BMCs recruited into the infarcted myocardium may contain various kinds of somatic stem cells, such as endothelial progenitor cells [20], bone marrow-derived stem cells [21], and bone marrow mononuclear cells [2], which have potent therapeutic effects in heart failure [22]. Furthermore, bone marrow-derived mesenchymal stem cells secrete prostaglandin [23], which may act like ONO-1301 and amplify the effects of the ONO-1301-mediated therapy. Kawabe et al. clearly showed that prostaglandin facilitates the recruitment of endothelial progenitor cells [24]. Although further analysis is needed, the enhanced accumulation of BMCs may predispose the damaged heart tissue to better restoration following MI.

Many reports have shown that granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) also induce BMC mobilization, with therapeutic effects in animal models [25]. However, G-CSF therapy in unselected patients with acute MI did not lead to functional improvements beyond those achieved with conventional therapy. In addition, the administration of GM-CSF in cancer patients has been shown to transiently increase the LV end-systolic dimensions and decrease cardiac contractility [25,26]. The lack of efficacy of G-CSF therapy in clinical trials may be due, at least in part, to its poor initiation and duration; such therapies are likely to be most beneficial during the early phase after acute MI. Although conventional prostacyclin and its analogs are chemically and biologically unstable, ONO-1301 is a long-acting prostacyclin agonist that exerts stable effects *in vivo*, because it lacks a prostanoid structure. Furthermore, we used a slow-release form of ONO-1301, made by polymerizing it with poly-lactic and glycolic acid; this ONO-1301 could still be detected in the blood 3 weeks after its administration (figure S4 in File S1).

Furthermore, in our *in vitro* analysis, although we used normal human dermal fibroblasts to examine the SDF-1/CXCR-4-dependent BMC migration, the reactivity to ONO-1301 stimulation will differ depending on the cell type. For example, the G-CSF expression was upregulated in some kinds of cells (unpublished data). Thus, together with the upregulation of multiple beneficial cytokines such as HGF and VEGF, because of the longer duration of its activity, ONO-1301 may be more potent than conventional protein-based therapies.

Our data showed that ONO-1301 treatment was a potent inducer of BMC homing. Of the BMCs that accumulated in the infarcted myocardium, 43 percent expressed isolectin, an endothelial cell marker, but the other BMCs had a fibroblastic morphology, and did not express cardiac-lineage or cardiofibroblast markers (Figure S3 in File S1). ONO-1301 administration resulted in the attenuation of cardiac dysfunction, with enhanced BMC accumulation. Further study is required to elucidate the mechanism, but we speculate that paracrine effects of factors released by the BMCs play pivotal roles in the therapeutic efficacy, rather than the transdifferentiation of the BMCs into the cardiac or vascular lineage. The effect of cardioprotective and angiogenic factors secreted by the accumulated BMCs and the direct stimulation of ONO-1301 itself may synergistically increase the angiogenesis and cardioprotection, leading to improved therapeutic results.

In summary, ONO-1301 may be a powerful, long-acting activator of multiple cytokines. In particular, SDF-1 may enhance the BMC accumulation in a SDF-1/CXCR4-signaling-dependent manner, leading to an attenuation of the cardiac dysfunction following MI. Our findings suggest that the method involving a sustained release of ONO-1301 may be adapted as a novel drug delivery system for treating heart failure.

Supporting Information

File S1.
(DOCX)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: YI SM Y. Sawa. Performed the experiments: YI KI NS. Analyzed the data: YI AS. Contributed reagents/materials/analysis tools: Y. Sakai. Wrote the paper: YI SM SF Y. Sawa.

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Synthetic prostacyclin agonist, ONO1301, enhances endogenous myocardial repair in a hamster model of dilated cardiomyopathy: A promising regenerative therapy for the failing heart

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Objectives: Remodeling of the left ventricle (LV) in idiopathic dilated cardiomyopathy (IDCM) is known to be associated with multiple pathologic changes that endogenous factors, such as hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF), protect against. Although a clinically relevant delivery method of these factors has not been established, ONO1301, a synthetic prostacyclin agonist, has been shown to upregulate multiple cardioprotective factors, including HGF and VEGF, in vivo. We thus hypothesized that ONO1301 may reverse LV remodeling in the DCM heart.

Methods: ONO1301 dose-dependently added to the normal human dermal fibroblasts and human coronary artery smooth muscle cells in vitro, to measure the expression of HGF, VEGF, stromal cell–derived factor (SDF)-1, and granulocyte-colony stimulating factor (G-CSF), assessed by real-time polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay. δ-Sarcoglycan-deficient J2N-k hamsters, which is an established DCM model, were treated by epicardial implantation of an atelocollagen sheet with or without ONO1301 immersion or sham operation.

Results: ONO1301 dose-dependently upregulated expression of these 4 factors in vitro. ONO1301 treatment, which induced dominant elevation of ONO1301 levels for 2 weeks, significantly preserved cardiac performance and prolonged survival compared with the other groups. This treatment significantly upregulated expressions of cardioprotective factors and was associated with increased capillaries, attenuated fibrosis, and upregulation of α-sarcoglycan in the DCM heart.

Conclusions: ONO1301 atelocollagen-sheet implantation reorganized cytoskeletal proteins, such as α-sarcoglycan, increased capillaries, reduced fibrosis, and was associated with upregulated expression of multiple cardioprotective factors, leading to preservation of cardiac performance and prolongation of survival in the δ-sarcoglycan-deficient DCM hamster. (J Thorac Cardiovasc Surg 2013;146:1516-25)

Idiopathic dilated cardiomyopathy (IDCM) is one of the most critical intractable diseases. The etiology and pathology of IDCM have therefore been intensively investigated to explore other treatment options.[1] Clinical and functional progression of IDCM has been shown to be closely correlated with the histopathology, such as apoptosis of cardiomyocytes, accumulation of fibrotic components, reduction of vascular density, and remodeling of sarcolemmal/cytoskeletal proteins. It has been recently suggested that cell transplantation into the IDCM heart positively modulates cellular behavior of native cardiac fibroblasts and/or coronary artery smooth muscle cells (CoASMCs), leading to upregulation of multiple cardioprotective factors in the heart.[2] Inasmuch as cell transplantation is clinically limited by the cell-culture procedure and the availability of a cell processing center, cell-free therapy that enhances cardiac regeneration has long been sought in the clinical arena.[3]

Prostacyclin and its analogs have been shown to upregulate expressions of various factors, such as hepatic growth factor (HGF) and vascular endothelial growth factor (VEGF) in vitro and in vivo.[4] Although previously generated prostacyclin agonists are chemically unstable, being limited by the delivery method, it has recently been shown that ONO1301 is a selective prostacyclin receptor (IPR) agonist having a unique, chemically stable structure, and polymerization of ONO1301 with polylactic-co-glycolic acid copolymer (PLGA) to form a microsphere (ONO1301-MS) upregulates multiple protective factors, represented by HGF, for 3 to 4 weeks in vivo.[5] We therefore

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Abbreviations and Acronyms

CoASMC = coronary artery smooth muscle cell
DCM = dilated cardiomyopathy
Dd/Ds = diastolic/systolic dimensions
EF = ejection fraction
ELISA = enzyme-linked immunosorbent assay
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
G-CSF = granulocyte colony stimulating factor
HCoASMC = human coronary artery smooth muscle cell
HGF = hepatic growth factor
IPR = prostacyclin receptor
IDCM = idiopathic dilated cardiomyopathy
LV = left ventricular (ventricle)
N group = atelocollagen sheet without ONO1301
NHDF = normal human dermal fibroblast
O group = atelocollagen sheet containing ONO1301
PCR = polymerase chain reaction
PLGA = polylactic-co-glycolic acid copolymer
S group = sham group
SDF-1 = stromal cell–derived factor-1
VEGF = vascular endothelial growth factor
vWF = von Willebrand factor

hypothesized that administration of ONO1301-MS into the IDCM heart might upregulate cardiac protective factors, leading to histologic and functional reverse left ventricular (LV) remodeling.

MATERIALS AND METHODS

Experimental procedures related to animal studies were carried out under the approval of the institutional ethics committee. The investigation conformed to the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication No. 85 to 23, revised 1996). All experimental procedures and evaluations were performed in a blinded manner.

Cell Culture

Normal human dermal fibroblasts (NHDFs) and human CoASMCs (HCoASMCs) were purchased from EIDIA Co, Ltd (Tokyo, Japan). The cells were cultured on 6-well plates with Dulbecco's modified Eagle's medium (Sigma-Aldrich, St Louis, Mo) supplemented with 10% fetal bovine serum (EIDIA) under 5% carbon dioxide. Next, 1- to 1000-nmol/L ONO1301 (Ono Pharmaceutical, Osaka, Japan), dimethyl sulphoxide (Sigma-Aldrich) or dibutyryl cyclic adenosine monophosphate (Sigma-Aldrich) was added to the culture medium for 72 hours, and then the culture supernatants and cells (n = 6, respectively) were harvested and stored at −80°C.

Procedure of ONO1301-MS Administration to the Dilated Cardiomyopathy (DCM) Hamster

Male 20-week-old δ-sarcoglycan-deficient J2N-k hamsters and J2N-n normal hamsters were purchased from Japan SLC (Shizuoka, Japan). Inasmuch as human DCM-like histopathologic features and associated functional deterioration develop in J2N-k hamsters, they have been used as an established IDCM model.[6] Each hamster underwent left lateral thoracotomy under 1.5% isoflurane anesthesia (n = 66). Subsequently, ONO-1301 was delivered into the heart using a novel drug delivery system, in which an atelocollagen sheet (Integran sheet; Koken Co, Ltd, Tokyo, Japan) shaped "hand-drum" containing ONO1301-MS (10 mg/kg) was placed to cover the entire ventricular free wall (O group, n = 22). Other hamsters underwent either ONO1301-free atelocollagen sheet implantation in the same manner (N group, n = 21) or sham operation (S group, n = 23). After the layered closure, the hamsters were housed in a temperature-controlled individual cage until spontaneous or scheduled death at 2 or 4 weeks after the operation (n = 5 each).

Measurement of ONO1301 Concentration in the Plasma and the Ventricular Tissue

Under isoflurane inhalation (5%), venous blood (1 mL) was sampled from the internal jugular vein, and the ventricle was then excised from the hamster at day 1 and weeks 1, 2, 4, and 8 after ONO1301 treatment (n = 3 each). The plasma was stored at −80°C, and the ventricle was thoroughly washed and stored at −80°C. The concentrations of ONO1301 in the plasma and the ventricle were measured by high-performance liquid chromatography with the tandem mass spectrometric (LC/MS/MS) detection.[7]

Transthoracic Echocardiography

Transthoracic echocardiography was performed using a system equipped with a 12-MHz transducer and SONOS 5500 (Agilent Technologies, Palo Alto, Calif) under isoflurane inhalation (1%). Diastolic/systolic dimensions (Dd/Ds) and ejection fraction (EF) of the LV were measured.[8]

Histopathology

The heart was excised under isoflurane anesthesia (5%) and immersion-fixed with ice cold 4% paraformaldehyde. The fixed heart was embedded with either paraffin or optimal cutting temperature compound (Funakoshi, Tokyo, Japan) and transversely sliced to generate paraffin or frozen sections, respectively. The paraffin sections were stained using picrosirius red or immunohistologically labeled using anti–von Willebrand factor (vWF) antibody (DAKO, Glostrup, Denmark). Frozen sections (7-μm thick) were immunohistologically labeled using anti–α-dystroglycan (clone: VIA4-1; Upstate Biotechnology, Lake Placid, NY), anti–α-sarcoglycan (clone: Ad1/20A6; Novocastra, Wetzlar, Germany), anti–β-sarcoglycan (clone: bSarc/5B1; Novocastra), anti-IPR (Abcam, Cambridge, United Kingdom), or anti–α-actin (Millipore, Billerica, Mass) antibodies. The sections were then labeled by corresponding AlexaFluor488/594-conjugated secondary antibodies counterstained with 6-diamidino-2-phenylindole (DAPI; Life Technologies, Calif). 3,3′-Diaminobenzidine (DAB) staining of IPR was performed using the LSAB2 kit (DAKO). Fluorescent-labeled sections were viewed under an ECLIPSE TE 200-U confocal microscope (Nikon, Tokyo, Japan). The percentage of the total area that was fibrotic, as determined by picrosirius red staining, was calculated by using a planimetric method with MetaMorph software (Molecular Device, Osaka, Japan). The number of capillaries per square millimeter was calculated by the BZ Analyzer (Keyence, Osaka, Japan) and was counted in 4 high-power fields per section (a total of 10-12 fields/heart).

Real-Time Polymerase Chain Reaction

Total RNA was isolated from the cultured cells and the free wall of the LV using the RNeasy Kit and reverse-transcribed using Omniscript Reverse

TABLE 1. Forward and reverse primers and probe

	F-primer	R-primer	Probe
Human			
GAPDH	GAA GGT GAA GGT CGG AGT C	GAA GAT GGT GAT GGG ATT TC	CAA GCT TCC CGT TCT CAG CC
HGF	ATG ATG TCC ACG GAA GAG GAG A	CAC TCG TAA TAG GCC ATC ATA GTT GA	TGC AAA CAG GTT CTC AAT GTT TCC CAG C
VEGF	GAA GTG GTG AAG TTC ATG GAT GTC T	CAC ACA GGA TGG CTT GAA GAT G	TTC CAG GAG TAC CCT GAT GAG ATC GA
SDF-1	CAT GCC GAT TCT TCG AAA GC	CTA CAA TCT GAA GGG CAC AGT TTG	TGT TGC CAG AGC CAA CGT CAA GCA
G-CSF	GCT GTG GCA CAG TGC ACT CT	CCC TGG ATC TTC CTC ACT TGC TC	CCT GCC CCA GAG CTT CCT GCT CA
Hamster			
GAPDH	CTG CAC CAC CAC CTG CTT AGC	GCC ATG CCA GTG AGC TTC C	CTG CAC CAC CAC CTG CTT AGC
HGF	AGG TCC CAT GGA TCA CAC AGA	GCC CTT GTC GGG ATA TCT TTC T	ACC AGC AGA CAC CAC ACC GGC A
VEGF	GCA CTG GAC CCT GGC TTT ACT	TCA TGG GAC TTC TGC TCT CCT T	ACC ATG CCA AGT GGT CCC AGG CT
α-Sarcoglycan	AAC TGA AGA GAG ACA TGG CCA CC	CAG TGC TGG TCC AGG ATG AGG	CCT CTC TCC ACC TTG CCC ATG TTC A
β-Sarcoglycan	TCC ACT GAG AGG ATT ACC AGC AAT	AGT TTG TAG CGC ACC CAG TCA C	TCC TCA ATG GAA CTG TGA TGG TCA GCC C
α-Dystroglycan	CAC ACA GTC ATT CCA GCT GTT GT	TCA TCC AGC TCG TCT GCA AAG	CCT TGA GGA CCA GGC CAC CTT TAT CAA

GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; *HGF*, hepatic growth factor; *VEGF*, vascular endothelial growth factor; *SDF-1*, stromal cell–derived factor-1; *G-CSF*, granulocyte colony stimulating factor.

transcriptase (Qiagen, Hilden, Germany). Quantitative polymerase chain reaction (PCR) was performed with the ABI 7500 Fast Real-Time PCR using TaqMan Universal Master Mix (Applied Biosystems, Division of Life Technologies Corporation, Carlsbad, Calif) and the designed primers/probes (Table 1). Expression of each mRNA was normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Western Blotting

The LV free wall was homogenized and centrifuged at 1000 g at 4°C for 10 minutes to retrieve protein. Subsequently, 10 to 20 μg of protein was subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis with 12.5% gels and transferred to polyvinylidene difluoride membranes (GE Healthcare, Little Chalfont, United Kingdom) using the Mini Trans-Blot system (170-3930; Bio-Rad Laboratories, Hercules, Calif). Protein blots in polyvinylidene difluoride membranes were incubated with each of the primary antibodies against α-sarcoglycan or GAPDH (Abcam, Plc, Cambridge, United Kingdom). The membranes were incubated with the corresponding horseradish peroxidase–labeled secondary antibody and then visualized using the ECL system (GE Healthcare Lifesciences, Cleveland, Ohio). The band intensities were quantified by Image-J software (Wine Rasband, Bethesda, Md). The values obtained were expressed as a percentage of the value in the J2N-n hamsters.

Statistical Analysis

Data are summarized as means ± SEMs. Comparisons among groups were made with the use of 1-way analysis of variance, followed by the Tukey honestly significant difference test. Differences across the whole time course of echocardiographic data and the concentration of ONO1301 were analyzed by repeated analysis of variance including main effects of the group, time, and their interactive effects, followed by pairwise comparisons at different times using a paired *t* test with the Bonferroni multiplicity correction. Survival curves were prepared by using the Kaplan-Meier method and compared using the log-rank test. All probability values are 2-sided. Statistical analysis was performed by using SPSS version 11.0 (SPSS, Inc, Chicago, Ill).

RESULTS

Effects of ONO1301 on the Secretion of Protective Factors in Vitro

Dose-dependent effects of ONO1301 on each component of the cells were assessed by cultivating commercially available NHDFs, HCoASMCs in vitro. Synthesis and extracellular secretion of protective factors were quantitatively assessed by real-time PCR and enzyme-linked immunosorbent assay (ELISA; Quantikine; R&D Systems, Minneapolis, Minn), respectively. ONO1301 treatment upregulated the expressions of HGF, VEGF, and stromal cell–derived factor-1 (SDF-1), but not granulocyte colony stimulating factor (G-CSF), in the NHDFs assessed by real-time PCR (Figure 1, *A*). In particular, a high concentration of ONO1301 significantly upregulated expressions of these factors. These findings were consistent with the results of ELISA for the culture supernatants (Figure 1, *B*). A high concentration of ONO1301 also significantly upregulated expressions of HGF, VEGF, and G-CSF, but not SDF-1, in the HCoASMCs, assessed by real-time PCR and ELISA (Figure 1, *C* and *D*).

Heart-Dominant Elevation of ONO1301 Concentration After ONO1301 Treatment

The difference in the ONO1301 concentration between the plasma and the LV after ONO1301 treatment was quantitatively and serially assessed by the LC/MS/MS method. The ventricular ONO1301 concentration was markedly greater than the plasma concentration at week 1 and week 2 after the treatment, although the plasma ONO1301 concentration

was higher than the detectable limit for this period (Figure 2, *A*). Both ventricular and plasma concentrations of ONO1301 were less than the detectable limit at weeks 4 and 8.

Presence of IPR in the Vasculature of the IDCM Heart

Localization of the IPR in the heart was assessed by immunohistolabeling. IPR was present in the microvasculature component, such as vascular SMCs and endothelial cells (Figure 2, *B* and *C*), but not in the cardiac fibroblasts or cardiomyocytes (Figure 2, *D*). Expression of the IPR was not different between the J2N-k and J2N-n hamsters.

Preserved Cardiac Performance in the IDCM Hamster With ONO1301 Therapy

The functional effects of ONO1301-atelocollagen sheet implantation on the IDCM heart were serially assessed by transthoracic echocardiography. LVDd/Ds and EF at 20 weeks of age, just before the treatment, were not significantly different among the 3 groups (Figure 2, *E*). After treatment, echocardiography showed that the LVDd/Ds and EF were significantly preserved until 4 weeks in the O group, compared with the N and S groups, which showed a progressive increase of LVDd/Ds and a progressive reduction of LVEF for the subsequent 8 weeks. However, even the O group showed progressively increased LVDd/Ds and reduced LVEF in the subsequent 4 weeks.

Upregulated HGF and VEGF in the Heart After ONO1301 Treatment

Real-time PCR was used to quantitatively assess the trend in expression of angiogenic factors, such as HGF and VEGF, in the hearts of the 3 groups and the normal hamster (n = 5, each). Intramyocardial mRNA levels of HGF and VEGF in the S and the N groups were not significantly different from those in the normal hamster at 2 or 4 weeks (Figure 3, *A*). In contrast, in the O group, both HGF and VEGF were significantly upregulated at 2 weeks compared with the other groups. VEGF significantly upregulated in the O group at 4 weeks, although the HGF level in the O group was not significantly different from that in the other groups at 4 weeks.

Increased Vasculature in the Heart After ONO1301 Treatment

The trend in the distribution and the number of arterioles and capillaries in the heart after ONO1301 treatment was assessed by vWF-labeled sections of the 3 groups and normal hamsters (n = 5, each). The number of vWF-positive arterioles and capillaries was significantly less in the N and the S groups than in the normal heart at 2 and 4 weeks. In contrast, vWF-positive arterioles and capillaries were homogeneously increased in the O group at 2 and 4 weeks compared with the other groups (Figure 3, *B*; $P < .05$).

Effect of ONO1301 on Myocardial Fibrosis

The distribution and the quantity of interstitial collagen in the heart after ONO1301 treatment was assessed by picrosirius red–stained sections (n = 5, each). Interstitial collagen was significantly accumulated in the J2N-k hamsters, regardless of the treatment, compared with the normal hamsters (Figure 3, *C*). However, collagen accumulation was significantly less in the O group than in the S and the N groups at 2 and 4 weeks.

Reorganization of Cytoskeletal Proteins After ONO1301 Treatment

The trend in the expression of α-sarcoglycan in the hearts after ONO1301 treatment was comprehensively assessed by immunohistolabeling, real-time PCR, and Western blotting analysis (Figure 4, *A-C*). α-Sarcoglycan was homogeneously expressed around the cardiomyocytes of the normal hamster, but it was rarely expressed in the heart of the N or S groups on immunoconfocal microscopy. In contrast, α-sarcoglycan expression was greater in the O group than in the N and S groups at 2 weeks, but not at 4 weeks. The mRNA of α-sarcoglycan was significantly greater in the O group than in the S and the N groups at 2 weeks, but not at 4 weeks. Consistently, Western blotting analysis in the heart showed significantly upregulated α-sarcoglycan expression at 2 weeks, but not at 4 weeks in the O group, compared with the S and the N groups. In addition, the trends in expressions of other cytoskeletal proteins, such as β-sarcoglycan and α-dystroglycan, after the ONO1301 treatment were assessed by immunohistolabeling and real-time PCR (Figure 4, *D* and *E*). β-Sarcoglycan was rarely expressed in the J2N-k hamster heart regardless of treatment, whereas expression of α-dystroglycan appeared to be greater in the O group than in the S and the N groups, although there were no significant differences.

Survival Benefit of ONO1301 Treatment in IDCM

No mortality or morbidity related to surgical procedure was identified in any of the groups. Survival of J2N-k hamsters after treatment was then assessed using the Kaplan-Meier method. Hamsters of the N and the S groups showed similar progression to death, primarily owing to congestive cardiac failure over the 4 weeks after treatment. In contrast, survival of hamsters in the O group was significantly prolonged compared with that of the other groups (Figure 5).

DISCUSSION

Summary of the Findings

In the present study, ONO1301 induced secretion of multiple cardiac protective factors such as HGF, VEGF, SDF-1, and G-CSF from NHDFs and HCoASMCs in a dose-dependent manner in vitro. Epicardial implantation of an ONO1301-immersed atelocollagen sheet, which was developed as a slow-releasing drug delivery system, induced heart-dominant elevation of ONO1301 for 2 weeks in

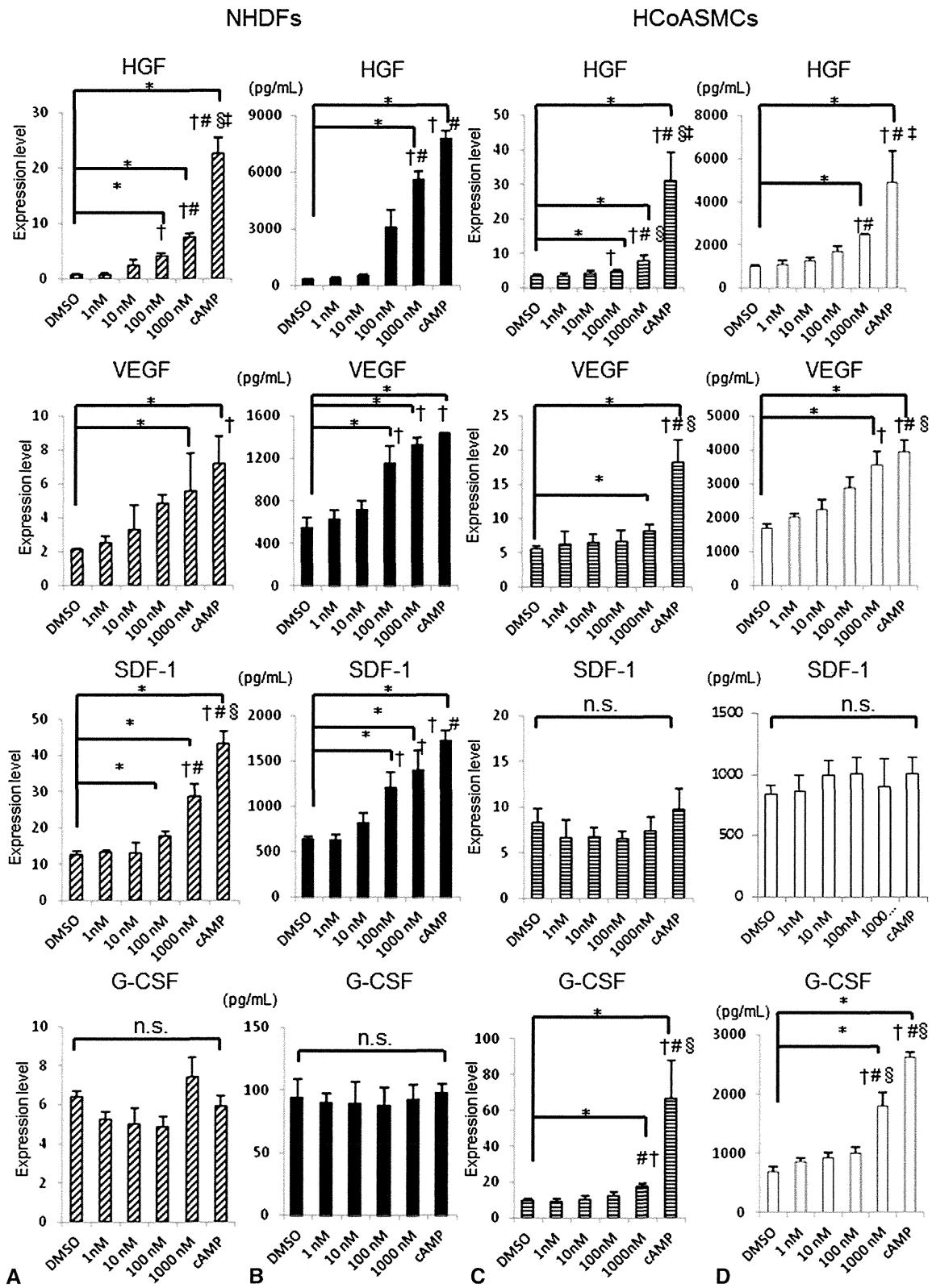

FIGURE 1. Effects of ONO1301 on the production of cardiac protective factors in NHDFs and HCoASMCs were assessed in vitro. ONO1301 treatment significantly upregulates expressions of HGF, VEGF, and SDF-1 in NHDFs, assessed by real-time PCR (A). HGF, VEGF, and SDF-1, but not G-CSF, are significantly secreted into the culture supernatant of NHDF after ONO1301 addition, measured by ELISA (B). ONO1301 addition significantly upregulates expressions of HGF, VEGF, and G-CSF, but not SDF-1, in HCoASMCs, assessed by real-time PCR and ELISA (C and D). *$P < .05$ versus control (DMSO);

the δ-sarcoglycan-deficient DCM hamsters. ONO1301-atelocollagen sheet implantation significantly upregulated expression of HGF, VEGF, increased vasculature, attenuated fibrosis, and upregulated α-sarcoglycan in the myocardium and, consequently, preserved cardiac performance and prolonged survival in this hamster DCM model.

Rationale, Feasibility, and Safety of an Atelocollagen Sheet–Based Therapy for IDCM

This study identified cell-dependent and dose-dependent effects of ONO1301 on the release of cardioprotective factors. The cells that were activated by ONO1301 in vitro included skin fibroblasts and CoASMCs. In addition, IPR, which is the sole receptor of ONO1301, was expressed in CoASMCs and endothelial cells, but not in cardiomyocytes or cardiac fibroblasts. These findings suggest that the target cells of ONO1301 may be the vascular SMCs and endothelial cells in the cardiac tissue. Local delivery of ONO1301 into the heart, directly targeting cardiac SMCs and endothelial cells, would thereby theoretically be useful in maximizing the therapeutic effects of ONO1301. In fact, it was shown that the ONO1301-immersed atelocollagen sheet implantation therapy induced marked heart-dominant elevation of the ONO1301 level in association with significantly positive functional effects, indicating rationale and feasibility of this treatment in the IDCM heart. In addition, both the ONO1301 and the atelocollagen sheet only groups did not produce procedure-related mortality despite the deteriorated cardiac function, suggesting the safety of this treatment for IDCM heart.

There are other possible clinically relevant methods for ONO1301 delivery to treat the DCM heart, such as injection of intramyocardial microbeads, systemic intravenous/subcutaneous injection, or oral intake. However, these methods are theoretically limited by possible local damage and/or poor efficiency in the drug delivery to the cardiac tissue[9] compared with the atelocollagen sheet–based drug delivery system as in this study. Intramyocardial injection of microbeads may also prove to be more efficacious, but further studies will be required to establish the optimal delivery methods of ONO1301 into the heart in preclinical and subsequent clinical studies.

Therapeutic Effects and Underlying Mechanisms of ONO1301 Sheet Therapy

Therapeutic efficiency of this treatment on this δ-sarcoglycan-deficient hamster IDCM model was assured in this study by comparing with the 2 control groups, in which the sham operation or placement of atelocollagen sheet only was performed. Global systolic cardiac function, assessed by echocardiography, was significantly preserved in the ONO1301-treated hamsters compared with the other groups, and most important, the life expectancy of the J2N-k hamsters was prolonged by this treatment. These important positive findings would be explained by multiple fundamental effects of this treatment, including increased myocardial blood flow, reduction of myocardial fibrosis, and reorganization of cytoskeletal proteins.

It has been reported that ONO1301 acts as an inducer of multiple cardioprotective factors in ischemic cardiac diseases.[5] Effects of ONO1301 on the IDCM heart, however, are poorly understood. Although the clinical manifestations of end-stage IDCM are similar to those of end-stage ischemic cardiomyopathy, typical IDCM is characterized by a decreased vascular network, increased fibrous components, and decreased expression of cytoskeletal proteins in a global and homogeneous manner.[10,11] This study also identified multiple endogenous factors upregulated by the ONO1301-atelocollagen sheet, such as HGF, a unique growth factor with antifibrosis and angiogenesis effects,[8,12] or VEGF, an important mediator of angiogenesis.[4] In addition, SDF-1 or G-CSF by the ONO1301 treatment in this study may have contributed to therapeutic stem cell homing and activation.[13,14] Further studies will be required to determine whether these agents induce regenerative responses.

The potential effects of these endogenous factors were well correlated with the pathologic changes in this study, such as increased vasculature, attenuated fibrosis, or upregulated α-sarcoglycan. Of them, increased blood flow may be one of the major mechanisms responsible for the positive therapeutic effects of the ONO1301 in this study. It has been shown that prostacyclin and prostacyclin-inducing HGF/VEGF bring a multiplier effect of vasodilation and new vessel formation responsible to regional ischemic insult.[15,16] In addition, genetic deletion of IPR had an important role on progression of cardiovascular disease.[15]

It is also interesting that cytoskeletal proteins were remodeled by the ONO1301 treatment in this study. Immunohistolabeling in this study demonstrated the transient reexpression of α-sarcoglycan and α-dystroglycan in the O group. It was speculated that α-sarcoglycan can be recycled from the plasma membrane differently from other sarcoglycans,[17] and inhibition of Smad3 associated with transforming growth factor β signal pathway suppressed by prostacyclin or HGF, brings to α-sarcoglycan gene expression.[18]

†$P < .05$ versus ONO1301 (1 nmol/L); #$P < .05$ versus ONO1301 (10 nmol/L); §$P < .05$ versus ONO1301 (100 nmol/L); ‡$P < .05$ versus ONO1301 (1000 nmol/L). *NHDF*, Normal human dermal fibroblast; *HCoASMC*, human coronary artery smooth muscle cell; *HGF*, hepatic growth factor; *VEGF*, vascular endothelial growth factor; *SDF-1*, stromal cell–derived factor-1; *PCR*, polymerase chain reaction; *G-CSF*, granulocyte colony stimulating factor; *ELISA*, enzyme-linked immunosorbent assay; *DMSO*, dimethyl sulphoxide; *cAMP*, cyclic aminophosphatase; *n.s.*, not significant.

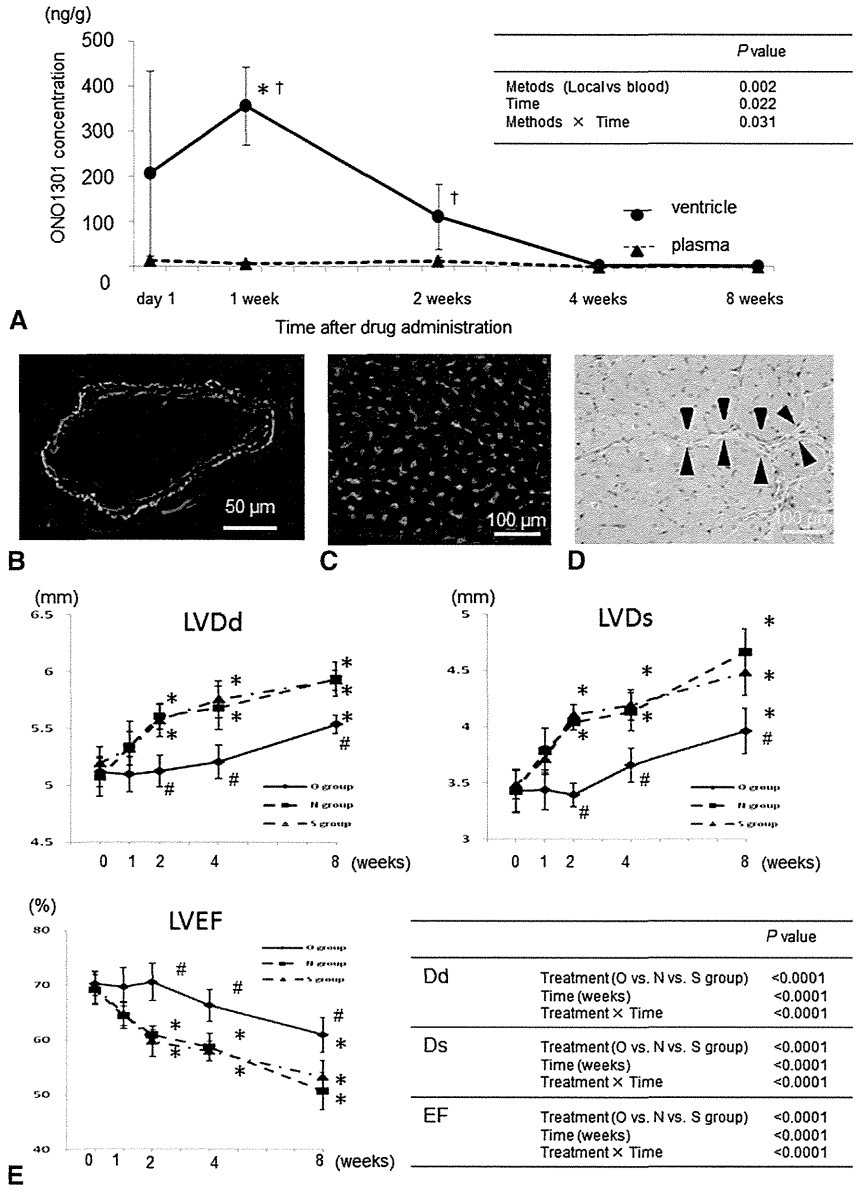

FIGURE 2. Levels of ONO1301 in cardiac tissue and plasma were serially quantified after implantation of ONO1301-eluted atelocollagen sheet for the DCM heart (A). ONO1301 is detected in both samples for 2 weeks after treatment, while the level of ONO1301 in the ventricle is significantly and markedly higher than in the plasma at weeks 1 and 2 after the treatment. *$P < .05$ versus 8 weeks; †$P < .05$ versus plasma concentration Immunofluorescence staining for IPR and alpha-actin in the DCM heart shows that IPR is positive in the vascular smooth muscle cells and the endotherial cells (B and C). *Green*, Filamentous-actin; *red*, IPR; *blue*, nuclei. 3,3′-diaminobenzidine staining, which produces a *brown color*, shows that IPR is expressed in the microvasculature, but not in cardiac fibroblasts and cardiomyocytes (*arrowhead*, cardiac fibroblast) (D). Changes in LVDd/Ds, and LVEF after treatment were serially measured by transthoracic echocardiography (E). These 4 parameters of the LV are preserved until 4 weeks after ONO1301 treatment compared with the other groups. However, ONO1301 treatment does not arrest the progression in dilatation of the dimensions and deterioration of the EF in the subsequent 4 weeks. #$P < .05$ versus N and S group; *$P < .05$ versus 0 weeks. *DCM*, Dilated cardiomyopathy; *LV*, left ventricular (ventricle); *Dd/Ds*, diastolic/systolic dimensions; *EF*, ejection fraction; *IPR*, prostacyclin receptor.

Moreover, regarding the transient reexpression of α-dystroglycan, Kondoh and associates[9] suggested that the reconstruction of α-dystroglycan may occur because the sarcoglycan might mask the matrix metalloproteinase cleavage site on dystroglycan and/or matrix metalloproteinase activity might be inhibited by HGF. In addition, β-sarcoglycan was rarely expressed after the ONO1301 treatment in our study. Kawada and colleagues[19] reported that both β- and δ-sarcoglycan were completely missing, but α- and γ-sarcoglycan were weakly expressed in the J2N-k hamster, and transfer of the δ-sarcoglycan gene could express not only δ- but the other 3 sarcoglycans. These findings might suggest the limitation of this drug therapy for reorganization of cytoskeletal proteins, but Hack and coworkers[20] reported that the

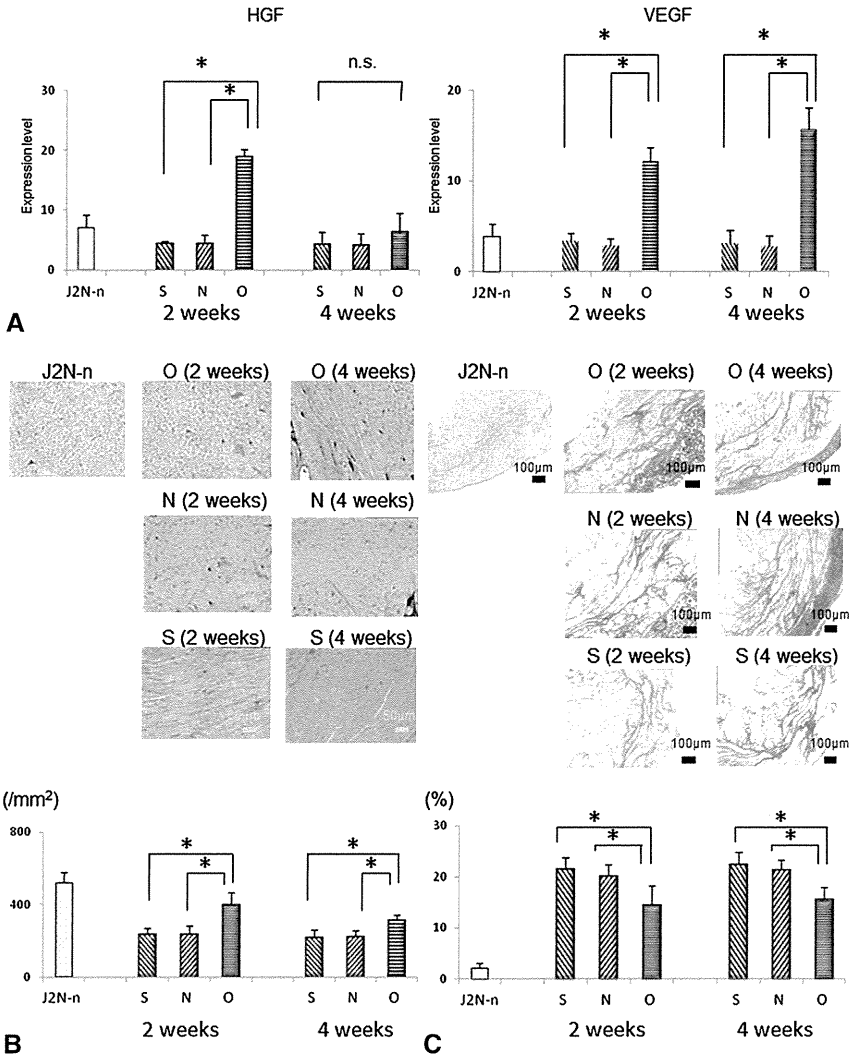

FIGURE 3. Expressions of HGF and VEGF in the cardiac tissue were assessed by real-time PCR, which shows greater expression of the 2 factors in the O group at 2 and 4 weeks compared with the other groups (n = 5 for each group) (A). Capillary density in the hearts was assessed by immunohistolabeling for vWF, which shows a greater number of capillaries in the ONO1301-treated hearts than in the other groups at 2 and 4 weeks (n = 5 for each group) (B). Interstitial fibrosis in the heart was assessed by picrosirius red staining, which shows less accumulation of fibrosis in the ONO1301-treated hearts than in the other groups at 2 and 4 weeks (n = 5 for each group) (C). *$P < .05$ versus O group. *HGF*, Hepatic growth factor; *VEGF*, vascular endothelial growth factor; *PCR*, polymerase chain reaction. *vWF*, von Willebrand factor.

expression of sarcoglycans, even in small amounts, prevented the damage of cardiomyocyte. Reorganization of α-sarcoglycan by the ONO1301 therapy might thus contribute to preserve cardiac function.

Although the level of prostacyclin in the heart in response to the ONO1301 treatment was not investigated in this study, it may be paradoxically elevated by the thromboxane synthase inhibitory activity of the ONO1301 on the heart,[21] possibly producing synergetic positive effects on the IDCM heart. In addition, it is interesting to research the involvement of the neurohormonal activations in the heart, such as adrenergic system, plasma renin activity, or endothelin, by the ONO1301 treatment.[22]

Clinical Perspectives

The atelocollagen sheet–based local ONO1301 delivery therapy globally reversed reduced vascular density, increased fibrosis, and reduced cytoskeletal proteins in the myocardium, all of which were the typical pathologic features in the human IDCM heart,[10,11] suggesting potential therapeutic benefits of this treatment for IDCM in the clinical scenario. In addition, safety of this treatment shown in this study warrants further preclinical study, including dose-response relation to explore minimum and maximal effective dose of the ONO1301 in the "GLP" standard. A very narrow dose necessary to achieve a positive response may prohibit this agent from clinical trials.

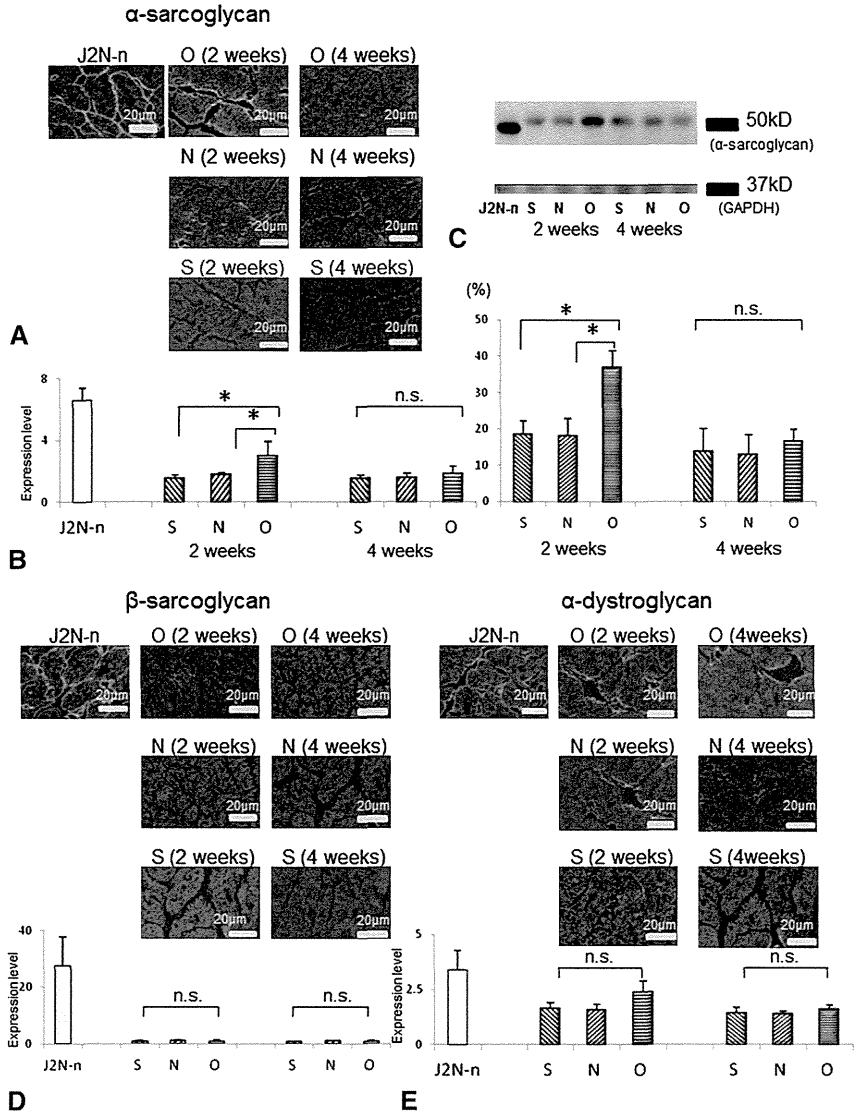

FIGURE 4. Expression of α-sarcoglycan, β-sarcoglycan, and α-dystoglycan in the heart after ONO1301 treatment was assessed by immunohistolabeling, real-time PCR, and Western blot analysis. Immunohistolabeling shows that α-sarcoglycan is clearly expressed around the cardiomyocytes in the normal hamsters, but not in the N or S group (A). Of note, α-sarcoglycan is expressed in the O group at 2 weeks but not at 4 weeks. *Green*, α-Sarcoglycan; *red*, filamentous-actin; *blue*, nuclei. Quantitative real-time PCR shows a significantly greater expression of α-sarcoglycan in the O group than in the N or S group at 2 weeks, but not at 4 weeks (n = 5 for each group at each time point, $*P < .05$ vs O group) (B). Consistently, Western blot analysis and the quantitative results of band intensities, which are expressed as a percentage of the value of the J2N-n hamsters, show significantly greater expression of α-sarcoglycan in the O group than in the N or S group at 2 weeks, but not at 4 weeks (n = 5 for each group at each time point, $*P < .05$ vs O group) (C). Expression of β-sarcoglycan in the DCM hamsters is not detected even after ONO1301 treatment (D). *Green*, β-sarcoglycan; *red*, filamentous-actin; *blue*, nuclei. α-Dystroglycan is rarely expressed in the N or S group, although its expression is upregulated in the O group (E). There are no significant differences between the O group and the other groups at 2 weeks. *Green*, α-Dystroglycan; *red*, filamentous-actin; *blue*, nuclei. *PCR*, Polymerase chain reaction; *n.s.*, not significant.

Re-treatment of epicardial implantation of the sheet containing ONO1301 might be technically challenging; however, technical modulation of microsphere generation, such as gelatin hydrogel, might induce further developments to generate a longer-release drug-delivery system than the method used in the present study.[23]

Study Limitations

This study was limited by use of a transgenic rodent model. The δ-sarcoglycan-deficient IDCM model used in this study is not completely relevant to human IDCM that shows a number of etiologic and pathologic variations. However, positive functional and pathologic effects

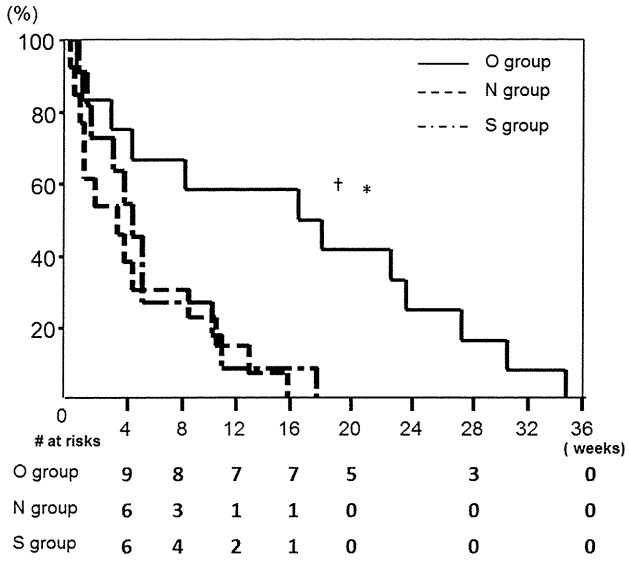

FIGURE 5. Survival after treatment was assessed by the Kaplan-Meier method. There is no significant difference between the N (n = 11) and S groups (n = 13), whereas the O group (n = 12) shows a significantly greater survival than the other groups (*$P < .05$ vs S group; †$P < .05$ vs N group).

associated with upregulated protective factors would be sufficient to prove the principal concept of this treatment. Agents that are beneficial in these mutant hamsters may not be beneficial in humans because the mechanisms responsible for the beneficial effects may be different in humans. However, further pathologic and functional studies for human DCM heart samples, in comparison with the deficient hamster, may be useful to strengthen the findings of this study.

Poor availability of the antibodies and genome sequences in the hamster limited in-depth evaluation of the mechanisms responsible for this treatment, which is warranted to be supplemented by murine IDCM model.[24]

CONCLUSIONS

ONO1301 reorganized cytoskeletal proteins, especially α-sarcoglycan, increased capillaries, and reduced fibrosis through the upregulation of cardiac protective factors, leading to functional recovery and prolonged survival in the δ-sarcoglycan-deficient IDCM hamster. A preclinical study to explore the optimal, clinically relevant protocol is warranted.

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Impact of cardiac support device combined with slow-release prostacyclin agonist in a canine ischemic cardiomyopathy model

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Background: The cardiac support device supports the heart and mechanically reduces left ventricular (LV) diastolic wall stress. Although it has been shown to halt LV remodeling in dilated cardiomyopathy, its therapeutic efficacy is limited by its lack of biological effects. In contrast, the slow-release synthetic prostacyclin agonist ONO-1301 enhances reversal of LV remodeling through biological mechanisms such as angiogenesis and attenuation of fibrosis. We therefore hypothesized that ONO-1301 plus a cardiac support device might be beneficial for the treatment of ischemic cardiomyopathy.

Methods: Twenty-four dogs with induced anterior wall infarction were assigned randomly to 1 of 4 groups at 1 week postinfarction as follows: cardiac support device alone, cardiac support device plus ONO-1301 (hybrid therapy), ONO-1301 alone, or sham control.

Results: At 8 weeks post-infarction, LV wall stress was reduced significantly in the hybrid therapy group compared with the other groups. Myocardial blood flow, measured by positron emission tomography, and vascular density were significantly higher in the hybrid therapy group compared with the cardiac support device alone and sham groups. The hybrid therapy group also showed the least interstitial fibrosis, the greatest recovery of LV systolic and diastolic functions, assessed by multidetector computed tomography and cardiac catheterization, and the lowest plasma N-terminal pro-B-type natriuretic peptide levels ($P < .05$).

Conclusions: The combination of a cardiac support device and the prostacyclin agonist ONO-1301 elicited a greater reversal of LV remodeling than either treatment alone, suggesting the potential of this hybrid therapy for the clinical treatment of ischemia-induced heart failure. (J Thorac Cardiovasc Surg 2014;147:1081-7)

Left ventricular (LV) remodeling in ischemic and nonischemic dilated cardiomyopathy is characterized by progressive dilatation and dysfunction of the left ventricle, leading to severe heart failure.[1,2] The cardiac support device is a mesh net designed to reduce diastolic ventricular wall stress by mechanical means and thus prevent LV dilatation. It has been shown to halt LV remodeling in dilated cardiomyopathy in preclinical studies.[3-5] Clinical trials undertaken on the basis of these favorable results showed beneficial effects on LV remodeling, including significantly decreased LV end-systolic (LVESV) and end-diastolic volumes (LVEDV), and a significant improvement in New York Heart Association functional class.[6-8] However, despite these positive effects, the device has not been associated with reductions in mortality and has not been approved for clinical use.[9]

The synthetic prostacyclin agonist ONO-1301 acts as a myocardial regenerative biological drug to enhance reversal of LV remodeling.[10-12] The beneficial effects of ONO-1301 on the heart are mediated by up-regulation of angiogenic and antifibrotic molecules, such as hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and stromal cell–derived factor-1 (SDF-1).[10-12] This mechanism has been shown to result in the active suppression of ischemic and fibrotic changes in the myocardium.[10-12]

We hypothesized that the biological effects of the slow-release form of the synthetic prostacyclin agonist ONO-1301 might complement the mechanical effects of the cardiac support device, thus enhancing its therapeutic effects in ischemic cardiomyopathy.

MATERIALS AND METHODS

All animals used in this study received care in compliance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85-23, revised 1996).

Animal Treatment

A total of 28 beagles (Oriental Yeast, Co, Ltd, Tokyo, Japan) weighing 9 to 11 kg were used. General anesthesia was administered with intramuscular ketamine (10 mg/kg) and intravenous propofol (5 mg/kg) for induction,

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Abbreviations and Acronyms

ANOVA = analysis of variance
dp/dt = delta pressure/delta time
Ees = end-systolic elastance
HGF = hepatocyte growth factor
LV = left ventricular
LVEDV = left ventricular end-diastolic volume
LVESV = left ventricular end-systolic volume
MDCT = multidetector computed tomography
MI = myocardial infarction
NT-proBNP = amino-terminal pro-brain natriuretic peptide
SDF-1 = stromal cell–derived factor-1
VEGF = vascular endothelial growth factor

and inhaled sevoflurane (1%–2%) for subsequent maintenance, with endotracheal intubation and mechanical ventilator support. After completion of the experiments, the animals were killed under general anesthesia, using an overdose of intravenous sodium pentobarbital (18 mg/kg) to achieve complete sedation, followed by administration of an intravenous potassium-based solution.

Myocardial Infarction Induction

With the animals under general anesthesia, a minimal left thoracotomy was performed through the fifth intercostal space, and the heart was exposed by pericardiotomy. The left descending artery and diagonal vessels were ligated both proximally and distally using 5-0 polypropylene sutures to produce an anterior myocardial infarction (MI). Akinesis of the anterior wall was confirmed by epicardial echocardiography and the chest was closed in layers. The animals were allowed to recover.

Cardiac Support Device

The cardiac support device (0.9–1.0 g), made from polyglycolic acid (Nipro Corporation, Osaka, Japan), was designed on the basis of data obtained from multidetector computed tomography (MDCT) and a heart excised at 1 week postinfarction.

Treatments

The animals were assigned randomly to 1 of 4 groups at 1 week after infarct induction as follows: cardiac support device alone, cardiac support device plus ONO-1301 (hybrid therapy), ONO-1301 alone, or sham control group. In the cardiac support device alone group, 2 sheets of atelocollagen (50 × 50 mm) (Integran; Nippon Zoki Pharmaceutical Co, Ltd, Osaka, Japan) immersed in suspended polylactic and glycolic acid (10 mg/kg) were fixed on the whole surface of the ventricles and the cardiac support device was placed as described previously.[3-5] The same procedure was used in the hybrid therapy group, with the addition of ONO-1301[10-12] (10 mg/kg) (ONO Pharmaceutical Co, Ltd, Osaka, Japan) instead of the polylactic and glycolic acid. In the ONO-1301 alone group, 2 sheets of atelocollagen (50 × 50 mm) immersed in suspended ONO-1301 (10 mg/kg) were fixed on the whole surface of the ventricles. The sham group was subjected to the same procedures as the ONO-1301 alone group, except for the use of polylactic and glycolic acid instead of ONO-1301.

Transthoracic Echocardiography

Transthoracic echocardiography was performed using a 5.0-MHz transducer (Altida; Toshiba Medical Systems Corporation, Tochigi, Japan) for 2-dimensional speckle-tracking echocardiography under general anesthesia. The data were analyzed using 2-dimensional Wall Motion Tracking software (Toshiba Medical Systems Corporation) as previously described.[13]

MDCT

Electrocardiography-gated MDCT was performed using a 16-row MDCT scanner (SOMATOM Emotion 16-Slice Configuration; Siemens, Munich, Germany) during an end-expiratory breath-hold under general anesthesia. MDCT was performed after intravenous injection of 30 mL of nonionic contrast medium (Iomeron; Bracco, Milan, Italy). All images were analyzed on a workstation (AZE VirtualPlace Lexus64; AZE, Tokyo, Japan). LVEDV and LVESV, LV ejection fraction, LV end-diastolic and end-systolic sphericity indices, and LV/right ventricular end-diastolic and end-systolic diameter values were obtained from the workstation.

Cardiac Catheterization

Under general anesthesia, a 3F micromanometer-tipped catheter (SPR-249; Millar Instruments, Houston, Tex) was inserted through the ventricular apex via a left thoracotomy to measure hemodynamic parameters and cardiac functions, including end-systolic pressure and end-diastolic pressure, delta pressure/delta time (dp/dt) maximum, dp/dt minimum, end-systolic elastance (Ees), and the time constant of relaxation in the left and right ventricles. LV volume was altered by occluding the inferior vena cava with tape via a left thoracotomy.

Wall Stress Calculation

LV wall stress was evaluated using specifically developed software (YD, Ltd, Tokyo, Japan) on an off-line personal computer. Global end-systolic and end-diastolic wall stresses were calculated on the basis of the data obtained from MDCT and cardiac catheterization.[14]

Cardiac Positron Emission Tomography

^{13}N-ammonia (200–300 MBq) positron emission tomography (PET) was performed using a HeadtomeV/SET2400W (Shimadzu, Co, Kyoto, Japan) under general anesthesia. Myocardial blood flow was quantitated using PMOD software (version 3.2) (PMOD Technologies, Ltd, Zurich, Switzerland) and divided into 17 segments as recommended by the American Heart Association.

Histologic Analysis

Paraffin-embedded transverse sections of the excised hearts were stained with periodic acid-Schiff to measure the short-axis diameter of the myocytes, and with Masson trichrome to assess the extent of fibrosis. The sections were immunostained with anti-CD31 antibody in LSAB kits (DakoCytomation, Glostrup, Denmark). Myocyte diameters and vascular density were measured in 10 different randomly selected fields using a Biorevo BZ-9000 fluorescence microscope (Keyence, Osaka, Japan), and percentage fibrosis was calculated using MetaMorph software (Molecular Devices, Tokyo, Japan).

Real-Time Polymerase Chain Reaction

Total RNA extracted from cardiac tissue was reverse-transcribed using TaqMan reverse transcription reagents (Applied Biosystems, Foster City, Calif), and assayed using the ABI PRISM 7700 (Applied Biosystems). The average copy number of gene transcripts was normalized to that of glyceraldehyde 3-phosphate dehydrogenase for each sample.

Statistical Analysis

All statistical analyses were performed using JMP software (JMP9; SAS institute, Inc, Cary, NC). Results are presented as the mean ± standard deviation. Cardiac catheterization and histologic data were compared by 1-way analysis of variance (ANOVA). MDCT, echocardiography, wall stress, and amino-terminal pro-brain natriuretic peptide (NT-proBNP)

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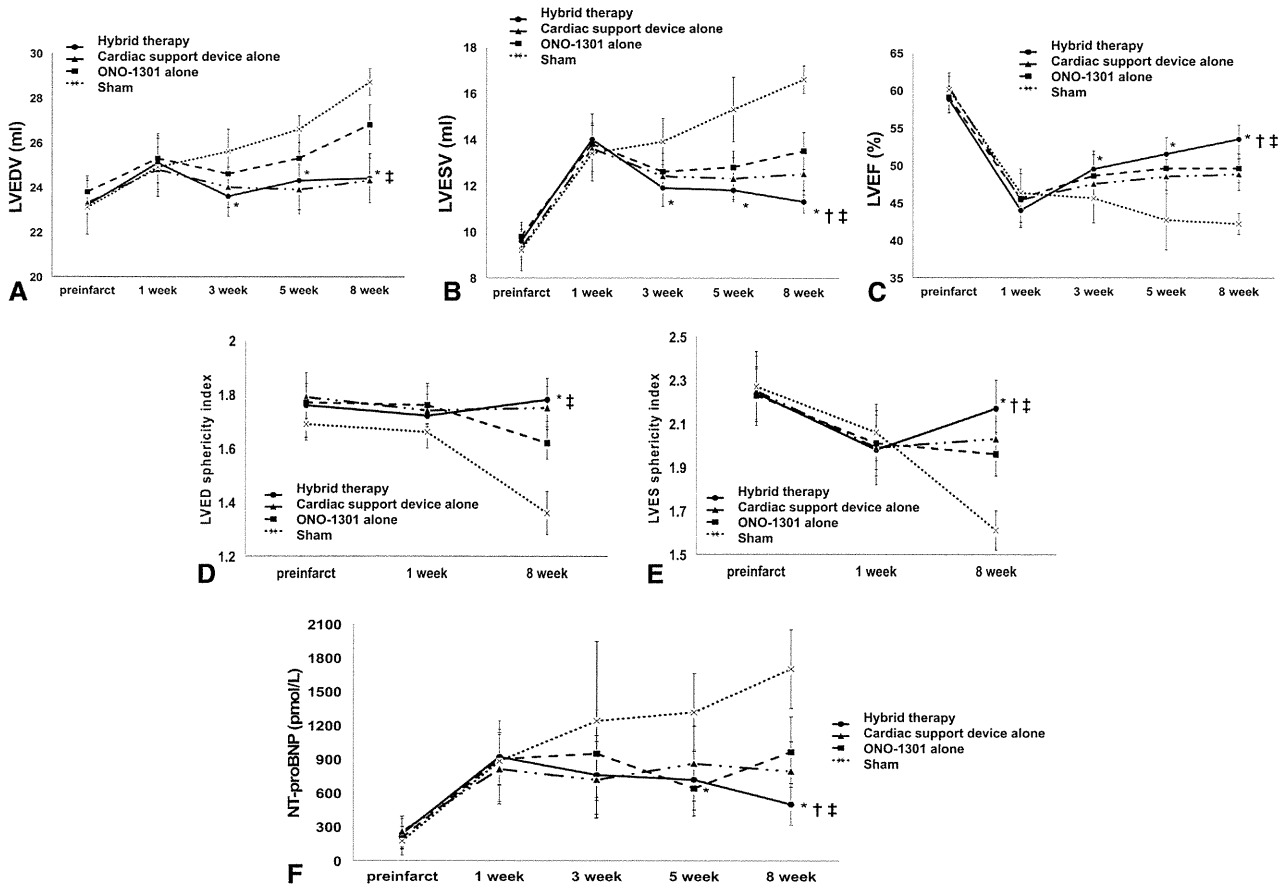

FIGURE 1. MDCT analysis. A, Changes in LVEDV. B, Changes in LVESV. C, Changes in LV ejection fraction. D, Changes in left ventricular end-diastolic and, (E) end-systolic sphericity indices. F, Changes in NT-proBNP. Hybrid therapy is shown by *circles with a solid line*, a cardiac support device alone is shown by *triangles with a dashed/dotted line*, ONO-1301 alone is shown by *squares with a dashed line*, and sham is shown by *crosses with a dotted line*. $*P < .05$ versus corresponding sham, $\dagger P < .05$ versus corresponding cardiac support device alone, $\ddagger P < .05$ versus corresponding ONO-1301 alone. *LV sphericity index*, LV long-axis diameter/LV short-axis diameter. *LVEDV*, Left ventricular end-diastolic volume; *LVESV*, left ventricular end-systolic volume; *NT-proBNP*, amino-terminal pro-brain natriuretic peptide.

data were compared by repeated ANOVA, using values obtained by subtracting the values at 1 week postinfarction from the values at each time point. Significant differences shown by ANOVA were subjected to post hoc analysis with Bonferroni correction. Sample size justification was not performed. A P value less than .05 was considered statistically significant.

RESULTS

Procedure-Related Morbidity and Mortality

Twenty-four animals completed the study. Three of the animals that failed to complete the study died within 1 week postinfarction and the remaining animal, which was a sham control, died at 7 weeks postinfarction. No dogs developed infections or had insufficient MI.

Recovery of Global Cardiac Performance With Hybrid Therapy

Global cardiac performance after the treatment was assessed serially and comprehensively by MDCT and cardiac catheterization. Both LVEDV and LVESV tended to increase after MI induction in the sham group (Figure 1, *A* and *B*). LVEDV was significantly smaller in the hybrid therapy group compared with the sham group at 3 and 5 weeks postinfarction, and significantly smaller than in both the ONO-1301 alone and sham groups at 8 weeks postinfarction. LVESV in the hybrid therapy group was significantly smaller than that in the sham group at 3 and 5 weeks, and was significantly smaller than that in the other groups at 8 weeks. As a result, LV ejection fraction was significantly greater in the hybrid therapy group compared with the sham group at 3 and 5 weeks, and significantly greater than in the other groups at 8 weeks (Figure 1, *C*).

The LV end-diastolic sphericity index was significantly greater in the hybrid therapy group compared with the ONO-1301 alone and sham groups at 8 weeks postinfarction (Figure 1, *D*). The LV end-systolic sphericity index decreased in all groups at 1 week postinfarction, whereas at 8 weeks the LV end-systolic sphericity index had decreased

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TABLE 1. Cardiac catheterization data

	Hybrid therapy	Cardiac support device alone	ONO-1301 alone	Sham
dp/dt maximum (mm Hg/s)				
LV	1822 ± 83*,†,‡	1584 ± 114	1601 ± 91	1238 ± 127
RV	547 ± 101	450 ± 53	539 ± 79	443 ± 86
Ees (mm Hg/mL)				
LV	10 ± 1*,†,‡	7 ± 1	8 ± 1	4 ± 1
RV	3 ± 1	3 ± 1	3 ± 1	2 ± 1
−dp/dt minimum (mm Hg/s)				
LV	1553 ± 61*,†,‡	1303 ± 71	1387 ± 64	1061 ± 107
RV	407 ± 59	378 ± 67	412 ± 88	333 ± 78
Time constant of relaxation (s)				
LV	33 ± 4*,†	42 ± 3	36 ± 3	47 ± 5
RV	39 ± 6	40 ± 2	38 ± 4	46 ± 6

Data are mean ± standard deviation. *RV*, Right ventricular; *LV*, left ventricular. *$P < .05$ versus sham. †$P < .05$ versus cardiac support device alone. ‡$P < .05$ versus ONO-1301 alone.

further in the sham group, remained the same in the cardiac support device alone and ONO-1301 alone groups, and recovered in the hybrid therapy group (Figure 1, *E*).

In addition, systolic function represented by LV dp/dt max and Ees at 8 weeks was greater in the cardiac support device alone and ONO-1301 alone groups compared with the sham group, whereas the hybrid therapy showed significantly greater dp/dt max and Ees than the other groups (Table 1). LV -dp/dt min, which represents diastolic function, also was significantly greater in the hybrid therapy group at 8 weeks than in the other groups. LV time constant of relaxation, which is also an index of diastolic function, was significantly smaller in the hybrid therapy group at 8 weeks postinfarction than in the cardiac support device alone and sham groups. There were no significant differences in any of these parameters in the right ventricle.

MI induction also resulted in an increase in plasma NT-proBNP, assessed by an enzyme-linked immunosorbent assay kit (Cardiopet proBNP; IDEXX Laboratories, Tokyo, Japan), at 1 week postinfarction (Figure 1, *F*). NT-proBNP continued to increase in the sham group, whereas the increase was suppressed in each of the other groups after treatment. NT-proBNP decreased gradually in the hybrid therapy group and was significantly lower than in the sham group at 5 weeks, and was significantly lower than in the other 2 groups at 8 weeks.

Functional Recovery of Infarct Border Area With Hybrid Therapy

Regional LV wall motion was evaluated using speckle-tracking echocardiography to dissect region-specific functional effects of the treatment. The infarct area showed a significant and marked reduction in the radial strain after induction of MI, with no significant differences among the 4 groups (Table 2). Radial strain levels in the border area decreased similarly in all groups at 1 week postinfarction, although at 8 weeks the hybrid therapy group showed the greatest recovery in this area. There was a marked decrease in radial strain in the remote area in the sham group at 8 weeks, but there was little change throughout the study in the other groups.

Reduction in Global End-Systolic/Diastolic Wall Stress With Hybrid Therapy

Changes in global end-systolic/end-diastolic wall stresses after treatment were assessed from MDCT and catheterization data (Table 2). Similar increases in global end-systolic wall stress were observed in all groups at 1 week postinfarction. At 8 weeks postinfarction, however, there was a further increase in the sham group, a slight reduction in the cardiac support device alone group, and almost no change in the ONO-1301 alone group, whereas global end-systolic wall stress was lowest in the hybrid therapy group. Similar increases in global end-diastolic wall stress were observed in all groups at 1 week postinfarction. The sham group showed a marked increase at 8 weeks postinfarction, whereas the hybrid therapy and cardiac support device alone groups showed notable reductions. Global end-diastolic wall stress was significantly lower in the hybrid therapy group compared with the ONO-1301 alone and sham groups at 8 weeks.

ONO-1301 Induced Angiogenic Myocardial Effects in Chronic MI

The angiogenic effects of the treatment were evaluated by assessing global myocardial blood flow at rest by ^{13}N-ammonia PET at 8 weeks postinfarction. Myocardial blood flow in the hybrid therapy group was similar to that in the ONO-1301 group, and both were significantly higher than in the cardiac support device alone and sham groups (Figure 2). Capillary densities in the border and remote areas at 8 weeks postinfarction, which was measured by immunostaining for CD31, was significantly greater in the hybrid therapy group than in the cardiac support device alone and sham groups (Figure 3, *A*).

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TABLE 2. Regional left ventricular wall motion and global left ventricular wall stress

	Hybrid therapy	Cardiac support device alone	ONO-1301 alone	Sham
Radial strain in the MI area (%)				
Pre-infarction	21.4 ± 2.3	20.9 ± 1.0	21.7 ± 2.4	22.4 ± 2.3
1 week post-infarction	7.1 ± 1.0	6.7 ± 0.7	7.5 ± 0.9	7.0 ± 1.0
8 weeks postinfarction	8.7 ± 1.2	7.3 ± 0.4	7.5 ± 1.3	6.7 ± 1.0
Radial strain in the border area (%)				
Pre-infarction	22.2 ± 2.6	21.8 ± 2.5	22.0 ± 1.6	21.3 ± 1.8
1 week postinfarction	10.4 ± 1.9	10.3 ± 1.9	11.2 ± 1.5	11.5 ± 1.9
8 weeks postinfarction	14.7 ± 1.1*,†,‡	10.8 ± 0.2	13.1 ± 1.7	8.1 ± 1.1
Radial strain in the remote area (%)				
Pre-infarction	20.7 ± 2.3	21.6 ± 2.0	21.0 ± 2.8	21.2 ± 2.7
1 week postinfarction	19.2 ± 2.1	20.5 ± 1.2	20.9 ± 2.2	19.6 ± 2.0
8 weeks postinfarction	20.2 ± 1.8*	19.7 ± 1.1	20.1 ± 1.5	14.8 ± 1.4
Global end-systolic wall stress (kdyne/cm^2)				
Pre-infarction	79.9 ± 6.8	84 ± 12.0	80.5 ± 8.1	87.6 ± 9.5
1 week postinfarction	108.1 ± 9.1	104.8 ± 11.9	102.7 ± 11.4	107.5 ± 9.6
8 weeks postinfarction	84 ± 5.7*,†,‡	97.7 ± 11.4	104.6 ± 10.0	161.9 ± 9.3
Global end-systolic wall stress (kdyne/cm^2)				
Pre-infarction	13.0 ± 1.5	11.5 ± 1.1	12.0 ± 1.3	12.0 ± 1.6
1 week postinfarction	17.9 ± 1.5	17.1 ± 1.0	17.0 ± 1.4	16.4 ± 2.5
8 weeks postinfarction	14.0 ± 2.5*,‡	14.0 ± 1.8	18.0 ± 1.5	24.4 ± 3.6

Data are mean ± standard deviation. *MI*, Myocardial infarction. *$P < .05$ versus sham. †$P < .05$ versus cardiac support device alone. ‡$P < .05$ versus ONO-1301 alone.

Histologic Evidence of Reversal of LV Remodeling With Hybrid Therapy

Pathologic cardiomyocyte hypertrophy and interstitial fibrosis in the border and remote areas at 8 weeks postinfarction were assessed by periodic acid-Schiff and Masson trichrome staining, respectively, to evaluate the degree of reversal of LV remodeling induced by each treatment (Figure 3, *B* and *C*). Cardiomyocyte diameters were significantly smaller in the border area in the hybrid therapy group compared with the ONO-1301 alone and sham groups, and were significantly smaller in the remote area compared with the sham group. In addition, there was significantly less interstitial fibrosis in the hybrid therapy group compared with the cardiac support device alone, ONO-1301 alone, and sham groups in the border area, and less than in the sham group in the remote area.

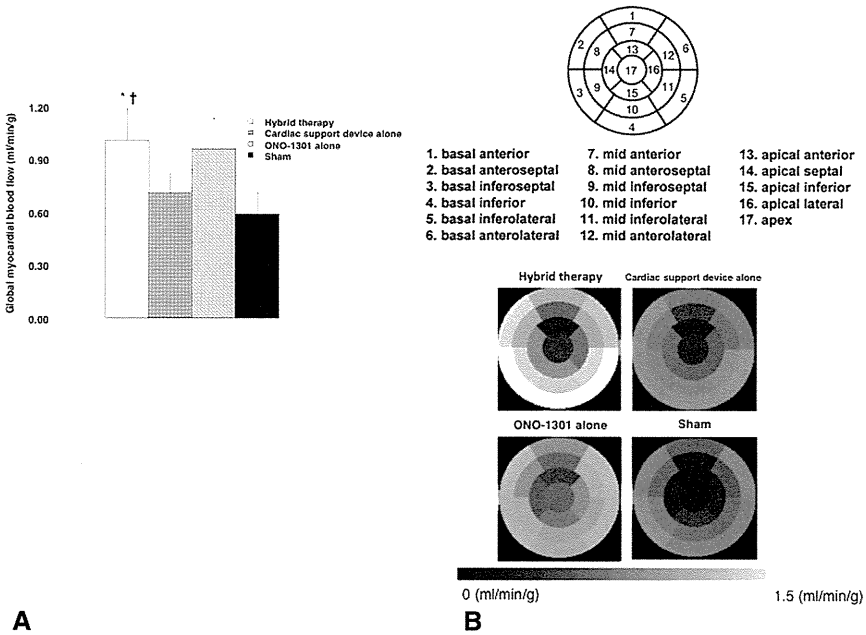

FIGURE 2. A, Global myocardial blood flow assessed by PET at 8 weeks postinfarction. B, Myocardial blood flow divided into 17 segments recommended by the American Heart Association. *$P < .05$ versus sham, †$P < .05$ versus cardiac support device alone.

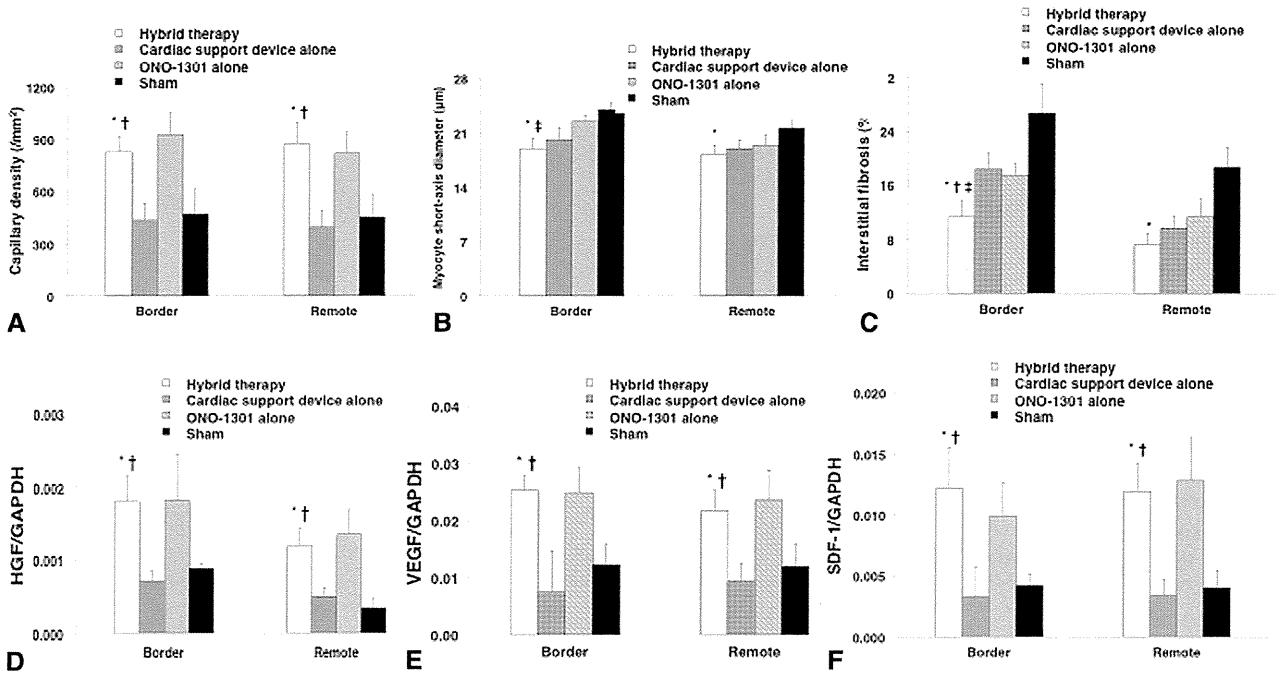

FIGURE 3. Histologic evaluation at 8 weeks postinfarction. A, Capillary density, (B) myocyte short-axis diameter, and (C) interstitial fibrosis in the border and remote areas. Expression levels of (D) HGF, (E) VEGF, and (F) SDF-1 in the border and remote areas quantified by real-time polymerase chain reaction at 8 weeks postinfarction. *$P < .05$ versus sham, †$P < .05$ versus cardiac support device alone. *GAPDH*, Glyceraldehyde 3-phosphate dehydrogenase; *HGF*, hepatocyte growth factor; *VEGF*, vascular endothelial growth factor; *SDF-1*; stromal cell–derived factor-1.

Up-Regulation of Cardiac Protective Factors

Real-time polymerase chain reaction was performed at 8 weeks postinfarction to determine the effects of the treatment on gene expression of major cardiac protective factors, such as HGF, VEGF and SDF-1 (Figure 3, *D-F*). Expression of HGF, VEGF, and SDF-1 in both the border and remote areas were similar in the hybrid therapy and ONO-1301 groups, and significantly higher in these 2 groups than in the cardiac support device alone and sham groups ($P < .05$).

DISCUSSION

This study examined the therapeutic efficacy of hybrid therapy, comprising a cardiac support device and a synthetic prostacyclin agonist (ONO-1301), in a canine model of ischemic cardiomyopathy, compared with the efficacy of either treatment alone. Hybrid therapy significantly improved both systolic and diastolic functions and reduced LV wall stress compared with the other treatments, and histologic examination indicated significantly greater reversal of LV remodeling in the hybrid therapy group. These results were reflected by a significantly greater reduction of NT-proBNP by hybrid therapy.

The cardiac support device used in this study comprised a net made of polyglycolic acid, which is a hydrolytically bioabsorbable polymer. This represents a major difference from the net used in previous studies,[3-5] and was designed to remain around the heart for approximately 10 weeks by adjusting the diameter of the thread. The cardiac support device remained in place at 8 weeks postinfarction, although it had become hydrolyzed to some extent. Our net was functionally equivalent to the nets used in previous studies; it prevented dilatation of the left ventricle, improved the LV sphericity index, and reduced diastolic LV wall stress, thus avoiding the positive feedback loop of cardiac dilatation, the change from an efficient ellipsoidal to a spherical LV chamber, interstitial fibrosis, and, ultimately, heart failure that occurs in ischemic dilated cardiomyopathy.[9] However, one disadvantage of this bioabsorbable net is that it could allow LV remodeling to progress after absorption. The present study did not investigate this aspect and further studies are needed to assess the relative advantages and disadvantages of bioabsorbable and nonabsorbable cardiac support devices.

ONO-1301 is a synthetic prostacyclin agonist that is not yet used in clinical practice. However, several experimental studies have shown its therapeutic efficacy in ischemic and nonischemic cardiomyopathy.[10-12] ONO-1301 was administered to the heart differently in the current study compared with previous studies,[10-12] but its plasma concentrations and reversal of LV remodeling were similar to those seen in previous studies, suggesting that this mode of administration was appropriate. In addition, ONO-1301 administration by incorporation in the cardiac support device could decrease

adverse effects such as hypotension, which may occur with systemic administration. Finally, LV remodeling generally progresses slowly, and long-term drug efficacy therefore is necessary. ONO-1301 has a slow-release time of approximately 4 weeks, and thus may be a suitable agent for the prevention of remodeling.

The favorable results of the current study regarding use of hybrid therapy may be attributed to the angiogenic and active antifibrotic effects of ONO-1301, acting via HGF, VEGF, and SDF-1, which complemented the mechanical effects of the cardiac support device with a consequent enhancement of therapeutic efficacy. Up-regulation of these cytokines and increased capillary density were observed in the hybrid therapy group, whereas PET examination showed significantly greater myocardial blood flow in the hybrid therapy group compared with the cardiac support device alone and sham groups. The additional benefits of ONO-1301 resulted in enhanced recovery of radial wall strain and the suppression of interstitial fibrosis in the border area in the hybrid therapy group, with consequent recovery of cardiac function.

This study was limited by the use of a canine model, which may not completely reflect clinical ischemic cardiomyopathy pathologies. In this experiment, there was no atherosclerosis, and no use of drugs such as β-blockers and angiotensin-converting enzyme inhibitors, which might be used in the clinical arena. However, a similar canine ischemic cardiomyopathy model has been established previously,[2,12] and it is possible to use this model to assess cardiac function and evaluate the therapeutic effects of interventions. Our model therefore was deemed adequate to show the therapeutic effects of the hybrid therapy with various modalities used in the clinical arena. However, this model may not be suitable for further studies of the mechanisms of hybrid therapy, and rodent models may be better suited for such investigations. This study also was limited in that it was not clear whether remodeling would remain suppressed even after complete absorption of the cardiac support net because the net remained at the end of this study. Therefore, longer-term studies lasting after absorption of the biodegradable net will be necessary.

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