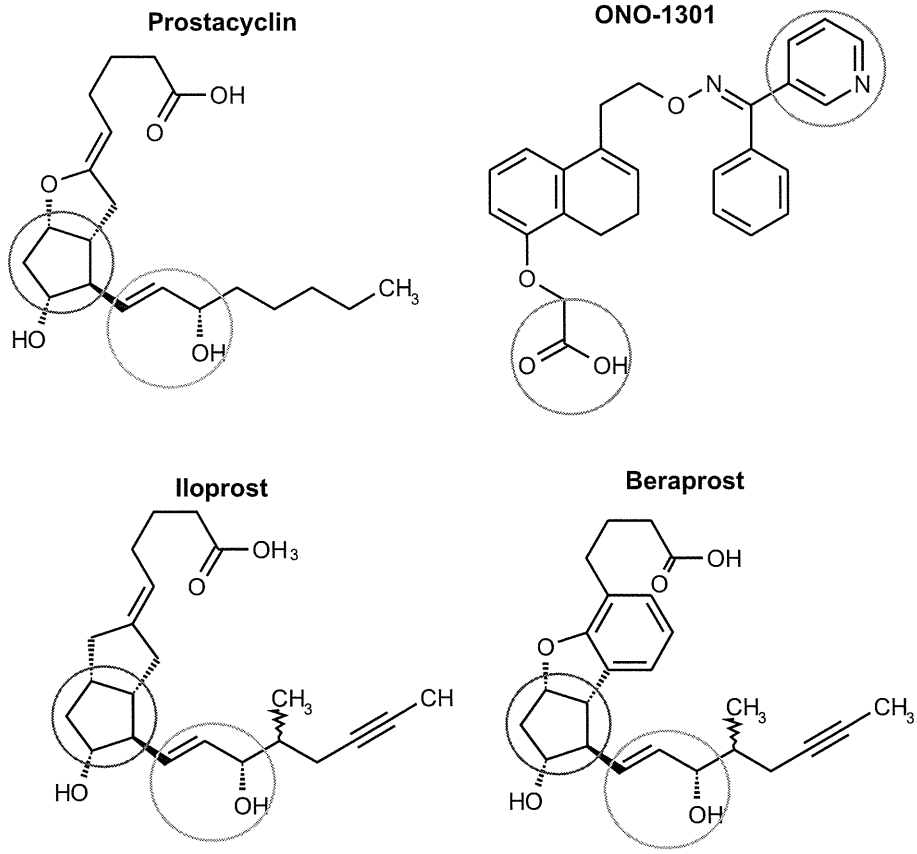

Fig. 1 Prostacyclin has the prostanoid structure including a five-membered ring (indicated as *blue circle*) and an allylic alcohol (indicated as *orange circle*), which are rapidly metabolised in vivo. In contrast, a synthetic selective agonist of prostacyclin, ONO-1301, lacks the prostanoid structure, while this reagent has the structure having a thromboxane A2 inhibitory activity (indicated as *purple circle*). Other selective prostacyclin agonists, such as iloprost or beraprost, have the prostanoid structure (indicated as *blue* and *orange circles*) without thromboxane A2 inhibitory activity

clinical settings [24], proposing the advantage of this new drug for acute and chronic pathologies that are related to ischaemia, inflammation and/or fibrosis. In addition, it was reported that ONO-1301 is inactivated by oxidation in the liver within 3–4 h [24], indicating a wide utility of this product as a drug in the clinical settings.

Pharmacological activity of ONO-1301

It has been shown that ONO-1301 agonises the IP receptor expressed in a variety of the cells, such as fibroblast, vascular smooth muscle cell or endothelial cell, to up-regulate expression of multiple factors, such as VEGF, HGF or SDF-1, in vitro [16]. The effects of ONO-1301 as a cytokine inducer were shown to be mediated at least in part by elevation of intracellular cyclic adenosine monophosphate (cAMP) [16, 26]. In addition, extracellularly released factors by ONO-1301 have been shown to enhance a tube-like formation of human umbilical vein endothelial cells (HUVECs) co-cultured with normal human dermal fibroblasts (NHDF) in vitro [27], indicating a pro-angiogenic property of ONO-1301. In addition, it was reported that NHDF stimulated by ONO-1301 enhanced migration of bone marrow (BM)-derived cells mediated by extracellularly released SDF-1, in vitro [20], suggesting that ONO-1301 may have an effect to enhance migration of circulating BM cells into the targeted territory contributing to BM cell-mediated tissue salvage and/or regeneration.

Development of ONO-1301SR to establish a sustained-release drug-delivery system

While ONO-1301 has been shown to have a long-lasting prostacyclin agonistic effect compared to the other prostacyclin agonists, it would be further useful and beneficial to develop a sustained-release drug-delivery system of ONO-1301 to achieve a further prolonged prostacyclin agonistic effects on the targeted territory of acute and chronic pathologies. For this purpose, ONO-1301 was polymerised with PLGA microspheres that are proven to be biocompatible and biodegradable, used as controlled delivery system for proteins or drugs in clinical settings [16, 25]. As a result, this ONO-1301SR product was shown to be hydrolysed at the site of administration to linearly release ONO-1301 into the adjacent tissue with a modest initial burst (Fig. 2). In addition, duration of ONO-1301 release can be adjusted by modifying the molecular weight of PLGA, the lactic/glycolic acids ratio or the particle size to achieve optimum effects, depending upon the targeted pathology or drug delivery mode [25].

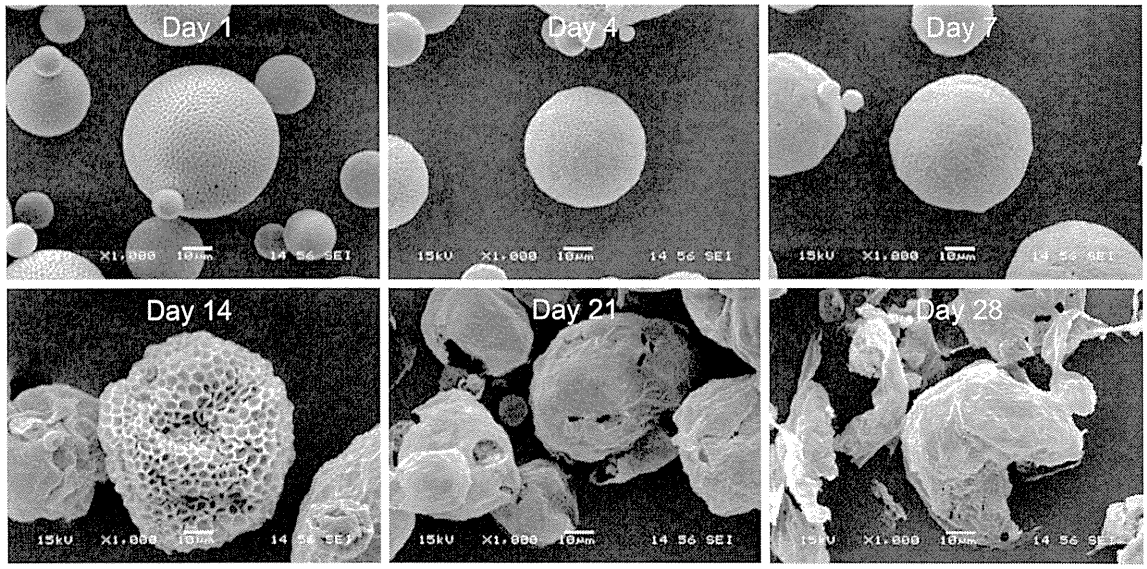

Fig. 2 Representative electron micrographic images of ONO-1301SR, which is a PLGA-polymerised form of ONO-1301, after production at 37 °C in vitro. Structure of the microspheres is gradually degraded over 28 days

Other prostaglandin agonists under development

Agonists of prostagrandins are theoretically therapeutic for acute and chronic pathologies associated with tissue ischaemia, inflammation and/or interstitial fibrosis. Xiao et al. [28] reported in 2004 that prostaglandin EP4 receptor agonist, ONO-4819, was effective in attenuating myocardial ischaemia–reperfusion injury via elevation of intracellular cAMP concentration in noncardiomyocytes. In addition, this product was shown to have a positive effect on bone regeneration [29–39] or nerve root angiogenesis [40], and have a protective effect against acute liver injury [41], skin injury [42] or renal tubulointerstitial fibrosis [43]. Of note, ONO-4819 is under the clinical study for treating medically refractory ulcerative colitis [44], though the result has not been reported. Another EP4 receptor agonist, EP4RAG, has been shown to have a protective effect against ischaemia–reperfusion myocardial injury [45], cardiac allograft transplantation-related inflammation [46] or experimental autoimmune myocarditis [47]. Despite several similar products to ONO-1301, it appears that sustained-release form of prostagrandin agonist has been developed only in ONO-1301 to date.

Evidence of cardiac tissue salvage/regeneration by ONO-1301SR

Therapeutic effects of ONO-1301SR have been tested on a variety of cardiac pathologies, such as acute and chronic myocardial infarction (MI), cardiomyopathy, cardiac allograft disease post-transplantation or myocarditis. As a result, ONO-1301SR treatment showed positive effects on these pathologies by different mechanisms from other existing treatments that are used in the clinical settings, indicating that ONO-1301SR is a potential new drug for a variety of cardiac diseases. This section summarises previous reports that document effects of ONO-1301 or ONO-1301SR on each cardiac pathologies.

Effects of ONO-1301SR on acute MI

Ischaemic insult against the heart by limiting coronary perfusion rapidly induces intracellular lactic acidosis in the cardiac myocytes, which leads to reduction of cellular contractility and subsequent necrotic cell death, consequently generating a state of "acute MI" [48, 49]. An array of debris from the dead cells or "danger signals" from cells that confront with the ischaemia activate inflammatory reactions, including accumulation of circulating cells or activation of residential cells in the cardiac tissue that consequently determines an area of "infarct region," where cardiac myocytes were lysed and replaced by fibrous components [50, 51]. In addition, border area between the infarct area and the area with sufficient blood supply confronts with persistent ischaemia that progressively widen the infarct region [51].

Treatment for the acute MI is therefore reperfusion of the ischaemic area to supply sufficient blood flow into the tissue [52]. It is, however, known that early reperfusion induces intracellular calcium overload, overproduction of superoxides and their derivatives and mitochondrial permeability transition pore opening in the cardiac myocytes, which consequently yields rapid cell death that often

causes lethal ventricular arrhythmia, and importantly exacerbates inflammatory response in the reperfused area [53, 54]. Despite a number of attempts, additional treatments that effectively reduce ischaemia–reperfusion cardiac injury have not been developed [53]. Among the agents that activate myocyte receptors, such as adenosine [55], bradykinin [56], opioids [57], glucagon-like peptide 1 [58], atrial natriuretic peptide (ANP) [59], erythropoietin [60] or insulin [61], intravenous infusion of adenosine and ANP showed positive, but not substantial, additional therapeutic effects to direct percutaneous coronary intervention for acute MI. In addition, effects of the agents that act intracellularly, such as volatile anaesthetics [62], nitrates [63], atorvastatin [64], delcasertib [65], nicorandil [59] or cyclosporine [66], have not been proven by large randomised studies. There are a number of other potential agents that were or were not tested by large-scale human studies, such as phosphodiesterase-5 inhibitors [67], superoxide dismutase [68] or neutralising antibodies against adhesion molecules such as P-selectin, intercellular adhesion molecule-1 [69]. Importantly, these treatments are targeted to effect on a single cellular and/or molecular process among a variety of complicated dynamic processes related to ischaemic-reperfusion cardiac injury, potentially limiting the overall therapeutic effects. In addition, delivery method of the agents needs to be optimised depending upon the underlying therapeutic mechanisms [68, 69].

In contrast, it has been shown that administration of ONO-1301SR directly activates endothelial cells and vascular smooth muscle cells through the IP receptor, to induce paracrinal release of protective factors, such as HGF, VEGF or SDF-1, into the damaged cardiac tissue. Nakamura et al. [27] first reported therapeutic effects of ONO-1301SR on acute MI in 2007. They directly injected ONO-1301SR into the myocardium that was subjected to ischaemia by left coronary artery ligation in mice. As a result, LV enlargement post-MI was ameliorated and survival was improved by ONO-1301SR treatment, in association with intramyocardially up-regulated HGF and VEGF. They concluded that angiogenesis by ONO-1301SR-induced up-regulation of multiple cytokines is the key therapeutic mechanisms of this treatment for acute MI [27]. In addition, the same team reported the angiogenesis-related positive effects of ONO-1301SR on acute MI with reperfusion using a rat model in 2012 [70]. Furthermore, our group reported that ONO-1301SR treatment enhances recruitment of bone marrow-derived cells into the ischaemic myocardium via enhanced SDF-1/C-X-C chemokine receptor type 4 interaction in a murine acute MI model [20]. It was thus concluded that accumulation of bone marrow-derived cells by ONO-1301SR treatment is an alternative therapeutic mechanisms of this treatment, though role of the accumulated cells needs to be clarified. Noticeably, our group delivered ONO-1301SR into the heart in an atelocollagen-based sheet form [20], since it was considered that direct injection of ONO-1301SR into the myocardium may induce myocardial injury.

All of these reports suggest mechanisms of cardiac salvage and/or regeneration in the ONO-1301SR treatment for acute MI, such as angiogenesis by up-regulation of multiple pro-angiogenic cytokines or recruitment of bone marrow-derived cells. However, further basic studies are necessary to thoroughly clarify the therapeutic mechanisms of this treatment for acute MI. Moreover, these reports indicate the therapeutic potential of ONO-1301SR for treating acute MI in clinical settings, whereas delivery method and dose of ONO-1301SR need to be optimised in basic studies by the good laboratory practice (GLP) standard.

Effects of ONO-1301SR on chronically failing heart

Chronic cardiac failure is a result of previous or continuous insult against the heart, such as ischaemia, valvular pathologies or genetic abnormalities. In this state, pressure and/or volume overload in the heart continuously activates a variety of cellular and molecular processes to remodel ventricular structure, by which pressure and/or volume overload is further exacerbated, to generate the viscous cycle, "left ventricular (LV) remodelling" [5]. In addition, humoral, hormonal and/or sympathetic nerve activities further exacerbates pressure and/or volume overload to affect progression of the LV remodelling.

Existing surgical treatments directly target pressure and/or volume overloading by intervening valvular pathologies or dilated ventricle, while existing medical treatments target hormonal and/or sympathetic nerve activities. On the other hand, treatments targeting responsible cellular and molecular processes for LV remodelling are under development as represented by cell transplantation therapy [9, 10, 12]. It has been shown that transplantation of somatic tissue-derived stem/progenitor cells, such as bone marrow-derived cells or skeletal muscle-derived cells, into the chronically failing heart enhanced native regeneration capacity by inducing constitutive expression of pro-angiogenic factors or anti-fibrotic factors, consequently to reverse LV remodelling, as reported by an array of pre-clinical studies [9, 10, 12]. Treatment by ONO-1301SR was also reported to induce similar therapeutic mechanisms to the cell transplantation therapy in chronic failing heart in pre-clinical studies as follows.

Iwata et al. [71] generated a chronic MI model in swine by placing the ameroid constrictor in the left circumflex artery (LCX) to induce MI. Four weeks later, they performed direct epicardial injection of ONO-1301SR into the infarct border area under LV electromechanical mapping

guidance using a transcatheter system. As a result, ONO-1301SR treatment enhanced collateral growth in relation to increased number of the capillaries, attenuated collagen fraction in the myocardial interstitium and reduced LV volume, indicating that this treatment reversed the LV remodelling. They discussed that intramyocardially delivered ONO-1301SR directly acted on the residential fibroblasts to induce up-regulation of cardiotherapeutic factors such as VEGF or HGF, which in turn activated the regeneration process in the chronic MI heart [71].

Our group generated a chronic MI model in canine by permanently ligating left coronary artery [22]. Subsequently, ONO-1301SR-immersed atelocollagen sheet was placed over the LV surface of this model. ONO-1301SR treatment induced functional recovery compared to sham treatment as assessed by standard and speckle-tracking echocardiography and cardiac catheterisation studies, in association with up-regulated HGF, VEGF or SDF-1. Importantly, this study showed increased myocardial blood flow by ONO-1301SR treatment as assessed by ^{13}N-ammonia positron emission tomography study [22], indicating that pro-angiogenic effects of ONO-1301SR augment myocardial blood flow to induce functional recovery in ischaemic cardiomyopathy (Fig. 3).

Effects of ONO-1301SR on dilated cardiomyopathy were tested by Hirata's group and our group. Hirata et al. [72] subcutaneously injected ONO-1301SR into the hamsters having genetically determined dilated cardiomyopathy. As a result, ONO-1301SR-treated hamsters showed a preserved cardiac function in relation to reduced fibrous components and increased capillary network in the myocardial interstitium, suggesting a therapeutic potential of systemic delivery of ONO-1301SR into the dilated cardiomyopathy-related chronic cardiac failure [72].

In contrast, our group used the rapid-pacing-induced canine model [23] and the delta-sarcoglycan-deficient hamster model [21] that was a different model from that of Hirata's group. Our group directly delivered into the heart in order to maximise the therapeutic effects of this reagent and minimise systemic complications. Firstly, ONO-1301SR was intramyocardially injected in rapid LV-paced canines with their LV ejection fraction being <40 % [23]. As a result, global and regional LV functions were recovered in 4 weeks after the treatment, in association with increased microvessel number, decreased myocyte diameter and decreased fibrous component in the LV myocardium [23]. However, the direct intramyocardial injection of ONO-1301SR used in this report was concerned by inconsistent delivery of the reagent and injection-related myocardial injury.

Therefore, in the subsequent study, ONO-1301SR immersed into the atelocollagen sheet was simply placed on the LV surface of hamster model of dilated cardiomyopathy [21]. This delivery method would achieve a consistent delivery of the reagent into the heart and minimum injury to the myocardium, whereas the myocardial territory that is

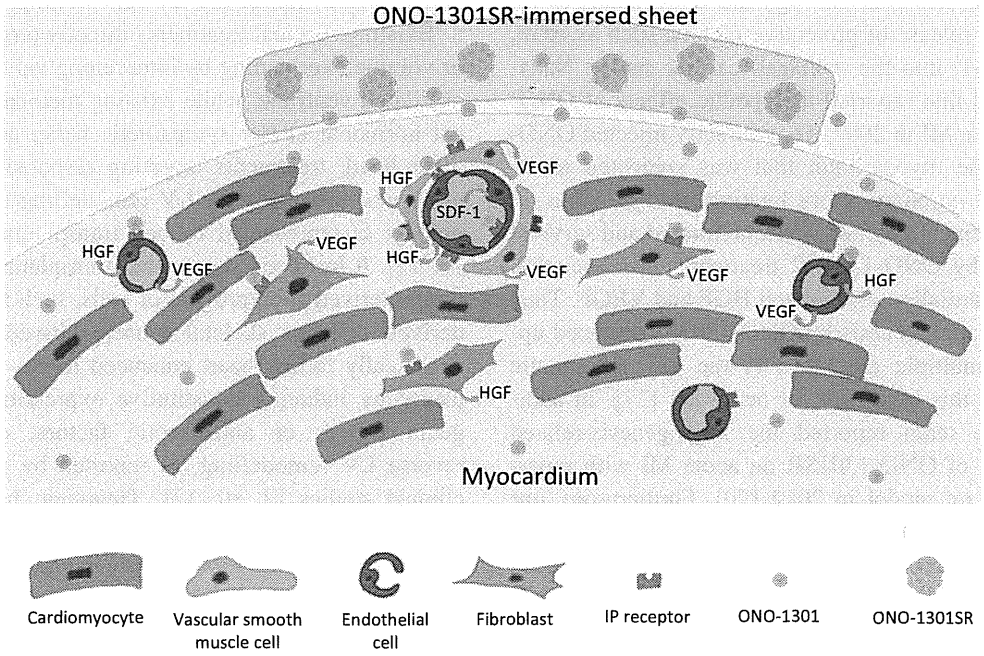

Fig. 3 Schematic representation of proposed mechanisms underlying ONO-1301SR-immersed sheet placement therapy for treating damaged cardiac tissue. ONO-1301 is linearly released from the ONO-1301SR by hydrolysis and infiltrated into the cardiac tissue. IP receptor-expressing cardiac cells, such as vascular smooth muscle cells, fibroblast and endothelial cells, are activated by ligation of ONO-1301 to paracrinally release protective factors, such as VEGF, HGF or SDF-1

affected by ONO-1301SR might be theoretically limited. As a result, myocardial vascular network was globally and homogeneously better developed in the ONO-1301SR-treated hamsters with a substantial survival benefit in association with up-regulated cardiotherapeutic factors such as VEGF, HGF or SDF-1 [21]. Importantly, this study showed a consistently heart-dominant elevation of ONO-1301 concentration until 4 weeks after the ONO-1301SR administration [21]. It was thus indicated that placement of ONO-1301SR-immersed collagen sheet over the LV surface act on the entire LV contributing to global functional recovery. These two studies proved the concept that local delivery of ONO-1301SR into the heart contributes to functional recovery and survival benefit in dilated cardiomyopathy-related chronic cardiac failure (Fig. 3).

Effects of ONO-1301SR on other cardiac pathologies

ONO-1301SR was thus shown to have anti-inflammatory, pro-angiogenic and anti-fibrotic effects on acute and chronic cardiac pathologies via up-regulation of a variety of cardiotherapeutic cytokines and chemokines. Furthermore, positive effects of ONO-1301SR were shown in other cardiac pathologies such as cardiac graft disease post-transplantation [73] or autoimmune myocarditis.

Suzuki et al. [73] subcutaneously injected ONO-1301SR into the mice that were subjected to heterotopic cardiac allograft transplantation in the aim to test the effects of ONO-1301SR on acute and chronic graft-host disease. This treatment was effective in chronic rejection as shown in the reduced myocardial fibrosis in a class II mismatch combination, but not effective in acute rejection in a full allomismatch combination, suggesting that ONO-1301SR might be a potential new drug for chronic rejection post-cardiac allograft transplantation. Hirata et al. [74] reported that daily intake of ONO-1301 (not PLGA-polymerised SR form, but bulk substance) suppressed a progression of LV remodelling chiefly via up-regulation of HGF in a rat autoimmune myocarditis model. It was indicated that HGF plays a critical role in LV remodelling in this model and that ONO-1301 may be an ideal inducer of HGF in the myocardium. Further studies are warranted to prove the positive effects of ONO-1301 on other cardiac pathologies related to acute/chronic inflammation, microvascular dysfunction or fibrous accumulation in the myocardium, such as hypertensive cardiac disease.

Towards clinical studies of ONO-1301SR for treating cardiac disease

Although positive effects of ONO-1301SR treatment were proven on a variety of cardiac pathologies, such as acute MI, idiopathic dilated cardiomyopathy, ischaemic cardiomyopathy, cardiac allograft disease post-transplantation or fulminant myocarditis, it is a key of success of this treatment to optimise delivery method of ONO-1301 for each target pathology in clinical study. This section discusses suitable target pathology and delivery method of this reagent, and studies necessary for the first-in-human study. In addition, potential methods to enhance the therapeutic effects of ONO-1301 are discussed in the prospect of clinical application.

Pathology-specific ONO-1301SR delivery for the clinical study

It has been shown that both systemic and local delivery of the ONO-1301SR potentially contributes to the therapeutic benefits for acute and chronic cardiac disease. However, optimal mode of the delivery in the clinical settings would be dependent upon the pathology and, most importantly, the standard treatment for the pathology in the routine clinical practice.

Since the standard treatment for the acute MI is the prompt reperfusion of the occluded coronary arteries by percutaneous transcatheter approach, direct intramyocardial injection of ONO-1301SR by transcatheter approach at the time of reperfusion may be ideal, although further basic studies using a large animal model are necessary. In addition, subcutaneous ONO-1301SR injection or oral intake of ONO-1301 would be optional mode of the ONO-1301SR delivery as an additional treatment for acute MI to the standard reperfusion therapy.

The standard treatment for ischaemic and non-ischaemic dilated cardiomyopathy is the intensive combination of medical and interventional treatments. Addition of subcutaneous ONO-1301SR injection or oral ONO-1301 intake to the optimal medical management would augment the therapeutic effects of the standard medical treatment. Placement of ONO-1301SR over the cardiac surface, which has been intensively developed by our laboratory, may be added at the time of coronary artery bypass grafting and/or mitral valve surgery for ischaemic and non-ischaemic dilated cardiomyopathy. Addition of the ONO-1301SR placement therapy at the time of ventricular assist device implantation surgery might be effective in enhancing functional recovery to achieve "bridge-to-recovery" for dilated cardiomyopathy or fulminant myocarditis.

Head-to-head comparison study for the therapeutic effects between placement over the heart and subcutaneous injection of ONO-1301SR has not been reported. However, it may be theorised that enhanced effects will be achieved by the local placement which maximise ONO-1301 delivery into the targeted area with minimal systemic effects, since paracrinal actions of the effector cells are augmented

in positive relation to the magnitude of the ONO-1301 stimuli [20, 21, 23].

Studies necessary for the clinical study

Once the target pathology and the delivery mode of the ONO-1301SR treatment were decided, dose-optimisation study ideally using a large animal model is necessary to launch the clinical study. Degree of therapeutic effects and systemic complications such as hypotension, bleeding or diarrhoea, in addition to plasma and cardiac ONO-1301 level, need to be investigated depending upon the dose of ONO-1301SR. Moreover, toxicity test in the GLP standard is necessary by using a large animal model over 3 months, since it was shown that ONO-1301 is extinguished from the body by 1 month [21].

Enhancing positive effects of ONO-1301SR treatment on cardiac pathologies

Therapeutic mechanisms of ONO-1301SR were to induce constitutive up-regulation of a variety of protective factors, such as VEGF, HGF or SDF-1, which are shared by somatic tissue-derived stem/progenitor cell transplantation therapy. One may be concerned by durability of the therapeutic efficacy, since all administered ONO-1301 as a SR form is inactivated by 4 weeks. Although newly formed vasculatures by 4 weeks might remain to contribute to the myocardial blood flow and thus the functional recovery despite extinction of paracrinal stimuli, as seen in the tissue-derived stem/progenitor cell-based therapy [75], additional concomitant treatments may augment the positive effects and prolong its durability. One approach would be combination with the treatments that contribute to cardiac function by a different mechanism from ONO-1301SR, while the other approach may be combination with treatments whose mechanisms are similar to ONO-1301SR.

Our group developed hybrid therapy by combination of ONO-1301SR delivery and cardiac support mesh net device placement [22]. It has been shown that placement of cardiac support net over the ventricles mechanically reduces diastolic LV wall stress to inhibit progression of the LV remodelling, whereas clinical studies of cardiac support net for treating chronically failing heart failed to show survival benefits despite positive effects on the LV volume [76]. This inconsistent result of cardiac support net device would be explained by a lack of biological effects in this treatment. In contrast, ONO-1301SR contributes to recovery of cardiac function by the biological effects, not by mechanical effects. It was therefore theorised that combination of ONO-1301SR and cardiac support net placement would augment the therapeutic effects of either treatment. To test this hypothesis, our laboratory developed a hybrid device consisting of biodegradable polyglycolic acid-based cardiac support net and ONO-1301SR-immersed atelocollagen sheet for treating a canine chronic MI model [22]. As a result, the hybrid device elicited a greater reversal of the MI-inducing LV remodelling than either single treatment, indicating the potential of this device for chronic cardiac failure [22].

Transplantation of somatic tissue-derived stem/progenitor cells has been shown to yield a functional recovery of the failing heart via a similar mechanism to the ONO-1301SR treatment, though therapeutic effects of the cell transplantation therapy are reportedly limited by poor initial retention and long-term survival of the transplanted cells [75, 77, 78]. One may claim that head-to-head comparison study in the therapeutic effects of the two treatments would be clinically important. This pre-clinical study may not be, however, justified by a large number of model animals used to gain statistical significance, since previous studies showed that both treatments improved ejection fraction by 5–10 % [22, 79]. Rather, addition of the ONO-1301SR placement therapy to the cell transplantation therapy may prolong the regenerative effects for the cardiac tissue, depending upon expression of IP receptor and subsequent intracellular signalling in the transplanted cells. Further studies are necessary to test this hypothesis.

Omentum is an abdominal organ, mobilised to be attached to the abdominal organ/tissue in response to the tissue damage/injury. Multiple pro-angiogenic factors are known to be abundantly expressed in the omentum, contributing to regeneration and/or healing of the damaged/injured tissue/organ. This unique character of the omentum was applied to develop a treatment for cardiac ischaemic disease by mobilising to the cardiac surface in a pedicle fashion [75, 80]. As a result, angiogenesis was induced in the ischaemic/infarct territory of the cardiac tissue. Of note, it was reported that omentum covering over the chronic MI-heart with local sustained-delivery of basic fibroblast growth factor (bFGF), but not without bFGF, induced a new vascular network formation between the pedicle omentum and the heart [75]. It is thus theorised that the omentum covering with local delivery of ONO-1301SR might be effective in augmenting regional blood flow in the ischaemic cardiac tissue via formation of new vascular networks in the heart.

ONO-1301/ONO-1301SR treatment for extracardiac pathologies

ONO-1301 is theoretically effective in treating any acute and chronic pathologies for which dysfunction of microvasculature or accumulation of fibrous components in

the tissue/organ is responsible, as shown in the studies for cardiac pathologies. In fact, use of this reagent was reported to be effective in pulmonary arterial hypertension (PAH), pulmonary fibrosis or chronic kidney disease. Moreover, the effects of ONO-1301SR as a potent protective cytokines-inducer might be applied to other pathologies, such as cerebral, liver or pancreatic pathologies. This section summarises previous reports and potential target of ONO-1301SR treatment for extracardiac pathologies. In addition, this section discusses a potential of ONO-1301SR in combination with medical devices, to accumulate further knowledge and information regarding this unique product, exploring further applications.

ONO-1301SR treatment for lung disease

Since several prostagrandin agonists are the standard treatment of primary and secondary PAH in the clinical practice [81, 82], an ideal target pathology of ONO-1301 and/or ONO-1301SR treatment might be PAH or associated lung diseases. In fact, Kataoka et al. reported in 2006 that repeated subcutaneous administration of ONO-1301 attenuated monocrotaline-induced PAH in rats via the long-lasting cAMP stimulation and thromboxane synthase inhibition [83, 84]. Subsequently, Obata et al. [85] reported in 2007 that a single subcutaneous injection of ONO-1301SR resulted in attenuated pulmonary arterial pressure, at least in part, through inhibition of vascular smooth muscle cell proliferation in a rat monocrotaline-induced PAH model. The same group reported in 2013 that oral administration of ONO-1301 was therapeutic in monocrotaline-induced PAH rats [86]. Moreover, Murakami et al. [87] reported in 2006 that repeated subcutaneous administration of ONO-1301 attenuated bleomycin-induced pulmonary fibrosis in mice.

Hayashi et al. [88] reported in 2010 that administration of ONO-1301 was more therapeutic for ovalbumin-induced asthma model in mice than beraprost. In addition, Kimura et al. [89] tested the hypothesis that ONO-1301SR treatment is effective in suppressing hyperresponsiveness, allergic inflammation and remodelling of the airway. As a result, they proved the anti-inflammatory and the reverse remodelling effects of ONO-1301SR on chronic house dust mite-induced asthma model in mice.

These results might warrant a potential of ONO-1301SR treatment for PAH or asthma, of which chronic inflammation is involved in the development of the pathologies, in clinical practice. Further basic studies should be focused on optimisation of the dose of ONO-1301SR or the administration mode of ONO-1301SR, such as a single subcutaneous injection, intermittent injections, or oral intake. It may be proposed that intravenous injection of ONO-1301SR would induce entrapment of the product in the pulmonary arterioles or capillaries to achieve sustained release of the ONO-1301, although intravenous injection may carry a substantial risk of pulmonary embolism that further exacerbates PAH or associated lung pathologies.

ONO-1301SR treatment for kidney diseases

Progression of chronic kidney disease is known to be regulated by chronic inflammatory and fibrotic process in the tubulointerstitium of the kidney. Anti-inflammatory effects of ONO-1301 on nephritis was first reported by Hayashi et al. in 1997 [90]. Subsequently, Yamasaki et al. [91] reported that repeated injections of ONO1301SR were effective in reducing renal fibrosis in diabetic nephropathy rat model. In addition, Nasu et al. [92] reported in 2012 that a single subcutaneous injection of ONO-1301SR into the mice that were subjected to unilateral ureteral obstruction yielded a suppression of interstitial fibrous components of the obstructed kidney partly via inhibition of transforming growth factor (TGF)-β, suggesting a potential of ONO-1301SR for the chronic kidney disease, though further studies to prove the safety, efficacy and further mechanisms underlie this treatment.

ONO-1301SR treatment for other organ pathologies

Although standard treatment of cerebral ischaemia is early reperfusion, additional medical treatments that ameliorate ischaemia–reperfusion injury would further improve the outcome of the intervention. Hazekawa et al. [93] reported in 2012 that a single subcutaneous injection of ONO-1301SR into the rats that were subjected to repeated induction of cerebral ischaemia yielded a short-term functional and histopathological recovery. The same groups reported in 2012 that repeated ONO-1301SR administration reduced ischaemic damage of rats that were subjected to middle cerebral artery occlusion [94].

Acute liver injury is a life-threatening disorder, initiated by a burst inflammation, followed by a complex inflammatory process. Since prompt treatment is known to improve the outcome of this pathology, new "shelf-stored drug" has been long sought. Xu et al. [95] reported in 2011 that intermittent oral administration of ONO-1301, not SR product, ameliorated CCl4-induced acute hepatic injury in mice partly via up-regulation of HGF. The same group reported in 2013 that ONO-1301SR was effective in treating CCl4-induced inflammatory chronic liver fibrosis in mice [96]. Inflammation plays a key role in clinical and pathological progression of chronic pancreatitis. Niina et al. [97] reported in 2014 that ONO-1301SR inhibited monocyte activity to suppress pancreatic fibrosis. These reports indicate that ONO-1301SR may be therapeutically

effective for acute and chronic pathologies related to ischaemia, inflammation and/or fibrosis in multiple organs.

A potential of ONO-1301SR in combination with medical devices

Of the implantable medical devices that have been recently developed, vascular stent has been widely used as the standard treatment for atherosclerotic arterial stenosis or aortic aneurysm [98]. In particular, stent graft implantation of aortic aneurysm has improved clinical outcomes of this pathology, though complications related to a poor attachment of the stent and the native aortic wall have not been fully resolved [99]. Since ONO-1301 has effects on tissue healing and/or regeneration, it is hypothesised that local delivery of ONO-1301 might strengthen the attachment between the stent graft and the native aortic wall. To test this hypothesis, our laboratory developed an aortic stent graft that was coated with ONO-1301SR and implanted in the thoracic aorta of canines [100]. As a result, the attachment was physiologically and histopathologically strengthened. This concept may be applicable to the transcatheter aortic valve replacement, in which aortic valve incompetence caused by suboptimal attachment of the prosthesis and the native aortic annulus yields a negative impact of this treatment [101].

Conclusions

Feasibility, safety and therapeutic efficacy of a synthetic prostacyclin agonist, ONO-1301, and a sustained-release form of ONO-1301, ONO-1301SR, have been tested in a variety of acute and chronic pathologies related to ischaemia, inflammation and fibrosis of multiple organs including the heart as pre-clinical studies. Major mechanisms underlying the therapeutic effects were consistently to induce release of multiple protective cytokines including HGF, VEGF or SDF-1 from targeted fibroblasts, vascular smooth muscle cells or endothelial cells, which enhance salvage and/or regeneration of the damaged tissue, including the heart.

Both acute and chronic cardiac failure related to ischaemic or non-ischaemic aetiologies would be a target of this novel treatment. Since direct placement of ONO-1301SR over the cardiac surface was suggested to be an optimal treatment for chronic cardiac failure using this product, launching the clinical study of this treatment is warranted. In addition, oral administration of ONO-1301 would be a potential drug for chronic cardiac failure, though further pre-clinical studies are needed in the GLP standard.

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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)

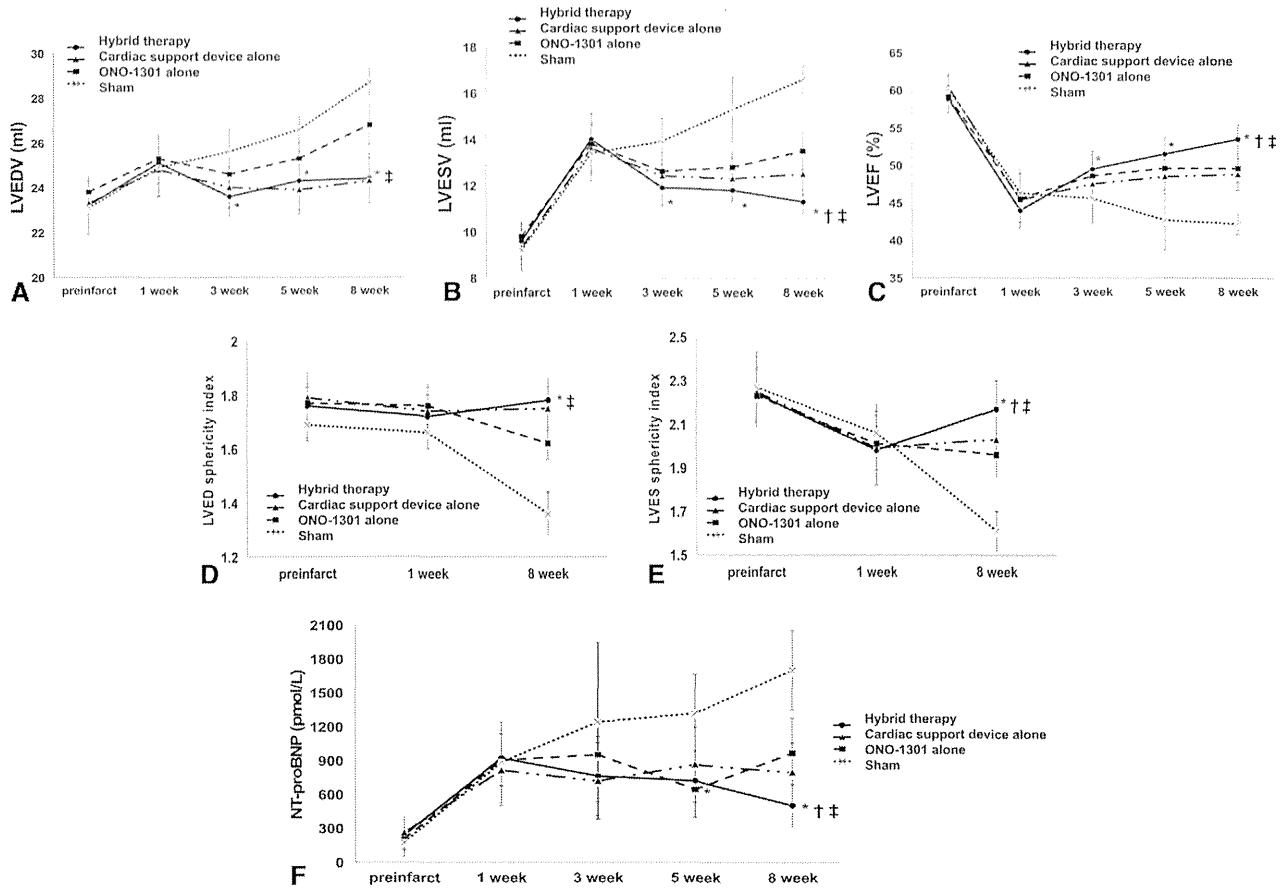

FIGURE 1. MDCT analysis. A, Changes in LVEDV. B, Changes in LVESV. C, Changes in LV ejection fraction. D, Changes in left ventricular end-diastolic and, (E) end-systolic sphericity indices. F, Changes in NT-proBNP. Hybrid therapy is shown by *circles with a solid line*, a cardiac support device alone 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, †$P < .05$ versus corresponding cardiac support device alone, ‡$P < .05$ 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

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.

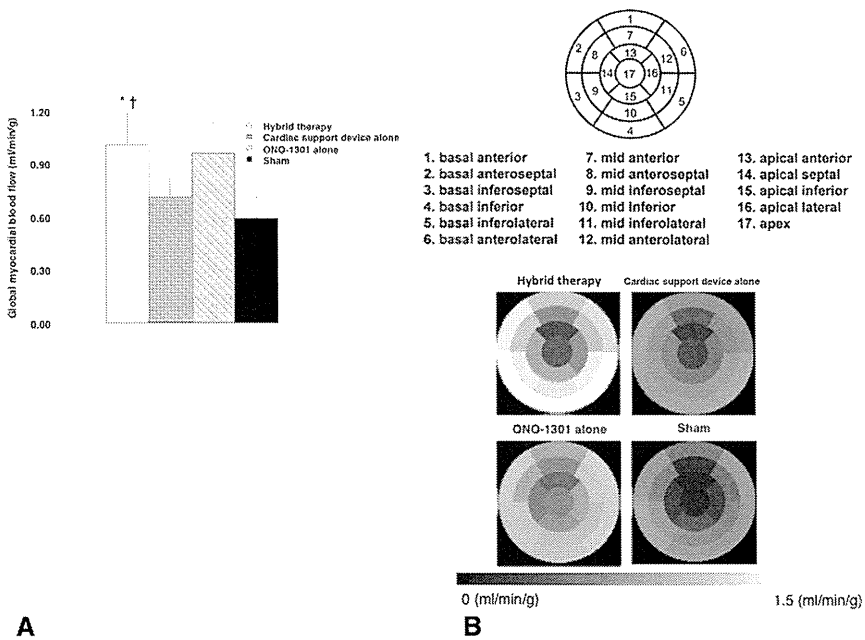

FIGURE 2. A, Global myocardial blood flow assessed by PET at 8 weeks postinfarction. B, Myocardial blood flow divided into 17 segments recommended by the American Heart Association. *$P < .05$ versus sham, †$P < .05$ versus cardiac support device alone.

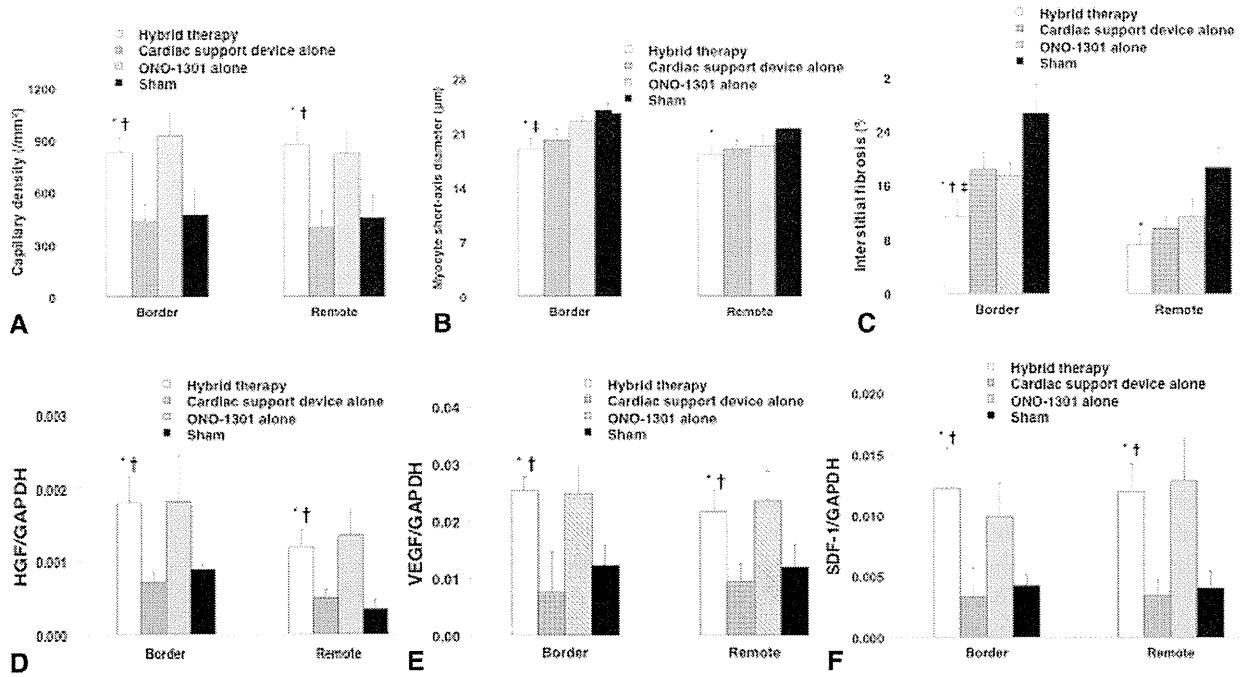

FIGURE 3. Histologic evaluation at 8 weeks postinfarction. A, Capillary density, (B) myocyte short-axis diameter, and (C) interstitial fibrosis in the border and remote areas. Expression levels of (D) HGF, (E) VEGF, and (F) SDF-1 in the border and remote areas quantified by real-time polymerase chain 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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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

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