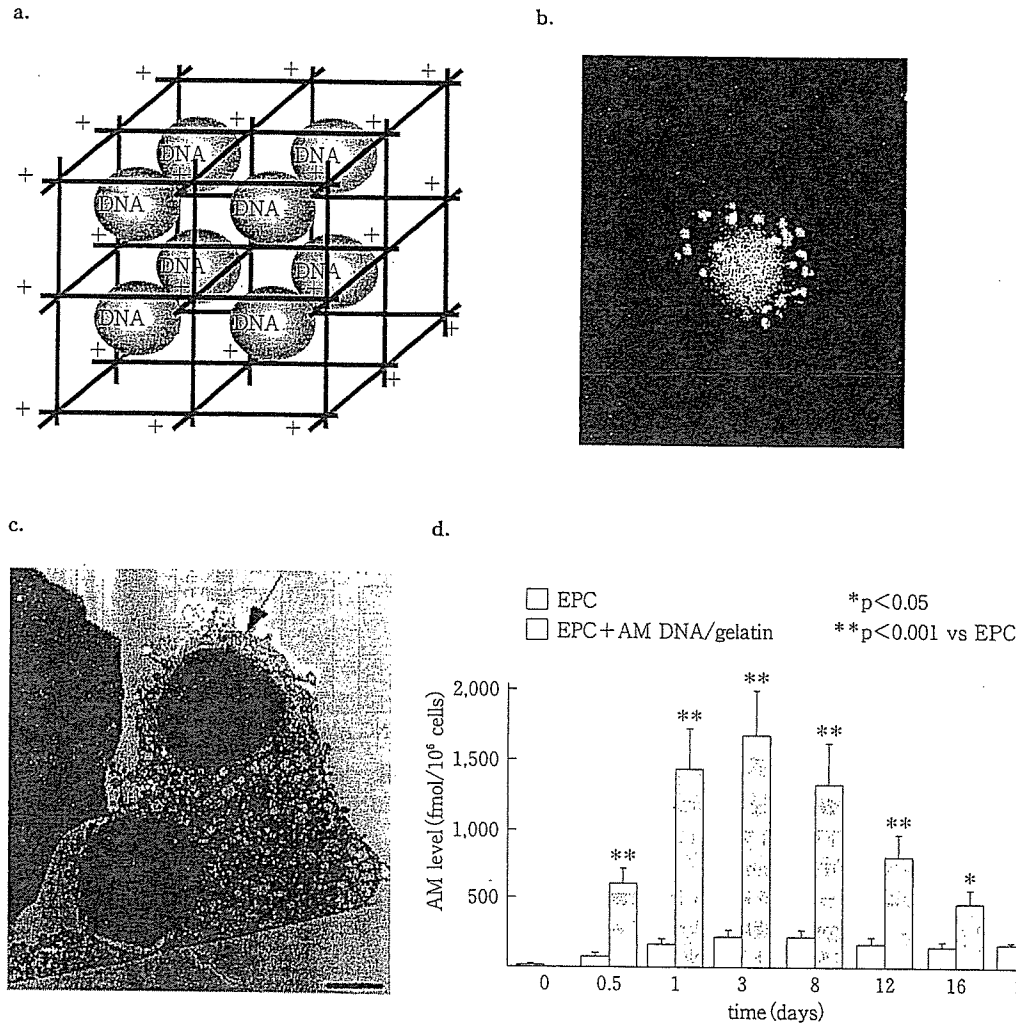

図1　ゼラチンを用いたEPCに対するAM遺伝子の導入[9]
DNA-ゼラチン複合体のシェーマ(a)とその実像(b).
電顕像でEPCがDNA-ゼラチン複合体を貪食するのが確認された(c).
ゼラチンによる遺伝子導入後のEPC培養液中のAM濃度(d).

上のAMを分泌し，2週間以上発現が持続した[8]．以上よりAM遺伝子をEPCに組み込むことで，①EPCの機能強化に結びつくのみならず，②ベクターとして局所まで運ばれることで(cell-based gene therapy)，AMの多彩な作用が効率良く局所で発揮されることが期待できると考えられた．

同様にMNCとAMの相互作用についても検討した．無血清培地にて24時間培養すると多数のMNCがアポトーシスを来すが，AMは用量依存性にMNCのアポトーシスを抑制した(図2-a)．一方，PI3K阻害薬であるwortmanninを加えるとAMの効果が抑制されることから，AMのMNCに対する抗アポトーシス作用はPI3K-Akt経路を介したものであると考えられた．更にAMはMNCの血管内皮細胞に対する接着を促進し，MNCからEPCへの分化を促進した．これらの結果よりAMは独自の血管新生作用のみならず，EPCおよびMNCの移植効果増強作用も有する可能性が示された．

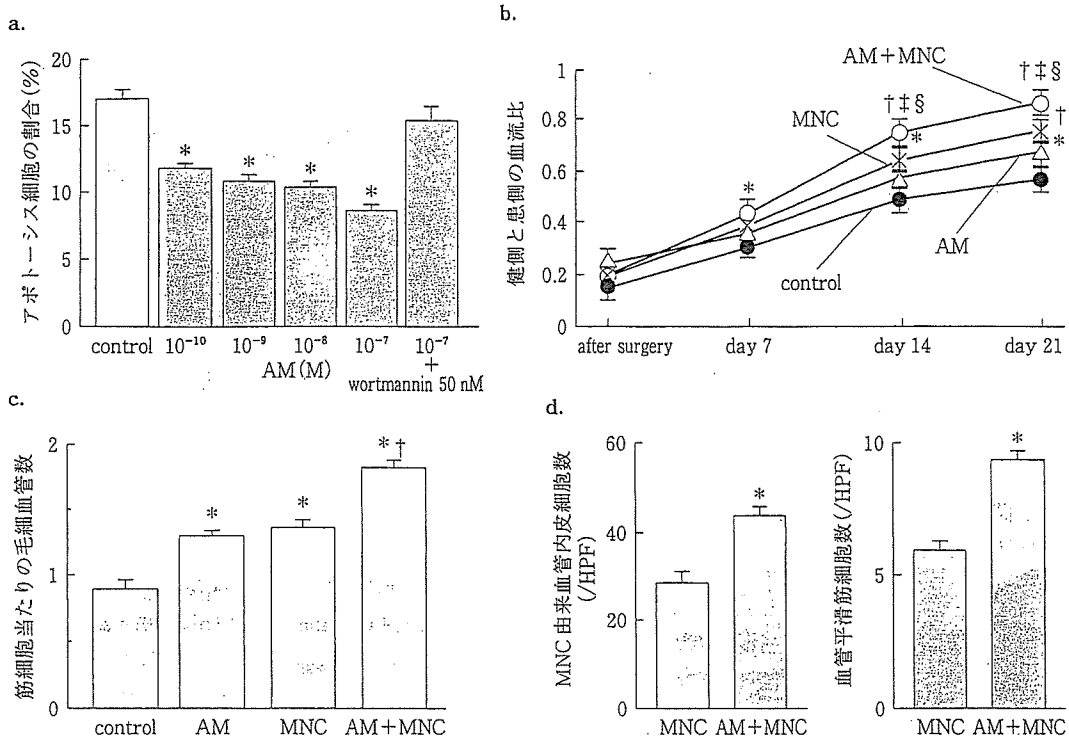

図2 AMとMNCの相互作用

AMのMNCに対する抗アポトーシス作用(a)，AM投与，MNC移植およびAMとMNC併用投与の下肢虚血ラットに対する治療効果(b-d).

a: *p<0.01 vs control.

b: レーザードプラによる下肢血流の評価

control: 下肢虚血ラットにvehicle投与，AM: 下肢虚血ラットにAM投与，MNC: 下肢虚血ラットにMNC移植，AM+MNC: 下肢虚血ラットにAMとMNC併用投与.

*p<0.05, †p<0.01 vs control, ‡p<0.01 vs AM, §p<0.05 vs MNC.

c: 毛細血管数の評価

*p<0.01 vs control, †p<0.01 vs AMおよびMNC.

d: AMの移植したMNCに対する分化促進作用

*p<0.01 vs MNC.

2. AM遺伝子とEPCを用いた肺高血圧治療

原発性肺高血圧症は原因不明の肺動脈性肺高血圧症であり，発病してから死亡するまで平均3年と非常に予後不良な疾患である．本疾患に対しては現在もなお決定的な治療法がなく，新たな治療法の開発が望まれている状況である．

近年，原発性肺高血圧症の発症原因として肺血管内皮の機能障害が報告されている[9]．一つは肺血管内皮機能の障害による血管作動物質のバランス破綻(収縮因子>拡張因子)である．また病的な肺血管内皮の異常増殖も機序の一つとして想定されている[10]．以上より，肺血管内皮機能障害の病態に着目した治療として，①肺血管内皮細胞で産生される拡張因子の補充と収縮因子の抑制，②正常な肺血管内皮細胞の再生促進という治療法が考えられる．

そこでAM遺伝子とEPCの移植併用による肺高血圧治療効果を，ラット肺高血圧モデルを用いて検討した[8]．ヒト臍帯血から単核球を分離し，VEGF下で培養することでEPCを得た．

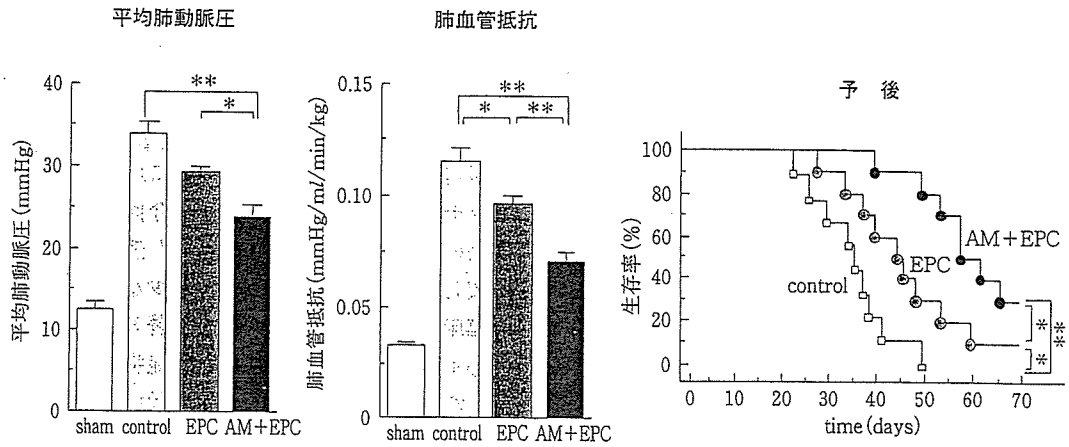

図3 AM遺伝子導入EPCの移植による平均肺動脈圧，肺血管抵抗，肺高血圧ラットの予後改善効果[8]

sham: 正常ラット，control: モノクロタリン肺高血圧ラットへvehicle投与，EPC: 肺高血圧ラットへEPC投与，AM+EPC: 肺高血圧ラットへAM遺伝子導入EPC投与．
$^{*}p<0.05$, $^{**}p<0.001$.

正常のラットにEPCを経静脈投与しても肺組織への付着は認められなかったが，モノクロタリンで肺血管内皮細胞や間質に障害を与えた後に投与すると，EPCは肺細動脈と間質に付着し成熟した血管内皮細胞となった．またEPCの移植は肺組織の血管数を増加させた．しかし血行動態的には平均肺動脈圧の有意な変化はなく，肺血管抵抗のわずかな改善が認められたのみであった(図3)．したがってEPCの移植のみでは肺高血圧治療効果には限界があると思われた．一方，AM遺伝子を導入したEPCを静脈内投与したところ[8]，コントロール群およびEPC移植単独群と比べて平均肺動脈圧を有意に低下させ，生存率を著明に改善させた．

AMは，特異的受容体が体血管よりむしろ肺血管に多数存在すること[11]，血管平滑筋細胞および血管内皮細胞に働き血管拡張を来すことから[12]，AM遺伝子導入EPC移植は正常な血管内皮の再生を促すのみならずAMによる肺血管拡張作用および血管新生作用があいまって肺高血圧の軽減に結びついたと思われる．既存の治療に抵抗性の原発性肺高血圧症患者が少なからず存在することを考えると，このAM遺伝子とEPCを用いた細胞移植治療は重症肺高血圧症に対する新たな治療法となる可能性がある．

3. 末梢動脈閉塞症に対するMNCを用いた細胞移植治療

続いて末梢動脈閉塞症(閉塞性動脈硬化症，Buerger病)に対するAMとMNC移植の併用療法の有効性を，下肢虚血動物モデルを用いて検討した．

ラットの左総腸骨動脈を結紮・切除して下肢虚血を作成し，AM単独投与，MNC移植単独およびAMとMNCの併用投与を行い，血管新生の程度について各群で比較検討した．3週間後の比較ではAM単独投与およびMNC移植単独においてコントロールと比較して有意な血流増加を認めた．更にAMとMNCの併用投与においては投与1週間後より顕著な血流増加を認め，3週間後の評価でも単独治療群よりも有意な血流増加を呈した(図2-b)．毛細血管の増加もAMとMNCの併用投与で最も著明であった(図2-c)．MNC移植単独およびAMとMNC併用投与はともに移植したMNCから血管内皮細胞への分化を認めたが，MNC単独と比較してAM併用投与では，より多くのMNC由来血管内皮細胞が確認された．更にAMとMNC併用

投与において血管の成熟に必要とされる血管平滑筋および壁細胞がMNC由来血管内皮細胞の周囲に多数確認された(図2-d). 以上のことよりAM単独投与, MNC単独投与でも血管新生作用があるが, 併用投与により血管新生効果が更に増強されることが明らかになった. またAM投与は移植したMNCの血管内皮細胞への分化を促進するのみならず, 成熟した血管の再生を促したものと考えられた. また心筋虚血モデルにおける検討においても同様の結果が得られた.

in vitro での検討およびこれらの動物実験の結果から, AMとMNCの併用投与は, AM独自の血管新生, 血管拡張作用に加え, 移植したMNCのアポトーシス抑制効果, 接着および内皮化を促進することで, 移植したMNCの組織への生着を保ち, 血管新生作用の増強および効果的な血管再生に結びついたと考えられた.

おわりに

今回, AMの遺伝子導入もしくはペプチド投与による細胞治療との併用療法の有効性について報告した. AMの遺伝子導入に用いた正帯電ゼラチンに関する詳細な記述は今回割愛するが, 従来のウイルスベクターを用いた遺伝子導入と比較して安全で倫理面の問題も少なく近い将来の臨床応用が期待される. また著者らは既に急性心不全症例や原発性肺高血圧症症例に対してAMのペプチドとしての投与を行い, 安全性を確認している[13,14]. 今回報告したMNCとAM併用療法に関しては, 末梢動脈閉塞症症例を対象とした臨床試験による有効性の確認が予定されており, 既に国立循環器病センターの倫理委員会で承認されている. AMは今回紹介した作用以外にも心筋細胞のアポトーシス抑制など幅広い生理作用を有しており, 様々な循環器疾患において細胞治療の効果を増強することが期待される.

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III. グレリン

病態と疾患からみたグレリン

循環器疾患

Ghrelin and cardiovascular disease

清水嘉人　永谷憲歳

Key words：悪液質，心機能障害，内皮機能障害

はじめに

グレリン(ghrelin)は，児島，寒川らにより発見された強力な成長ホルモン(GH)分泌ペプチドであり[1]，視床下部から分泌されるGH放出ホルモンとともに脳下垂体のGH分泌調節をしている．グレリンはG蛋白共役型のオーファン受容体GHS-R(growth hormone secretagogue receptor，成長ホルモン分泌促進因子受容体)[2]の内因性リガンドとして胃組織から単離された．グレリンはGHの強力な分泌活性を有するが，GHを介さない独自の作用として，摂食亢進作用や体重増加作用をもち，エネルギー代謝の調節に関与することが明らかとなった[3]．グレリンの受容体であるGHS-RのmRNAは視床下部，下垂体に多く発現しているが，心房，心室，血管などにもその発現が確認された[4]ことからグレリンの循環器系への関与が示唆された．循環器疾患，特に重症慢性心不全患者においては，心機能障害をはじめ悪液質や内皮機能障害などがその病態としてしばしば認められる．

本稿では，これらの病態におけるグレリンの病態生理学的意義に関して概説する．

1. 心不全患者に伴う悪液質におけるグレリンの意義

様々な疾患の末期では，体重減少，筋力低下を来す症例が存在する．このような病態は悪液質(cachexia)といわれ，エネルギーバランスの不調和により異化の亢進した状態となっている．特に，末期の慢性心不全患者では，cardiac cachexiaと呼ばれる心機能低下に伴う悪液質のため負のエネルギーバランスを呈しており，その存在は心不全の独立した予後規定因子と考えられている[5]．cardiac cachexiaの原因として，血行動態の悪化のみならず，異化作用を有するTNF-α，アンジオテンシン，ノルエピネフリンなどのサイトカインまたは神経体液性因子が関与している．cardiac cachexiaを伴う慢性心不全患者では，血漿グレリン濃度の上昇が認められた(図1)[6]．血漿グレリン濃度は心不全の重症度の指標であるNYHA機能分類および左室駆出率とは相関しなかったが，体格指数(BMI)と負の相関を示し，更に血中TNF-α値と正の相関を示したことより血漿グレリン濃度とcardiac cachexiaの関連が示唆された．そのほか，例えば肺癌[7]や神経性食思不振症[8]など循環器疾患以外の悪液質を有する疾患においても血漿グレリン濃度の上昇が認められた．

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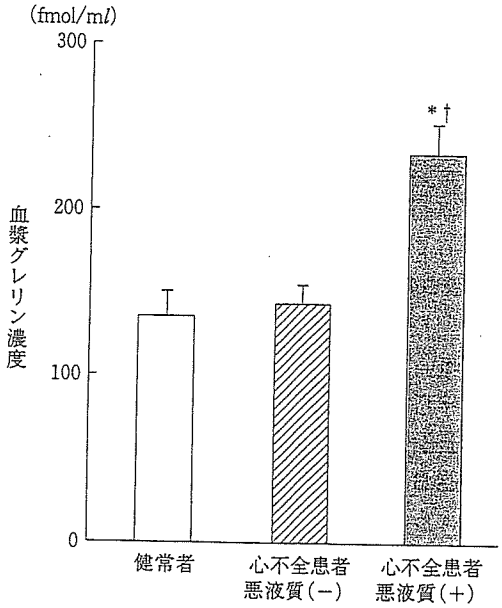

図1 心不全患者における悪液質の有無による血漿グレリン濃度[6]

*$p<0.05$ vs 健常者, †$p<0.05$ vs 悪液質を伴わない心不全患者.

悪液質における血漿グレリン濃度の上昇は，グレリンの有するエネルギー代謝調節作用を考慮すると，負のエネルギーバランスに対する代償的機序によるものと考えられる．すなわち，悪液質における異化亢進時に，胃から分泌されたグレリンは消化管内ではなく血管内に分泌され，増加した血中のグレリンがホルモンとして循環することにより摂食亢進が起こり，更には同化/異化のエネルギーバランスの不調和を改善すると考えられる．またグレリンはGHの分泌促進を介した蛋白同化作用や，更にグレリン独自の作用としての末梢での脂肪蓄積作用も併せ持っており[9]，これらの作用も悪液質の是正に関与していると思われる．

2. 心機能障害に対するグレリンの意義

グレリンはエネルギー代謝調節作用のほかに，循環動態における生理的作用として，GHおよびその mediator である insulin-like growth factor-1(IGF-1)を介した蛋白同化作用による代償性心肥大の促進や血管拡張作用があり，またGHを介さないグレリン独自の作用としての血管拡張作用[10]や交感神経抑制作用[4]を有する．GHS-Rは心臓や血管にも発現がみられていることから，グレリンはGHS-Rを介して循環動態の調節にかかわっている可能性がある．特に心機能低下時には，前述の悪液質の際と同様に，グレリンが代償的機序により循環動態の維持に関与する可能性があり，今後のメカニズムの解明が待たれる．またグレリンには細胞内シグナル伝達の刺激，特にERK(extracellular signal-regulated kinase)-1/2およびPI(phosphatidylinositol)3-kinase/Aktの活性化を介して内皮細胞や心筋細胞のアポトーシスを抑制する効果がある[11]ことが報告されており，グレリンの心保護作用が心機能低下予防，更には心不全の進展の抑制に重要な役割を果たしている可能性が示唆される．

3. 内皮機能障害に対するグレリンの意義

慢性心不全の病態の一つとして血管内皮機能の低下があげられる．内皮機能障害は動脈硬化の初期段階でもあり，心血管障害の発症に影響する．GHはIGF-1を介して内皮機能を改善することが知られている[12]．またGHS-Rに対する外因性リガンドである合成GH分泌促進物質hexarelinもまた，内皮機能障害を改善することが報告されている[13]．動脈硬化を呈したヒトの血管ではGHS-Rが高発現されていることが報告されており[14]，このことからもGHS-Rの内因性リガンドであるグレリンが，内皮機能障害更には動脈硬化の進展予防に関与している可能性が考えられる．グレリンをGH欠損ラットに投与すると，アセチルコリン負荷に対する単離大動脈の弛緩の改善がみられ，グレリンはGHを介さない独自の作用として血管内皮機能改善効果を有することが示唆された[15]．更にグレリンを投与されたラット大動脈では内皮型一酸化窒素合成酵素(eNOS)蛋白の高発現がみられており(図2)[15]，グレリンの内皮機能改善効果はeNOS/NO経路の改善によるものと考えられた．内皮由来のNOは抗動脈硬化作用を有することから，グレリンはGHS-Rを介して内皮

図2 グレリンを皮下投与されたラットにおける内皮型一酸化窒素合成酵素(eNOS)蛋白の発現[15]

機能の調節に重要な役割を果たし，血管の恒常性の維持や動脈硬化の進展予防に関与する可能性が示唆された．

おわりに

これまでの研究から，グレリンは悪液質を有する慢性心不全において，代償的に増加し，異化に傾いたエネルギーバランスの不調和の改善に関与していると考えられる．またその強力なGH分泌作用またはグレリン独自の作用により，心機能および内皮機能の維持にも関与していると考えられ，グレリンは循環器系における恒常性の維持に重要な役割を担っていることが示唆された．

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特　論

グレリンのトランスレーショナルリサーチ
—循環器疾患—

Ghrelin in the treatment of cardiovascular disease

永谷憲歳[1]　寒川賢治[2]

Key words：グレリン，心不全，心機能，交感神経

はじめに

グレリン(ghrelin)は，1999年，児島，寒川らにより発見された成長ホルモン(GH)分泌ペプチドである[1]．グレリンの受容体GHS-R(growth hormone secretagogue receptor，成長ホルモン分泌促進因子受容体)[2]は視床下部，下垂体に多く発現しているが，心房，心室，血管などにもその発現が確認され[3]，グレリン独自の心血管作用の存在が示唆された．これまでに明らかとなったグレリンの心血管作用にはGH/IGF-1を介したもの(蛋白同化作用，強心作用，血管拡張作用)[4,5]とGHS-Rを介したグレリン独自の働き(血管拡張作用，心血管のアポトーシス抑制，交感神経抑制作用，エネルギー代謝改善作用)がある．

本稿では，グレリンの循環器領域におけるトランスレーショナルリサーチ，特にグレリンによる難治性心不全治療の可能性について述べる．

1. 心不全の病態とグレリン

慢性心不全患者ではその末期において，体重減少，筋力低下を来す症例が少なからず存在する．このような状態は心悪液質(cardiac cachexia)と呼ばれ，その存在は心不全の独立した予後規定因子であると考えられている[6]．心悪液質の原因としては，血行動態の悪化のみでなく，異化作用を有するTNF-α，アンジオテンシンII，ノルエピネフリンなどのサイトカイン，神経体液性因子が関与している[7]．著者らはグレリンのエネルギー代謝改善作用に着目し，心不全，心悪液質の病態におけるグレリンの意義に関して検討した．慢性心不全患者74人の血中グレリン濃度を測定し，臨床データと比較検討した．興味深いことに，心悪液質を伴う心不全患者24人において，血漿GH濃度とともにグレリン濃度が上昇していた(図1)[8]．血漿グレリン濃度は心不全の重症度の指標であるNYHA機能分類および左室駆出率とは相関しなかった．グレリンは蛋白同化作用を有するGHの分泌を刺激することや，GHを介さずに摂食促進[9]，エネルギー代謝改善[10]に働くことを考慮すると，心悪液質の病態における代償機序として胃組織でのグレリン分泌が促進されたと考えられる．以上よりグレリンは，慢性心不全に伴う異化に傾いたエネルギー代謝の是正に重要な役割を担っている可能性がある．

2. グレリンの心血管作用

健常人や慢性心不全患者にグレリン(10μg/

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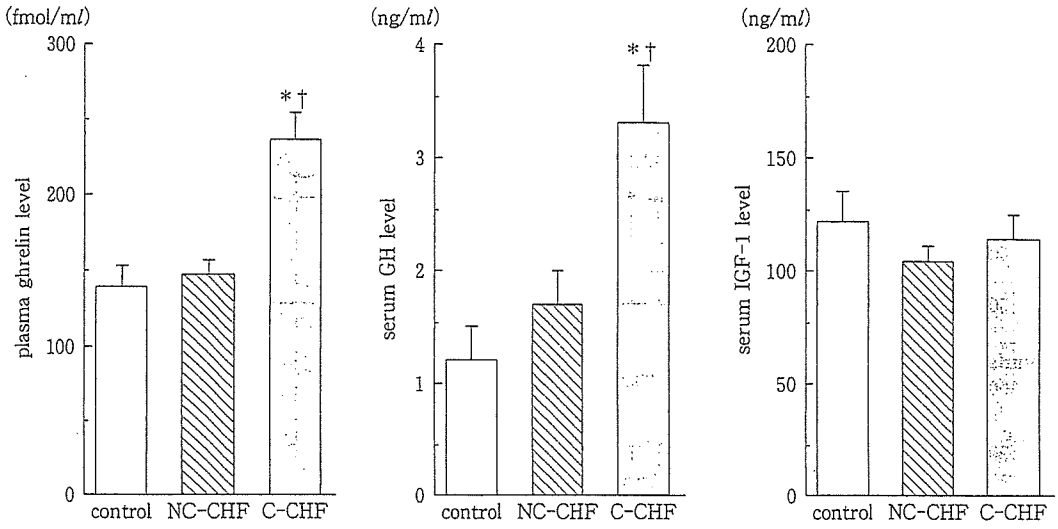

図1 心不全患者における心悪液質の有無による血中グレリン，成長ホルモン(GH)，インスリン様成長因子(IGF-1)濃度[8]

NC-CHF: 心悪液質を伴わない心不全患者，C-CHF: 心悪液質を伴った心不全患者．
*$p<0.05$ vs control, †$p<0.05$ vs NC-CHF.

kg)を静脈内投与すると，比較的長時間持続する降圧効果がみられる(図2)[11]．GHS-Rは血管に多数存在すること，前腕動脈への局所投与ではGH/IGF-1を増加させずに血流を増加させること[12]，GH欠損のdwarfラットへの静脈内投与においても血圧を低下させる[13]などの結果から，グレリンによる血管拡張作用が示唆された．Wileyらは単離血管を用いた研究で，グレリンはエンドセリンによって収縮した血管を内皮非依存性に拡張させることを証明した[14]．

興味深いことに，グレリンの静脈内投与によって著明に動脈圧が低下するにもかかわらず心拍数の増加は認められなかった(図2)[11]．近年，Matsumuraらは，グレリンの脳室内投与の結果からグレリンは交感神経を有意に抑制することで血圧を調節していることを明らかにした[15]．

グレリンは生体にとっては交感神経抑制の方向に働くにもかかわらず，グレリンの静脈内投与は心拍出量を有意に増加させた[3,11]．単離心筋細胞に対するグレリンの直接の心筋収縮作用はみられないことから[13]，この心拍出量の増加はグレリンの血管拡張作用による心後負荷の軽減，GHによる強心作用が一部関与していると考えられた．そのほか，グレリンは心筋細胞，血管内皮細胞のアポトーシスを抑制することが報告された．そのメカニズムとしてERK1/2とPI3-kinase/Aktの活性化が関与する[16]．これらの実験結果より，グレリンは心血管の保護に働くことが示唆された．

3. グレリンによる新たな心不全治療

GHはインスリン様成長因子I(insulin like growth factor I: IGF-1)を介して心筋収縮増大，血管拡張，心筋構築促進に働く[4,5]．実際，GHを冠動脈結紮後の心不全ラットに2週間皮下投与すると，心拍出量の増大，血管抵抗の低下，代償性左室肥大の促進による心筋壁応力の低下が得られる[4]．したがって強力なGH分泌促進作用をもつグレリンが慢性心不全の治療薬として応用できる可能性がある．またグレリン独自の心血管作用，エネルギー代謝改善作用が，心機能不全や心悪液質の是正に有効である可能性がある．そこで著者らは，悪液質を伴った慢性心不全ラットにグレリン(100μg/kg)を1日2回，3週間の皮下投与を行い，心機能および心悪液質の改善効果を検討した[13]．グレリン投与によ

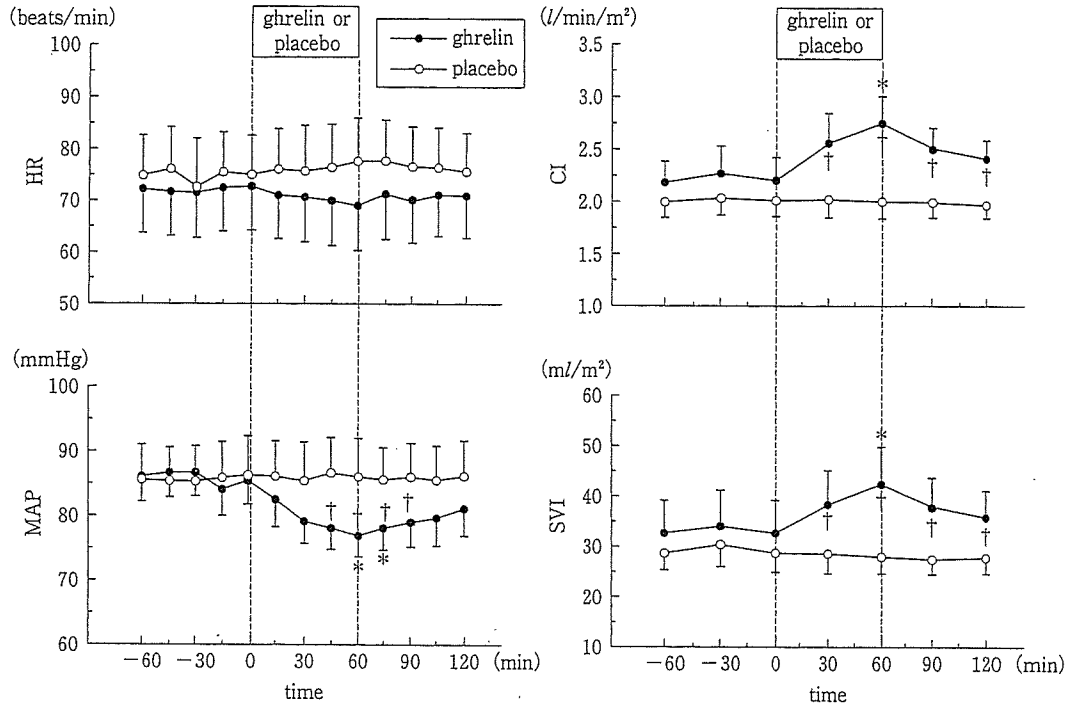

図2 心不全患者へグレリンを静脈内投与したときの心拍数(HR)，平均動脈圧(MAP)，心係数(CI)，一回駆出量係数(SVI)の変化[11]
*p<0.05 vs time 0, †p<0.05 vs placebo.

り血中GH，IGF−1濃度は上昇し，心拍出量および心筋収縮性の指標であるMax dp/dtは増加した(図3)．またグレリン投与群では，これまでに報告されたGH補充療法と同様に非梗塞部の代償性心肥大が促進された．グレリンはGH/IGF−1分泌促進を介して蛋白同化作用を発揮し，心筋構築を促進させ壁応力を軽減させることで心機能改善に働いた可能性がある．またグレリン投与により上昇したGH/IGF−1が心筋収縮を増強させた可能性がある．グレリン慢性投与により末梢血管抵抗の有意な低下が認められたことから，心拍出量の増加は血管拡張作用による心後負荷の軽減が一部関与していると考えられる．また慢性心不全ラットにグレリンを投与すると，体重および骨格筋量の有意な増加が観察された(図3)．GHを介した蛋白同化作用以外に，摂食促進，末梢での脂肪蓄積作用が心悪液質の是正に関与した可能性がある．

グレリンのトランスレーショナルリサーチを推し進めるべく，これらの動物実験をもとに心不全患者へのグレリン投与の臨床試験を開始した．グレリン(0.10μg/kg/min)を経静脈的に1時間投与すると，血中GH濃度の上昇(基礎値の16倍)以外に，平均動脈圧の低下(−9 mmHg)，心拍出量の増加(+25%)が認められた(図2)[11]．更に慢性心不全患者を対象にグレリンの長期投与(1日2回，3週間)を行った．明らかな副作用は出現せず，代償性心肥大を促し(心筋構築の改善)，心機能を改善させた．またグレリン投与によって骨格筋量が増加した．これらの効果にはグレリンによるGH分泌促進作用が関与していると考えられた．興味深いことに，繰り返しのグレリン投与は血中ノルアドレナリンを全例で有意に低下させた．交感神経の抑制は慢性心不全患者の心機能改善・生命予後改善に働くと考えられているため，グレリンは交感神経抑制作用を介して心機能改善に働いた可能性がある．またグレリン投与により運動耐