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Research Correspondence

Improvement of Left Ventricular Dysfunction and of Survival Prognosis of Dilated Cardiomyopathy by Administration of Calcium Sensitizer SCH00013 in a Mouse Model

To the Editor: Dilated cardiomyopathy (DCM) is a myocardial disease with poor prognosis, characterized by progressive ventricular dilation and systolic dysfunction. Underlying etiologies include idiopathic, viral (myocarditis), toxic agents and mitochondrial and metabolic disorders, but mutations in genes encoding components of sarcomere, sarcolemma, cytoskeleton, or nuclear envelope—including lamin A/C (*LMNA*)—also cause DCM (1). Therefore, treatment and prevention of DCM are important especially for the individuals who carry the disease-causing mutation. We previously reported an animal model of DCM, *Lmna* knock-in mouse carrying H222P mutation in homozygous state ($Lmna^{H222P/H222P}$), which developed progressive contractile dysfunction (2). To explore a therapeutic strategy for DCM, we treated $Lmna^{H222P/H222P}$ mice with a pyridazinone derivative calcium (Ca^{2+}) sensitizing agent SCH00013 (3). We found that SCH00013 ameliorated systolic dysfunction (Table 1), prolonged life expectancy, decreased cardiac interstitial fibrosis, and modulated the expression of genes involved in cardiac remodeling (see also the Online Appendix for experimental details and additional discussion).

As a therapeutic strategy for heart failure, medication with angiotensin converting enzyme inhibitors, angiotensin II type I receptor blockers, and beta-blockers has been employed. Cardiotonic agents could be useful for treatment of systolic dysfunction, because the agents augment cardiac contractility via increased level of cyclic adenosine-mono-phosphate (cAMP) in cardiomyocytes, leading to an increase in intracellular Ca^{2+} concentration. Classical cardiotonic agents could provide short-term hemodynamic benefits, but a long-term administration was correlated with poor survival rates and often accompanied by adverse effects, presumably due to the elevated Ca^{2+} concentrations leading to cardiotoxic and arrhythmogenic effects. However, Ca^{2+} sensitizers are novel cardiotonic agents that elicit a positive inotropic effect via increasing Ca^{2+} sensitivity of muscle contraction without increasing the concentrations of cAMP and intracellular Ca^{2+} (4).

We tested the effect of the long-term oral administration of a Ca^{2+} sensitizer SCH00013 in DCM model $Lmna^{H222P/H222P}$ mice, because SCH00013 was reported to ameliorate the impaired contractility without superfluous expenditure of energy in the cardiac muscles (5). Body weight and water intake were not different between SCH00013-untreated and -treated $Lmna^{H222P/H222P}$ mice (Online

Fig. S1). Plasma concentrations of SCH00013 in the treated $Lmna^{+/+}$ and $Lmna^{H222P/H222P}$ male mice were 580.0 ± 23.5 ng/ml and 552.3 ± 26.1 ng/ml, respectively, whereas those in female mice were 456.0 ± 107.6 ng/ml and 411.5 ± 43.8 ng/ml, respectively (n = 4 to 5 in each group). Untreated $Lmna^{H222P/H222P}$ male mice started to die at 4 months of age, and they all died by 11 months of age (Online Fig. S2). A 50% survival time was prolonged by the treatment (untreated mice 7.90 ± 0.26 months vs. treated mice 8.70 ± 0.22 months, p < 0.05), although a Kaplan-Meier analysis of the overall mortality showed no statistical difference between them (p = 0.14). By contrast, untreated $Lmna^{H222P/H222P}$ female mice died between 6 and 13 months of age, and SCH00013 significantly improved survival prognosis, evidenced by the prolonged 50% survival time (9.80 ± 0.26 months vs. 11.14 ± 0.31 months, p < 0.01) and reduced overall mortality (p < 0.05, log-rank test).

Echocardiographic examination (Online Fig. S3) revealed that the untreated mice developed left ventricular dilation and contractile dysfunction, evidenced by decreased left ventricular fractional shortening and left ventricular ejection fraction, which were overt after 2 months of age in males and 4 months of age in females. The agent SCH00013 significantly improved left ventricular fractional shortening and left ventricular ejection fraction in males at 4 and 6 months of age and in females at 6, 8, and 10 months of age (Table 1). Systolic blood pressure in $Lmna^{H222P/H222P}$ mice at 4 months of age was not significantly modified by administration of SCH00013 for 2 months: treated 124.0 ± 3.4 mm Hg versus untreated 121.0 ± 2.2 mm Hg in males, and treated 112.9 ± 7.7 mm Hg versus untreated 120.3 ± 3.8 mm Hg in females (n = 8 and n = 4 for treated and untreated mice, respectively), demonstrating that SCH00013 improved the left ventricular contractile dysfunction in $Lmna^{H222P/H222P}$ mice without affecting systolic blood pressure.

The $Lmna^{H222P/H222P}$ mice showed dilation of both ventricles with enlargement of atrial cavity often accompanied by atrial thrombosis, which were prevented by the treatment (Online Fig. S4). Microscopic analyses demonstrated that the interstitial fibrosis, degeneration, and necrosis of cardiomyocytes in the ventricles of $Lmna^{H222P/H222P}$ mice were ameliorated by SCH00013 (Online Fig. S4). Moreover, the increased collagen deposition in the hearts of $Lmna^{H222P/H222P}$ mice was significantly suppressed by the treatment (Online Fig. S5).

Table 1 Echocardiographic Data for Untreated and SCH00013-Treated $Lmna^{+/+}$ and $Lmna^{H222P/H222P}$ Mice

	IVSd (mm)	PWd (mm)	LVM (mg)	LVEDD (mm)	LVESD (mm)	LVFS (%)	LVEF (%)	Heart Rate (beats/min)
Data for male mice age 2, 4, 6, and 8 months								
2 months of age								
Untreated $Lmna^{+/+}$ (n = 15)	0.49 ± 0.01	0.50 ± 0.01	45.2 ± 1.18	3.28 ± 0.04	1.85 ± 0.03	43.7 ± 0.59	81.5 ± 0.59	500 ± 7
SCH $Lmna^{+/+}$ (n = 22)	0.50 ± 0.01	0.54 ± 0.01	46.7 ± 1.36	3.21 ± 0.03	1.80 ± 0.03	43.9 ± 0.75	81.7 ± 0.76	523 ± 9
Untreated $Lmna^{H222P/H222P}$ (n = 21)	0.50 ± 0.01	0.49 ± 0.01	46.0 ± 1.17	3.30 ± 0.03	1.98 ± 0.03*	39.6 ± 0.93*	76.5 ± 1.19*	524 ± 7
SCH $Lmna^{H222P/H222P}$ (n = 17)	0.49 ± 0.02	0.51 ± 0.02	46.0 ± 1.76	3.28 ± 0.03	1.92 ± 0.02†	41.1 ± 0.60†	78.4 ± 0.77†	536 ± 10
4 months of age								
Untreated $Lmna^{+/+}$ (n = 12)	0.48 ± 0.02	0.53 ± 0.02	48.3 ± 1.90	3.38 ± 0.06	1.94 ± 0.05	42.8 ± 0.82	80.5 ± 0.82	542 ± 12
SCH $Lmna^{+/+}$ (n = 22)	0.52 ± 0.01	0.54 ± 0.01	51.7 ± 1.36	3.38 ± 0.03	1.95 ± 0.03	42.5 ± 0.68	80.0 ± 0.72	526 ± 7
Untreated $Lmna^{H222P/H222P}$ (n = 21)	0.47 ± 0.01	0.47 ± 0.01*	55.4 ± 2.05	3.80 ± 0.07‡	2.64 ± 0.08‡	30.9 ± 1.07‡	66.7 ± 1.44‡	560 ± 10
SCH $Lmna^{H222P/H222P}$ (n = 17)	0.49 ± 0.01	0.50 ± 0.01	55.8 ± 2.09	3.69 ± 0.06§	2.33 ± 0.06‖	37.0 ± 0.92§¶	72.9 ± 1.97†#	543 ± 7
6 months of age								
Untreated $Lmna^{+/+}$ (n = 12)	0.49 ± 0.01	0.53 ± 0.02	56.1 ± 2.10	3.64 ± 0.04	2.10 ± 0.03	42.3 ± 0.46	79.9 ± 0.63	532 ± 9
SCH $Lmna^{+/+}$ (n = 21)	0.50 ± 0.01	0.50 ± 0.01	56.1 ± 1.47	3.68 ± 0.05	2.19 ± 0.05	40.6 ± 0.65	78.2 ± 0.64	537 ± 7
Untreated $Lmna^{H222P/H222P}$ (n = 17)	0.43 ± 0.01**	0.42 ± 0.02‡	57.5 ± 2.28	4.34 ± 0.12‡	3.36 ± 0.15‡	23.0 ± 1.29‡	53.9 ± 2.59‡	516 ± 18
SCH $Lmna^{H222P/H222P}$ (n = 17)	0.46 ± 0.01††	0.43 ± 0.01§	58.8 ± 2.30	4.10 ± 0.10§	3.01 ± 0.12§#	27.0 ± 1.29§#	60.6 ± 2.08§#	533 ± 7
8 months of age								
Untreated $Lmna^{+/+}$ (n = 12)	0.50 ± 0.01	0.49 ± 0.02	54.1 ± 2.57	3.62 ± 0.07	2.07 ± 0.06	43.0 ± 0.64	80.8 ± 0.62	535 ± 11
SCH $Lmna^{+/+}$ (n = 21)	0.49 ± 0.01	0.49 ± 0.01	58.1 ± 2.20	3.80 ± 0.08	2.19 ± 0.08	39.9 ± 0.81	77.7 ± 0.92	544 ± 8
Untreated $Lmna^{H222P/H222P}$ (n = 7)	0.37 ± 0.01‡	0.36 ± 0.02‡	52.0 ± 5.27	4.37 ± 0.12‡	3.63 ± 0.10‡	15.9 ± 0.88‡	41.0 ± 1.65‡	566 ± 20
SCH $Lmna^{H222P/H222P}$ (n = 7)	0.38 ± 0.01§	0.37 ± 0.01§	54.4 ± 3.65	4.38 ± 0.15§	3.50 ± 0.16§	19.5 ± 0.79§	48.3 ± 3.08§	552 ± 11
Data for female mice age 2, 4, 6, 8, and 10 months								
2 months of age								
Untreated $Lmna^{+/+}$ (n = 7)	0.47 ± 0.02	0.52 ± 0.01	42.9 ± 1.93	3.14 ± 0.03	1.80 ± 0.05	41.9 ± 1.52	82.5 ± 0.95	535 ± 13
SCH $Lmna^{+/+}$ (n = 14)	0.49 ± 0.01	0.51 ± 0.01	40.9 ± 1.38	3.10 ± 0.02	1.77 ± 0.03	42.7 ± 0.80	80.5 ± 0.84	521 ± 10
Untreated $Lmna^{H222P/H222P}$ (n = 18)	0.51 ± 0.01	0.49 ± 0.01	43.7 ± 0.91	3.18 ± 0.02	1.84 ± 0.03	42.7 ± 0.67	79.1 ± 0.74	544 ± 7
SCH $Lmna^{H222P/H222P}$ (n = 14)	0.50 ± 0.02	0.50 ± 0.01	41.2 ± 0.80	3.12 ± 0.03	1.85 ± 0.04	40.9 ± 0.77	78.2 ± 0.77	551 ± 10
4 months of age								
Untreated $Lmna^{+/+}$ (n = 7)	0.50 ± 0.02	0.56 ± 0.02	43.1 ± 2.23	3.24 ± 0.03	1.72 ± 0.02	43.2 ± 0.43	81.0 ± 0.42	521 ± 15
SCH $Lmna^{+/+}$ (n = 14)	0.50 ± 0.01	0.52 ± 0.02	47.1 ± 1.35	3.28 ± 0.04	1.88 ± 0.03	42.3 ± 0.73	80.3 ± 0.71	530 ± 16
Untreated $Lmna^{H222P/H222P}$ (n = 18)	0.47 ± 0.01	0.46 ± 0.01‡	45.9 ± 1.27	3.48 ± 0.05‡	2.24 ± 0.07‡	36.0 ± 0.84‡	72.8 ± 1.06‡	553 ± 8
SCH $Lmna^{H222P/H222P}$ (n = 14)	0.49 ± 0.01	0.50 ± 0.02	46.5 ± 2.04	3.32 ± 0.04#	2.05 ± 0.030††#	38.2 ± 0.49§#	75.2 ± 0.61††	557 ± 11
6 months of age								
Untreated $Lmna^{+/+}$ (n = 7)	0.49 ± 0.01	0.50 ± 0.02	43.0 ± 2.09	3.28 ± 0.05	1.82 ± 0.04	42.7 ± 0.44	80.5 ± 0.44	519 ± 12
SCH $Lmna^{+/+}$ (n = 14)	0.49 ± 0.01	0.49 ± 0.01	45.8 ± 1.91	3.33 ± 0.04	1.87 ± 0.03	43.8 ± 0.39	81.2 ± 0.35	521 ± 8
Untreated $Lmna^{H222P/H222P}$ (n = 16)	0.46 ± 0.01	0.44 ± 0.01*	46.5 ± 1.38	3.66 ± 0.07‡	2.57 ± 0.10‡	30.0 ± 1.43‡	64.9 ± 2.06‡	555 ± 9
SCH $Lmna^{H222P/H222P}$ (n = 14)	0.46 ± 0.02	0.45 ± 0.01†	45.2 ± 2.54	3.44 ± 0.06#	2.23 ± 0.07§‖	35.4 ± 1.05§‖	71.6 ± 1.22§‖	558 ± 7
8 months of age								
Untreated $Lmna^{+/+}$ (n = 7)	0.47 ± 0.03	0.49 ± 0.02	42.4 ± 1.67	3.24 ± 0.05	1.80 ± 0.03	44.4 ± 0.76	82.3 ± 0.84	510 ± 16
SCH $Lmna^{+/+}$ (n = 14)	0.47 ± 0.01	0.47 ± 0.01	44.3 ± 1.33	3.36 ± 0.04	1.91 ± 0.03	43.0 ± 0.52	80.8 ± 0.45	539 ± 8
Untreated $Lmna^{H222P/H222P}$ (n = 16)	0.40 ± 0.01**	0.38 ± 0.01‡	47.4 ± 1.41	3.99 ± 0.07‡	3.16 ± 0.09‡	21.2 ± 1.15‡	51.1 ± 1.84‡	572 ± 9
SCH $Lmna^{H222P/H222P}$ (n = 14)	0.42 ± 0.01†	0.43 ± 0.01†‖	48.8 ± 2.60	3.80 ± 0.13††	2.72 ± 0.17§#	29.3 ± 2.03§‖	63.2 ± 3.20§‖	542 ± 9
10 months of age								
Untreated $Lmna^{+/+}$ (n = 7)	0.49 ± 0.01	0.47 ± 0.01	44.3 ± 1.45	3.32 ± 0.04	1.88 ± 0.03	43.6 ± 0.58	81.4 ± 0.38	529 ± 13
SCH $Lmna^{+/+}$ (n = 13)	0.49 ± 0.01	0.50 ± 0.01	46.6 ± 1.05	3.36 ± 0.03	1.93 ± 0.03	42.5 ± 0.41	80.4 ± 0.38	540 ± 11
Untreated $Lmna^{H222P/H222P}$ (n = 7)	0.35 ± 0.02‡	0.37 ± 0.03**	52.1 ± 4.09	4.40 ± 0.11‡	3.74 ± 0.14‡	15.1 ± 1.72‡	38.9 ± 3.83‡	506 ± 28
SCH $Lmna^{H222P/H222P}$ (n = 7)	0.42 ± 0.02††	0.43 ± 0.02††	54.4 ± 4.51	4.05 ± 0.16§	3.11 ± 0.21§#	23.6 ± 2.36§#	54.4 ± 4.21§#	547 ± 21

Cardiac function was evaluated by transthoracic echocardiographic analyses of the left ventricle (LV). The left ventricular mass (LVM) and the percentage of left ventricular fractional shortening (LVFS) were calculated as follows: [(IVSd + PWd + EDD)3 − EDD3] × 1.055 and (LVEDD − LVESD)/LVEDD × 100, respectively. *$p < 0.05$, **$p < 0.01$, and ‡$p < 0.001$, versus age-matched untreated $Lmna^{+/+}$ mice. †$p < 0.05$, ††$p < 0.01$, and §$p < 0.001$, versus age-matched SCH00013-treated $Lmna^{+/+}$ mice. #$p < 0.05$, ‖$p < 0.01$, and ¶$p < 0.001$, versus age-matched untreated $Lmna^{H222P/H222P}$ mice.

IVSd = interventricular septal wall thickness in diastole; LVEDD = left ventricular end-diastolic diameter; LVEF = left ventricular ejection fraction; LVESD = left ventricular end-systolic diameter; LVM = left ventricular mass; PWd = posterior wall thickness in diastole.

We next investigated the gene expression in the hearts from untreated and treated $Lmna^{+/+}$ and $Lmna^{H222P/H222P}$ female mice, because the beneficial effect of SCH00013 was prominent in female mice. In the hearts from untreated $Lmna^{H222P/H222P}$ mice, $Nppa$, $Nppb$, $Myh7$, and $Myl7$ messenger ribonucleic acids were significantly increased, and the upregulation of $Nppa$ and $Myl7$ was significantly reduced in the treated mice (Online Fig. S6). We also found increased messenger ribonucleic acid expression of proto-oncogene Fos and extracellular matrix remodeling-related genes $Tgfb1$, $Tgfb2$, and $Col1a2$ in the untreated $Lmna^{H222P/H222P}$ mice, whereas these changes were suppressed by the treatment (Online Fig. S6). Left ventricles from the untreated $Lmna^{H222P/H222P}$ mice showed 2.2-fold and 1.7-fold increases of Nppa and Mlc2 proteins, respectively, as compared with the untreated $Lmna^{+/+}$ mice, and the increased expression was suppressed by the treatment (Online Fig. S7). In addition, we investigated whether the apoptotic signal was induced by the $Lmna$ mutation, because there is an association among apoptosis, cardiac myocyte drop-out, ventricular remodeling, and deterioration of systolic performance in various experimental models of heart failure. However, the number of transferase-mediated dUTP nick-end labeling–positive cells was not increased in the hearts of $Lmna^{H222P/H222P}$ mice, and western blot analyses showed no or little expression of Fas-L or Fas proteins, respectively, in the $Lmna^{H222P/H222P}$ mice (Online Fig. S8). These results demonstrated that the apoptosis was not associated with the cardiac phenotypes in $Lmna^{H222P/H222P}$ mice and suggested that loss of cardiomyocytes was caused by cell death mechanisms other than the apoptosis.

The molecular mechanisms for the beneficial effect of SCH00013 remained unclear, but it might be related to the phosphodiesterase III activity. This possibility is unlikely, however, because SCH00013 inhibited the phosphodiesterase III activity at much higher concentration ($IC_{50} = 64.9$ μmol/l) than the concentration at which it produced the positive inotropic effect ($IC_{50} = 9.2$ μmol/l) in guinea pig hearts (3); and we showed that the plasma concentration of SCH00013 in the $Lmna^{H222P/H222P}$ mice ranged from 1 to 2 μmol/l, although we did not measure the concentration in the hearts. By contrast, because the Ca^{2+} sensitivity of cardiac muscle contraction was not decreased in the $Lmna^{H222P/H222P}$ mice at 3 months of age (Online Fig. S9), the Ca^{2+} sensitizing effect might not play a major role at the early stage, but the Ca^{2+} sensitizing effect of SCH00013 was enhanced in the stretched muscles (5), raising a possibility that the Ca^{2+} sensitivity in the failed heart might be different. Although the molecular mechanisms should be clarified, our findings implied that the Ca^{2+} sensitizer could be a plausible option for preventing disease progression of DCM.

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▶ **APPENDIX**

For supplementary information and supplementary figure legends, please see the online version of this article.

Letters to the Editor

A Meta-Analysis of Remote Monitoring of Heart Failure Patients

Structured disease management improves the prognosis of patients with chronic heart failure and has already been included in the current treatment guidelines. Along with better medication and increased use of defibrillators, planned periodic visits have also become routine in clinical practice. Remote patient monitoring (RPM) is a different type of structured disease management. Although the RPM systems (telephone support, network care, device-assisted monitoring) and health care environments are heterogeneous, the crucial difference from usual care is that RPM enables daily contact with healthcare experts and thus facilitates regular short-term evaluation of the disease status and early intervention. The elaborate meta-analysis by Klersy et al. (1) pointed out considerable benefits to be gained from RPM in terms