A slow-releasing form of prostacyclin agonist (ONO1301SR) enhances endogenous secretion of multiple cardiotherapeutic cytokines and improves cardiac function in a rapid-pacing–induced model of canine heart failure

Tomonori Shirasaka, MD,^a Shigeru Miyagawa, MD, PhD,^b Satsuki Fukushima, MD, PhD,^b Atsuhiko Saito, PhD,^b Motoko Shiozaki, PhD,^b Naomasa Kawaguchi, PhD,^c Nariaki Matsuura, MD, PhD,^c Satoshi Nakatani, MD, PhD,^d Yoshiki Sakai, PhD,^e Takashi Daimon, PhD,^f Yutaka Okita, MD, PhD,^a and Yoshiki Sawa, MD, PhD^b

Objectives: Cardiac functional deterioration in dilated cardiomyopathy (DCM) is known to be reversed by intramyocardial up-regulation of multiple cardioprotective factors, whereas a prostacyclin analog, ONO1301, has been shown to paracrinally activate interstitial cells to release a variety of protective factors. We here hypothesized that intramyocardial delivery of a slow-releasing form of ONO1301 (ONO1301SR) might activate regional myocardium to up-regulate cardiotherapeutic factors, leading to regional and global functional recovery in DCM.

Methods and Results: ONO1301 elevated messenger RNA and protein level of hepatocyte growth factor, vascular endothelial growth factor, and stromal-derived factor-1 of normal human dermal fibroblasts in a dose-dependent manner in vitro. Intramyocardial delivery of ONO1301SR, which is ONO1301 mixed with polylactic and glycolic acid polymer (PLGA), but not that of PLGA only, yielded significant global functional recovery in a canine rapid pacing–induced DCM model, assessed by echocardiography and cardiac catheterization (n = 5 each). Importantly, speckle-tracking echocardiography unveiled significant regional functional recovery in the ONO1301-delivered territory, consistent to significantly increased vascular density, reduced interstitial collagen accumulation, attenuated myocyte hypertrophy, and reversed mitochondrial structure in the corresponding area.

Conclusions: Intramyocardial delivery of ONO1301SR, which is a PLGA-coated slow-releasing form of ONO1301, up-regulated multiple cardiotherapeutic factors in the injected territory, leading to region-specific reverse left ventricular remodeling and consequently a global functional recovery in a rapid-pacing–induced canine DCM model, warranting a further preclinical study to optimize this novel drug-delivery system to treat DCM. (J Thorac Cardiovasc Surg 2013;146:413–21)

Dilated cardiomyopathy (DCM) is characterized by progressive and severe deterioration of cardiac function, eventually leading to advanced heart failure necessitating surgical interventions such as cardiac transplantation¹ or mechanical assist device implantation,² despite maximum currently available medical therapy including angiotensin-converting enzyme inhibitor³ or beta-blocker.⁴ Despite a variety of etiologies in DCM, the diseases consistently include pathologic

hypertrophy of cardiomyocytes associated with mitochondrial dysfunction, increased interstitial fibrosis, and limited regional blood flow.^{5–7} Pathologic left ventricular (LV) remodeling is reportedly reversed, at least in part, by cell transplantation that intramyocardially up-regulates multiple cardiotherapeutic cytokines in a constitutive manner.^{8,9} However, cell therapy is limited in the clinical arena owing to availability of cell processing center or ethical issues. Therefore, synthetic reagents that yield similar cardiotherapeutic effects to cell transplantation have been sought.

Prostacyclin is an endogenous factor released by endothelial cells, activating endothelial cells, fibroblasts, or smooth muscle cells in an autocrine and paracrine manner to release multiple growth factors or cytokines, consequently producing local and systemic anti-inflammatory, antifibrotic, proangiogenic, and antithrombotic effects. However, clinical use of synthetic prostacyclin or prostacyclin analogs, such as epoprostenol and beraprost, for chronic diseases is hampered by its chemical instability^{10,11} and therefore the delivery method.

ONO1301 is a synthetic prostacyclin analog having a unique structural feature to maintain chemical stability,

From the Division of Cardiovascular Surgery,^a Kobe University Graduate School of Medicine, Kobe; the Division of Cardiovascular Surgery,^b Osaka University Graduate School of Medicine, Suita; the Department of Molecular Pathology,^c Graduate School of Medicine and Health Sciences, Division of Functional Diagnostics,^d Department of Health Sciences, Osaka University Graduate School of Medicine, Osaka; Ono Pharmaceutical Company Ltd,^e Osaka; and the Division of Biostatistics,^f Hyogo College of Medicine, Hyogo, Japan.

Disclosures: Yoshiki Sakai is an employee of ONO pharmaceutical Co Ltd. All other authors have nothing to disclose with regard to commercial support.

Received for publication July 27, 2012; revisions received Sept 8, 2012; accepted for publication Oct 2, 2012; available ahead of print April 1, 2013.

Address for reprints: Yoshiki Sawa, MD, PhD, Osaka University Graduate School of Medicine, Suita, Japan, E1: 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan (E-mail: sawa@surg1.med.osaka-u.ac.jp).

0022-5223/\$36.00

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<http://dx.doi.org/10.1016/j.jtcvs.2012.10.003>

Abbreviations and Acronyms

DCM	= dilated cardiomyopathy
Dd	= end-diastolic left ventricular dimension
Ds	= end-systolic left ventricular dimension
E	= early transmitral filling wave
E'	= early diastolic velocity of the mitral annulus
EF	= ejection fraction
EDWT	= end-diastolic wall thickness
ELISA	= enzyme-linked immunosorbent assay
ESWT	= end-systolic wall thickness
HGF	= hepatocyte growth factor
LV	= left ventricular (ventricle)
mRNA	= messenger RNA
ONO1301SR	= slow releasing form of ONO1301
PCR	= polymerase chain reaction
PLGA	= polylactic and glycolic acid polymer
SDF-1	= stromal-derived factor-1
VEGF	= vascular endothelial growth factor

possibly allowing slow-releasing system.¹² Of note, ONO1301 reportedly activates fibroblasts to release multiple factors such as hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF),¹³ both of which are known to be cardiotherapeutic.^{14,15} Nakamura and associates¹³ reported that direct intramyocardial injection of ONO1301 yielded cardiotherapeutic effects in a model of acute myocardial infarction in the mouse. On the other hand, Hirata and colleagues¹⁶ reported that subcutaneous injection of ONO1301 improves global cardiac function associated with globally reduced fibrosis and increased capillaries in a hamster DCM model. However, it remains unclear that intramyocardial delivery of ONO1301 would produce therapeutic effects on a model of DCM heart failure in a large animal.

We therefore hypothesized that intramyocardial injection of ONO1301 might activate regional interstitial cells including fibroblasts in the injected area to locally up-regulate multiple therapeutic factors, leading to region-specific functional recovery in DCM. Thus, we investigated therapeutic effects of local administration of a slow-releasing form of ONO1301 on regional cardiac function of DCM heart by using the canine rapid-pacing induction that is an established DCM model.^{17,18}

METHODS**Animal Care**

All studies were performed with the approval of the institutional ethics committee in Osaka University Graduate School of Medicine. All

animals were treated in compliance with the “Principles of Laboratory Animal Care” (the National Society for Medical Research) and the “Guide for the Care and Use of Laboratory Animals” (National Institutes of Health publication). Human dermal fibroblasts were treated in compliance with the principles outlined in the Declaration of Helsinki. All procedures and analysis were carried out in a blinded manner. We had full access to and take full responsibility for the integrity of data and agree to the manuscript as written.

Culture of Human Dermal Fibroblasts With ONO1301 Added

Human dermal fibroblast cell line (NHDF; CryoNHDF Neo, Lonza, Switzerland) was cultured in fine bubble mixing culture (FGM-2 Bulletkit, Lonza) containing 2% fetal bovine serum. ONO1301 (0.1–1.0 $\mu\text{mol/mL}$) was added for 72 hours after serum-free culture for 24 hours.

Generation of a Slow-Releasing Form of ONO1301

A slow releasing form of ONO1301 (ONO1301SR; Ono Pharmaceutical Co Ltd, Osaka, Japan) was created by polymerization of ONO1301 with polylactic and glycolic acid polymer (PLGA) as described previously.¹⁹ In brief, ONO1301 (5 mg) was mixed with 100 mg of PLGA in 0.1% polyvinyl alcohol with an equal molar ratio of lactic acid/glycolic acid. Releasing time of ONO1301SR in vitro was between about 14 days to 25 days, as determined by measuring residual ONO1301 in the pellets by liquid chromatography.

Generation of Canine DCM Model and Intramyocardial ONO1301SR Injection

Beagles weighing 10 kg (Oriental Yeast Co Ltd, Tokyo, Japan) were endotracheally intubated and supported by mechanical ventilation under general anesthesia using intravenous sodium pentobarbital (6 mg/kg) for induction and inhaled sevoflurane (1%–2%) for subsequent maintenance. We maintained the adequacy of anesthesia evaluated by giving the dogs electrical stimuli every 30 minutes. This evaluation was performed during each operation for each procedure. The heart was exposed via the left fifth intercostal space, and 2 bipolar pacing leads (FINELINE II EZ STEROX; Boston Scientific, Boston, Mass) were attached on the free wall of the right ventricle, connected to a pulse generator (INSIGNIA I, Boston Scientific) placed in subcutaneous pocket. The ventricle was continuously paced at 240 beats/min for 8 weeks.¹⁸

Four weeks after rapid pacing commenced, either ONO1301SR or PLGA polymer only was injected with a 26-gauge needle at 5 points of lateral wall of the LV at regular intervals (total 15 mg of ONO1301 or PLGA polymer was injected, ONO1301SR group and control group, $n = 5$ each). Rapid pacing was temporally discontinued during the injection procedure, and it was set back at 240 beats/min the day after each operation. Dogs kept on rapid pacing for 8 weeks were humanely killed under general anesthesia with an overdose of intravenous sodium pentobarbital (18 mg/kg) to achieve complete sedation followed by administration of potassium-based solution intravenously to assure that they were completely dead. The hearts were retrieved at 4 weeks after injection of either ONO1301SR or PLGA only. We here defined lateral LV wall where ONO1301SR was directly injected as the “target site” and the septal wall as the “remote site.”

Conventional and Speckle-Tracking Echocardiography and Cardiac Catheterization

Transthoracic echocardiography (Altida; Toshiba Medical Systems Corporation, Tochigi, Japan) was performed under general anesthesia by 1% sevoflurane inhalation. End-diastolic and end-systolic LV dimensions (Dd and Ds, respectively) and end-systolic and end-diastolic wall thickness (ESWT and EDWT, respectively) of the target site and remote site were measured at mid-LV short axis view by conventional echocardiography. LV ejection fraction (EF) was calculated with biplanar Simpson’s rule

from the apical 4-chamber view. E/E' , an indicator of diastolic function, was calculated by measuring peak Doppler velocities of early transmitral filling wave (E) and the peak early diastolic velocity of the mitral annulus (E').

Speckle-tracking echocardiography and an offline software (Altida Extend; Toshiba Medical Systems Corporation) were used to measure radial, circumferential, transverse, and longitudinal strains to quantitatively assess regional LV wall motion.²⁰ Radial and circumferential strains were measured from the mid-LV short-axis view, whereas transverse and longitudinal strains were from the apical 4-chamber view.

Cardiac catheterization was performed under general anesthesia using 1% sevoflurane inhalation. A 3F micromanometer-tipped catheter (SPR-249; Millar Instruments, Inc, Houston, Tex) was inserted through the LV apex to measure heart rate, LV maximal systolic pressure, maximal rate of the LV pressure change evaluating systolic preload-dependent LV function, and time constant of LV relaxation (τ) evaluating diastolic load-dependent function.

Real-Time Polymerase Chain Reaction

Total RNA was retrieved from NHDF by using RNeasy Mini kit (Qiagen, Venlo, The Netherlands) and treated with RNase-Free DNase Set (Qiagen). TaqMan probes were designed using Primer Express software (Applied Biosystems, Carlsbad, Calif). Real-time polymerase chain reaction (PCR) was performed using a 7500 Fast Real-Time PCR System with TaqMan Universal PCR Master Mix (Applied Biosystems). Concentration of HGF, VEGF, and stromal-derived factor-1 (SDF-1) in the culture supernatant of NHDF was measured by using an enzyme-linked immunosorbent assay (ELISA) kit (Procarta Cytokine Assay kit, Panomics, Santa Clara, Calif).

Histologic Analysis and Electron Microscopy

The extracted dog hearts were transversely cut, fixed with 10% buffered formalin, and embedded in paraffin. The heart sections of 10- μ m thickness were stained with hematoxylin and eosin, Masson-trichrome, picro-sirius red, and periodic acid-Schiff. The heart sections were also labeled by anti-von Willebrand factor antibody (Dako EPOS) visualized by horseradish peroxidase (DakoCytomation, Glostrup, Denmark). Fibrotic area was calculated in the picro-sirius red-stained sections by using a planimetric method with a morphometry analyzer (NIS elements D, Nikon, Japan) on 5 optical fields that were selected randomly for each sample. Extracted dog heart tissues were fixed with 2.5% glutaraldehyde, stained with uranyl acetate and lead citrate, and examined with a Hitachi H-7100 electron microscope (Hitachi High-Technologies, Tokyo, Japan).

Statistical Analysis

All data are presented as the mean $\pm$ standard error of the mean. The analyses were performed using nonparametric methods because the sample sizes were too small to allow checking of the assumptions of parametric methods. Expression of messenger RNA (mRNA) in vitro analyzed by PCR and ELISA was analyzed by Jonckheere-Terpstra test for assuring dose-dependent effect of ONO1301. Hemodynamic data obtained from conventional echocardiography, cardiac catheterization, and speckle-tracking echocardiography, as well as histopathologic findings such as percent fibrosis, cell diameter, and vascular density at the target and remote sites of control group or ONO1301SR group, were analyzed by nonparametric repeated-measures analysis. Statistical analyses were performed with the R program (R Development Core Team 2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing; Vienna, Austria).

RESULTS

Effects of ONO1301 on Expression of Endogenous Cytokines In Vitro

Effects of ONO1301 on expression of HGF, VEGF, and SDF-1 in the NHDF in vitro were examined by real-time

PCR and ELISA. Relative expression of mRNA for HGF, VEGF, and SDF-1 was up-regulated in the NHDF with ONO1301 added in a dose-dependent manner (Figure 1, A-C), which was consistent with the release of HGF, VEGF, and SDF-1 into the supernatants (Figure 1, D-F).

Global Recovery of the DCM Heart by Injection of ONO1301SR

Serial changes in global systolic and diastolic cardiac function were assessed under general anesthesia by conventional echocardiography at 3 time points: 0 weeks (before commencement of rapid pacing), 4 weeks after the commencement of rapid pacing (just before injection of either ONO1301SR or PLGA only), and 4 weeks after injection of either ONO1301SR or PLGA only. Cardiac performance was markedly deteriorated, including increased Dd/Ds and E/E' and decreased EF, ESWT, and EDWT at 4 weeks, when either ONO1301MS or PLGA only was intramyocardially injected.

At 4 weeks after PLGA injection, both systolic and diastolic cardiac functions had further deteriorated.

On the other hand, EF and ESWT/EDWT at both the target site and remote site at 4 weeks after ONO1301SR injection were significantly greater than those at 4 weeks after PLGA injection (EF, $39\% \pm 1.7\%$ vs $19\% \pm 2.0\%$; $P < .05$; Figure 2, E; ESWT/EDWT at target site, $1.3 \pm 3.0 \times 10^{-2}$ vs $1.1 \pm 2.0 \times 10^{-2}$; $P = .01$; Figure 2, C; ESWT/EDWT at remote site, 1.2 ± 0.1 vs $1.1 \pm 3.0 \times 10^{-2}$; $P = .04$; Figure 2, D), although the impact of the recovery was stronger in the target site. Ds was significantly smaller after ONO1301SR injection than after PLGA injection (Ds, 23 ± 2.4 vs 31 ± 1.7 mm; $P < .05$; Figure 2, B), whereas Dd also showed a trend to be smaller after ONO1301SR injection than after PLGA injection (Dd, 29 ± 2.3 vs 34 ± 1.4 ; $P < .05$; Figure 2, A). E/E' after ONO1301SR injection was significantly smaller than that after PLGA injection (E/E' , 11 ± 1.2 vs 16 ± 0.5 ; $P < .05$; Figure 2, F).

Cardiac catheterization, carried out at 4 weeks after either ONO1301SR or PLGA injection, revealed that τ was significantly smaller after ONO1301SR injection than after PLGA injection (τ , 32 ± 0.9 vs 55 ± 5.8 ; $P < .05$). Heart rate, LV maximal systolic pressure, and maximal rate of the LV pressure change did not show any significant difference at 4 weeks after injection of either ONO1301SR or PLGA.

Regional Functional Recovery After ONO1301SR Injection

Serial changes of regional systolic cardiac function were assessed under general anesthesia by speckle-tracking echocardiography at the same 3 time points as conventional echocardiography. At 4 weeks after the commencement of rapid pacing, all strain values at both target and remote sites were decreased compared with those

FIGURE 1. PCR and ELISA analysis in vitro showed that messenger RNA levels for HGF (A and D), VEGF (B and E), and SDF-1 (C and F) increased in a dose-dependent manner in NHDF cultured with ONO1301. *PCR*, Polymerase chain reaction; *ELISA*, enzyme-linked immunosorbent assay; *NHDF*, normal human dermal fibroblast; *HGF*, hepatocyte growth factor; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *SDF-1*, stromal-derived factor-1; *VEGF*, vascular endothelial growth factor. Mean $\pm$ standard error of the mean, respectively.

before rapid pacing. At 4 weeks after the PLGA injection, the absolute values of peak systolic radial, circumferential, transverse, and longitudinal strains at both target and remote sites further decreased compared with those before the PLGA injection. In contrast, at 4 weeks after the ONO1301SR injection, strain values of radial, transverse, and circumferential strains were greater at the target site than those after the PLGA injection (radial strain, $36\% \pm 4.7\%$ vs $8.3\% \pm 1.4\%$; $P < .05$; transverse strain, $39\% \pm 9.3\%$ vs $9.5\% \pm 2.1\%$; $P < .05$; circumferential strain, $-11\% \pm 1.3\%$ vs $-3.9\% \pm 0.6\%$; $P < .05$; Figure 3, A-C), although longitudinal strain was not different between the hearts with and without ONO1301SR treatment (Figure 3, D). On the other hand, only radial strain was significantly improved at the remote site after ONO1301SR injection compared with that after PLGA injection (Figure 3, E-H).

Histologic Findings of Reverse LV Remodeling After ONO1301SR Injection

Gross myocardial structure, assessed by hematoxylin and eosin staining and Masson trichrome staining, showed a thicker LV wall and a smaller LV cavity 4 weeks after ONO1301SR administration (Figure 4, H-K) than that after PLGA injection (Figure 4, D-G).

Quantity of interstitial fibrosis at the target site, evaluated by picro-sirius red staining, was significantly less at 4 weeks after ONO1301SR administration compared with that after PLGA injection (percent fibrosis at the target site, $9.9\% \pm 0.7\%$ vs $23\% \pm 0.9\%$; $P < .01$; Figure 4, A). Of note, distribution of interstitial fibrosis was significantly more restricted at the target site than that at the remote site after ONO1301SR administration ($9.9\% \pm 0.7\%$ vs $16\% \pm 1.2\%$), whereas PLGA injection did not produce such an uneven distribution ($23\% \pm 0.9\%$ at the target site vs $23\% \pm 0.8\%$ at the remote site).

Mean transverse cellular diameter of cardiomyocytes (Figure 4, B) at the target site, measured by periodic acid-Schiff-stained sections, was also significantly smaller at 4 weeks after ONO1301SR administration compared with that after PLGA injection (12 ± 0.6 mm vs 15 ± 0.8 mm; $P < .01$). The diameter of cardiomyocytes at the target site was smaller after ONO1301SR administration compared with that at the remote site (12 ± 0.6 vs 14 ± 0.3 mm; $P < .01$), whereas such an uneven distribution in the myocyte size was not observed after PLGA injection.

Vascular density (Figure 4, C), assessed by counting the number of factor VIII-positive cells in the fields, was significantly greater at the target site at 4 weeks after ONO1301SR administration compared with that after

FIGURE 2. Echocardiography (A-F) showed ONO1301SR significantly improved distressed cardiac function. Note that ESWT/EDWT, reflecting on the radial strain of myocardium, was significantly more recovered in the ONO1301SR group than in the control group and that the clinical impact was more prominent in the target site after ONO1301SR treatment. *Dd*, End-diastolic dimension of the left ventricle; *Ds*, end-systolic dimension of the left ventricle; *EF*, ejection; *ESWT*, end-systolic wall thickness; *EDWT*, end-diastolic wall thickness. Mean $\pm$ standard error of the mean, respectively. *Ind*, induction of rapid pacing; *Pre*, before treatment of polylactic and glycolic acid polymer (PLGA) or ONO1301SR; *Post*, after treatment of PLGA or ONO1301SR.

PLGA injection ($998 \pm 70/\text{mm}^2$ vs $467 \pm 33/\text{mm}^2$; $P < .01$). The vascular density at the target site was greater after ONO1301SR administration compared with that at the remote site ($998 \pm 70/\text{mm}^2$ vs $491 \pm 24/\text{mm}^2$), whereas such an uneven distribution of vascular density was not observed after PLGA injection.

Electron microscopy revealed that the cardiomyocytes at 4 weeks after PLGA injection showed a prominent swelling or disruption of mitochondria, intracellular or perinuclear edema, and sarcoplasmic vacuoles resulting from dilation of sarcoplasmic reticulum (Figure 5, A). However, marked loss of myofilaments and alterations of characteristic sarcomeric structure were not observed in any groups. Although the interfibrillar space in the myocardium after ONO1301SR injection was slightly widened, the mitochondria were compact and showed densely packed cristae (Figure 5, B) compared with those after PLGA injection.

DISCUSSION

We here demonstrate that ONO1301 dose-dependently up-regulated expression of multiple cytokines, such as HGF, VEGF, and SDF-1, in fibroblasts in vitro. Histologic reverse LV remodeling, such as attenuated fibrosis and swelling of cardiomyocytes, increased vascular density, and recovered mitochondrial structure, in the target area but not significantly in the remote area, were consistent to the regional functional recovery, assessed by speckle-tracking echocardiography, which was more prominent at the target area than that at the remote area after the ONO1301SR injection. Such regional recovery at the target area after ONO1301SR injection resulted in recovery of global function, including systolic and diastolic function.

Iwata and associates²¹ reported that local administration of prostacyclin analog may induce HGF production followed by VEGF expression via cyclic adenosine monophosphate-mediated pathway and that elevation of HGF

Speckle tracking echocardiography

(upper; target site, lower; remote site)

FIGURE 3. Speckle-tracking echocardiography showed that the absolute values of peak radial, circumferential, and transverse strains at the target site in the ONO1301SR group were significantly higher than in the control group (A-D) while all of them but radial strains at the remote site in the ONO1301SR group and that in the control group were not significantly different (E-H), which implied that ONO1301SR had influence on the cardiac performance especially at the very site where ONO1301SR was administered; *Radial*, radial strain; *Circumferential*, circumferential strain; *Transverse*, transverse strain; *Longitudinal*, longitudinal strain. Target site is defined as the area in which ONO1301SR or glycolic acid polymer (PLGA) is injected while remote site as noninjection area. *Ind.*, Induction of rapid pacing; *Pre.*, before treatment of PLGA or ONO1301SR; *Post.*, after treatment of PLGA or ONO1301SR. Mean $\pm$ standard error of the mean, respectively.

or VEGF may mediate the favorable effect in the treatment of ischemic heart failure. We here showed that ONO1301 directly activates fibroblasts in vitro and releases not only HGF and VEGF, as reported previously,^{13,16,21} but also SDF-1, which has been thought to be a representative therapeutic stem cell homing factor in ischemic heart.²² In the present in vivo study, we used the slow-releasing form to deliver ONO1301 and, importantly, deliver ONO1301SR directly into the myocardium of the canine DCM heart in the aim to elevate regionally ONO1301 level, thus maximizing the effects on the cardiac fibroblasts to release cardiotherapeutic factors. Consequently, pathologic and functional effects of intramyocardial ONO1301SR injection were markedly prominent in the target area (area surrounding the injection sites) compared with the remote area, suggesting that cardiac fibroblasts residing in the target area might have played a key role in locally up-regulated cardiotherapeutic cytokines.

In addition, it was noted that the typical structural features of cardiomyocytes in the severely ischemic heart,

such as swelling of mitochondria, intracellular or perinuclear edema, and sarcoplasmic vacuoles referred to by a phenomenon, “permeability transition,”²³ were reversed after ONO1301SR injection in this study. On the basis of these findings, targeted injection of ONO1301SR into the damaged myocardial area might maximize therapeutic effects of ONO1301 that up-regulates cardiotherapeutic cytokines in a regional concentration-dependent manner.

Use of slow releasing form in administrating ONO1301 directly into the heart includes concerns related to the initial burst that might have an adverse effect on hemodynamics.²⁴ In this study, there is no hemodynamic compromise during or immediately after the procedure despite the poor cardiac function, suggesting that the protocol used here in injecting ONO1301SR might be appropriate in treating the DCM heart. Further study for dose-dependent hemodynamic change immediately after ONO1301SR administration would be needed in translating this treatment into the clinical arena.

Intramyocardial delivery of ONO1301 might be achieved by direct injection, intracoronary artery injection,

FIGURE 4. Histopathology; quantitative evaluation of interstitial fibrosis (A), mean cell diameter (B), vascular density (C), and representative micrograph of the Control group (D-G) and the ONO1301SR group (H-K). D and H, Hematoxylin and eosin staining. E and I, Masson trichrome staining. F and J, Sirius red staining. G and K, Staining with anti-human-von Willebrand factor. At the target site in the ONO1301SR group, the amounts of fibrosis and mean cell diameter were significantly smaller and vascular density was significantly higher than those in the control group and those at the remote site in the ONO1301SR group. Mean $\pm$ standard error of the mean, respectively. LV, Left ventricle; RV, right ventricle.

or attachment on the epicardial surface. Injection area-specific recovery, demonstrated in this study, would suggest that direct injection of ONO1301 might be more effective in the myocardium that has heterogeneous disease, such as ischemic cardiomyopathy, as reported by Iwata and associates.²¹ Combination with coronary artery bypass grafting would also be a clinically applicable strategy for this purpose. On the other hand, homogeneous disease such as DCM might gain more therapeutic benefits by diffusely attaching ONO1301 on the epicardial surface compared with direct injection, although further basic investigation will be needed to establish this strategy. Intracoronary injection is known to diffusely deliver reagents or cells into the myocardium²⁵; however, intracoronary injection of ONO1301SR whose diameter is more than 20 μm will cause coronary embolism and ischemic myocardial damage.

This study is limited by the use of a canine model, which is not exactly relevant to the clinical DCM diseases and has

limited reagents for mRNA or protein investigations available.

However, a large animal model is essential in investigating cardiac performance by the latest technology used in the clinical arena, such as speckle-tracking echocardiography used in this study, whereas rodent models with or without genetic modifications would be useful in showing the mechanistic insights of this treatment. As mechanistic insights have been reported by several studies, the main focus of this study was to test the hypothesis that intramyocardial injection of ONO1301 induces region-specific and global functional recovery in dilated cardiomyopathy. In addition, this study investigated the mechanisms of this treatment to show the consistency with the previous studies that used rodent models to prove the mechanisms of this treatment.

Injection to the anterior wall and use of the posterior wall as the control was an option; however, in the surgical view, injection into the lateral wall produced consistent,

FIGURE 5. Electron microscopy revealed that the myocardium of the control group (A) showed prominent swelling or disruption of mitochondria, intra-cellular or perinuclear edema, and sarcoplasmic vacuoles resulting from dilation of sarcoplasmic reticulum (arrowhead). On the other hand, mitochondria in the ONO1301SR group (B) were compact and showed densely packed cristae.

reproducible, and safe injections compared with that into the anterior wall. Therefore, injection of the reagent into the lateral wall and septal wall was used as the control in this study. However, pathophysiology of the septum are substantially influenced by the performance of the RV.

In summary, we quantitatively evaluated region-specific pathologic and functional effects of ONO1301SR, a slow-releasing form of prostacyclin agonist, on a rapid-pacing canine DCM model. Multitherapeutic endogenous cytokines induced by intramyocardial ONO1301SR injection may be responsible for the improved cardiac performance and ultrastructure. ONO1301SR is a promising therapeutic drug for enhancing myocardial regeneration on the impaired myocardium.

We thank Masako Yokoyama, Yuka Fujiwara, and Shigeru Matsumi for their excellent technical assistance.

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