Animal preparation. Male C57BL/6J mice (8 wk old, weighing 22–24 g) were purchased from CLEA Japan, (Tokyo, Japan). After 1 wk of observation, either transverse aortic constriction (TAC) or a sham operation was performed as previously described (24). In brief, the transverse aorta was isolated between the carotid arteries and constricted by a 7-0 silk suture ligature tied firmly against a 30-gauge needle. Sham-operated mice underwent a similar surgical procedure without aortic constriction. The needle was promptly removed, and the chest was closed with a 5-0 silk suture. Each surgical procedure was completed within 30 min to maintain the body temperature at 37°C.

Experimental protocol. Vildagliptin was gifted by Novartis Pharmaceuticals (Basel, Switzerland). The sham-operated or TAC mice were randomly divided into two subgroups; the sham-operated group with (n = 10) or without vildagliptin (n = 10) and TAC with (n = 40) or without vildagliptin (n = 41). The vildagliptin treatment subgroups were provided with drinking water containing vildagliptin (10 mg·kg body wt^{-1}·day^{-1}) (39, 58), and the other groups received unsupplemented drinking water from 1 day postsurgery. The mice were allowed to drink ad libitum, and the drinking volumes were measured. The mice were fed a normal chow diet for 4 wk.

Echocardiography. Transthoracic echocardiography was performed before euthanasia as previously described (24). In brief, at 4 wk postsurgery, the mice were placed in a supine position without anesthesia. Short-axis, two-dimensional guided M-mode Doppler echocardiograms were captured and analyzed offline using a Vevo 770 High-Resolution in vivo Micro-Imaging System (VisualSonics, Toronto, Canada) equipped with a 15- to 45-MHz transducer. Left ventricular (LV) end-diastolic diameter (Dd), end-systolic diameter (Ds), and fractional shortening (FS) were measured. All measurements were made from leading edge to leading edge, according to the American Society of Echocardiography guidelines (22). Percentage FS was calculated as follows: %FS = [(LVDd − LVDs)/LVDd] × 100. The investigator performing and interpreting the echocardiograms was blinded to the subgroups.

Hemodynamic assessment. To confirm pressure overload, four to five mice in each group were randomly selected for LV pressure measurement, as previously described (27). In brief, under pentobarbital anesthesia, an endotracheal tube was inserted and connected to a volume-cycled rodent ventilator. A 1.4-Fr micromanometer-tipped catheter (Millar Instruments, Houston, TX) was inserted into the right carotid artery, blood pressure and heart rate were measured simultaneously, and data were acquired using the PowerLab Data Acquisition System (AD Instruments, Bella Vista, NSW, Australia).

Analysis of intraperitoneal glucose tolerance test. Four weeks after either TAC or sham operation, about half of the surviving mice, namely five mice in each sham-operated group and 12 and 8 mice in TAC with and without vildagliptin groups, were randomly selected for an intraperitoneal glucose tolerance test following overnight fasting (12–16 h). As overnight fasting and glucose injection might be stressful, we enrolled only half of the surviving mice to reduce the effect on additional deaths. Glucose (1 mg/kg body wt) was injected into the intraperitoneal cavity, as previously described (54). Blood was sampled from the tail prior to and at 30, 60, 90, and 120 min after glucose administration. Blood glucose concentrations were measured by a glucose meter using the glucose oxidase method (Glutest Ace R; Sanwa Kagaku Kenkyusho, Nagoya, Japan).

GLP-1 measurement [ELISA]. Four weeks after treatment, their chests were opened under anesthesia 1 h after feeding following overnight fasting for GLP-1 measurement by enzyme-linked immunosorbent assay (ELISA). Blood samples were obtained from the hearts and immediately collected in BD P700 tubes (Becton Dickinson, Franklin Lakes, NJ) containing EDTA and DPP-IV protease inhibitor cocktail. The tubes were centrifuged at 1,200 *g* for 10 min to extract plasma. The plasma samples were then stored at −80°C in a freezer until GLP-1 assay. Plasma GLP-1 levels were measured using a Glucagon-Like Peptide-1 (Active) ELISA Kit (Millipore, Billerica, MA) according to the manufacturer's instructions (52). The GLP-1 ELISA measures biologically active GLP-1-(7–37) and GLP-1-(7–36)-NH_2 but does not cross-react with glucagon, GLP-2, inactive GLP-1-(9–37) or GLP-1-(9–37)-NH_2.

Histology and immunohistochemistry. Histochemical analysis was performed as previously described (25). Briefly, the surviving mice were euthanized after 4 wk of observation. The hearts were harvested, and cardiac tissues were fixed with 4% paraformaldehyde. The fixed samples were embedded in paraffin and sectioned at 4-μm thickness for picrosirius red staining. The extent of myocardial collagen was analyzed in five hearts from each group (30). The original images were digitized and transformed into binary images, and each area was calculated using ImageJ software (NIH, Bethesda, MD). The total myocardial collagen index was defined as the total area of collagen content in the entire microscopic field divided by the total connective tissue area plus the myocardial area. The terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay was performed using an ApopTag Peroxidase In Situ Apoptosis Detection Kit (Millipore), according to the manufacturer's instructions. The number of TUNEL-positive cells was expressed as a percentage of total cells, as previously described (35).

Real-time quantitative polymerase chain reaction. Four weeks after TAC, murine ventricles were processed for total RNA isolation using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. First-strand cDNA was synthesized from 1 μg total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). The primers and probes used to quantify *transforming growth factor-1β (Tgf-1β)* and *glyceraldehyde 3-phosphate dehydrogenase (Gapdh)* were recommended by the manufacturer (Applied Biosystems). Real-time quantitative reverse transcriptase polymerase chain reaction (RT-PCR) was performed in a StepOne Real-Time PCR System (Applied Biosystems). From each amplification plot, a threshold cycle (Ct) value was calculated, representing the PCR cycle number at which fluorescence was detectable above an arbitrary threshold. Each sample was analyzed in duplicate, and the results were systematically normalized to GAPDH expression using the ΔΔCt method (29).

Western blot analysis. LV samples frozen at −80°C were placed on ice, homogenized, and lysed with lysis buffer [1% NP-40, 150 mM NaCl, 20 mM Tris pH 7.5, 2 mM EDTA, 50 mM NaF, 1 mM Na_3VO_4, plus protease inhibitor cocktail (Nacalai tesque, Kyoto, Japan)]. The supernatant was loaded onto 10%–15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels. Immunoblotting was performed as previously described (41). The ChemiDoc XRS System (Bio-Rad Laboratories, Hercules, CA) was used for chemiluminescence imaging. Primary antibodies against phospho-Smad2 (p-Smad2), p-Smad3, caspase-3, and cleaved caspase-3 primary antibodies were purchased from Cell Signaling Technology (Beverly, MA); anti-Smad2/3 primary antibody was purchased from BD Transduction Laboratories (Franklin Lakes, NJ); and anti-GAPDH (used as a loading control) primary antibody was purchased from Millipore. Target bands were identified using ECL prime and ECL Select Western blotting reagents (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Protein bands were quantified by densitometry.

Statistical analysis. All of the data are expressed as means ± SE and were analyzed by repeated-measures analysis of variance (ANOVA) followed by Bonferroni test and Student's t-test for paired and nonpaired data as appropriate. The differences in the number of surviving mice were analyzed by Kaplan-Meier method. P values of <0.05 were considered significant using JMP 8.0.1 software (SAS Institute, Cary, NC).

RESULTS

Hemodynamic measurements. The blood pressures and heart rates 4 wk after TAC were similar in the sham-operated groups with and without vildagliptin (71.2 ± 3.1 vs. 74.5 ± 3.2

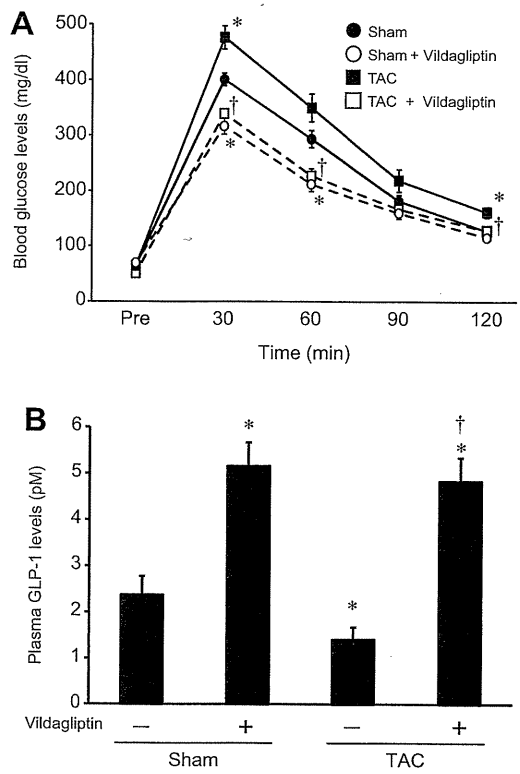

Fig. 1. *A*: plasma glucose levels measured by intraperitoneal glucose tolerance test. Sham-operated mice (n = 5), sham-operated mice with vildagliptin (n = 5), mice with transverse aortic constriction (TAC) (n = 8), and TAC mice with vildagliptin (n = 12) were enrolled. *B*: plasma levels of glucagon-like peptide-1 (GLP-1) 4 wk after TAC or sham operation. All blood samples were collected 1 h after feeding following overnight fasting. Sham-operated (n = 8), sham-operated mice with vildagliptin (n = 10), TAC mice (n = 15), and TAC mice with vildagliptin (n = 21) were measured. The data shown are means ± SE. *P < 0.05 vs. sham operated, †P < 0.05 vs. TAC.

mmHg; 459 ± 36 vs. 451 ± 22 beats/min; P = 0.482 and P = 0.830, respectively; n = 5 in each group), and in the TAC groups with and without vildagliptin (101.4 ± 9 vs. 121.4 ± 10 mmHg; 394 ± 80 vs. 463 ± 27 beats/min; P = 0.193 and P = 0.400; n = 5 and n = 4, respectively).

Intraperitoneal glucose tolerance test and plasma GLP-1 levels. Figure 1*A* shows the results of the intraperitoneal glucose tolerance test. Blood glucose levels at 30 and 120 min after intraperitoneal glucose injections were higher in the TAC mice than in the sham-operated mice (TAC vs. sham operated: 476.4 ± 20.9 vs. 400.5 ± 11.2 mg/dl at 30 min, and 161.5 ± 9.4 vs. 127.3 ± 8.3 mg/dl at 120 min, n = 8 and 5; P < 0.05). This was consistent with our previous report (27) in which TAC mice exhibited impaired glucose tolerance. Vildagliptin administration decreased blood glucose levels at each time point after glucose injection in TAC mice (with vs. without vildagliptin: 345.1 ± 7.9 vs. 476.4 ± 20.9 mg/dl at 30 min, 245.5 ± 13.1 vs. 349.6 ± 25.3 mg/dl at 60 min, and 127.9 ± 8.5 vs. 161.5 ± 9.4 mg/dl at 120 min, n = 12 and 8; P < 0.05).

We evaluated the GLP-1 levels in the TAC mice with or without vildagliptin. Because ad libitum feeding could have affected the plasma GLP-1 levels, we conducted a preliminary experiment to identify the optimal conditions for GLP-1 measurement. Nine-week-old C57BL6/J mice were fed a normal chow diet, divided into two groups, and treated with or without vildagliptin for 4 wk, as described above. To evaluate whether feeding affected the plasma GLP-1 levels, the mice were fasted 12 h before blood sampling. We randomly separated each group into two subgroups; the two subgroups were fasted further, and the others were allowed to feed 1 h before sampling. Under fasting conditions, vildagliptin produced a statistically insignificant increase in GLP-1 levels (with vs. without vildagliptin: 5.19 ± 1.04 vs. 3.93 ± 0.70 pM, n = 5 each; P > 0.05). In the mice sampled 1 h after feeding, GLP-1 levels were elevated with in the vildagliptin group (with vs. without vildagliptin: 9.13 ± 2.35 vs. 4.47 ± 0.87 pM, n = 4 and 5; P < 0.05), suggesting that this sampling time schedule, i.e., 1 h after food intake, was suitable for plasma GLP-1 measurement. Figure 1*B* shows the plasma GLP-1 levels under this time schedule. The GLP-1 levels were decreased in the TAC mice (sham operated vs. TAC: 2.37 ± 0.40 vs. 1.41 ± 0.26 pM, n = 8 and 15; P < 0.05), but elevated in TAC mice with vildagliptin to the levels of sham-operated mice with vildagliptin (with vs. without vildagliptin: 4.83 ± 0.50 vs. 1.41 ± 0.26 pM, n = 21 and 15; P < 0.05).

Echocardiography. Representative echocardiographic images are shown in Fig. 2. Echocardiographic analysis revealed enlarged Dd and Ds in the TAC mice both with and without vildagliptin (n = 27 and 17). Both LV dilatation and dysfunction in the TAC group were ameliorated by vildagliptin treatment (Fig. 3).

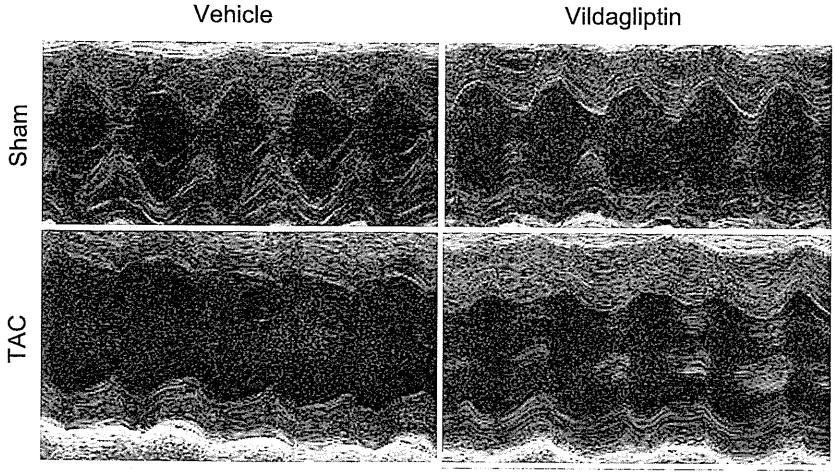

Fig. 2. Representative M-mode echocardiograms of mice 4 wk after TAC or sham operation. Top left, sham operated; top right, sham operated with vildagliptin; bottom left, TAC; bottom right, TAC with vildagliptin.

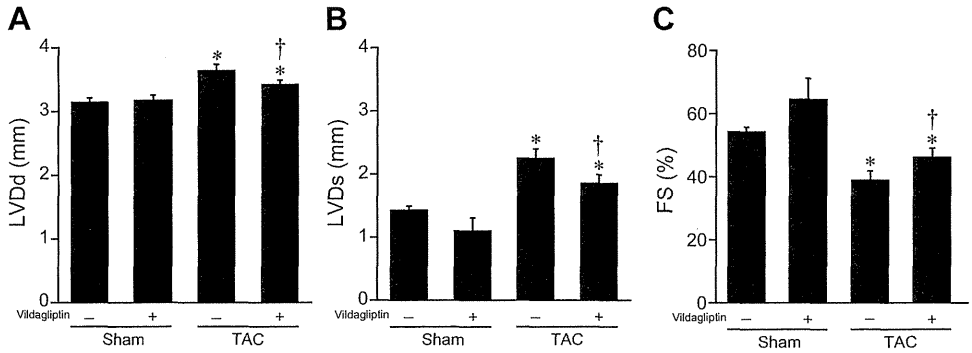

Fig. 3. *A* and *B*: left ventricular (LV) dimensions. *C*: fractional shortening (FS) 4 wk after TAC or sham operation. LV end-diastolic diameter (Dd), end-systolic diameter (Ds), and FS measured by echocardiography at 4 wk after TAC or sham operation. Echocardiographic analysis revealed enlarged LV end-diastolic and end-systolic diameters in both of the TAC groups compared with the sham-operated mice. Both LV dilatation and dysfunction in the TAC group were ameliorated by vildagliptin treatment. Sham-operated mice (n = 5), sham-operated mice with vildagliptin (n = 5), TAC mice (n = 17), and TAC mice with vildagliptin (n = 27) were examined. LVDd, LV end-diastolic dimension; LVDs, LV end-systolic dimension. Data shown are means ± SE. *$P < 0.05$ vs. sham operated, †$P < 0.05$ vs. TAC.

Water uptake and heart weight. Body weight was not statistically different between the groups: 24.3 ± 1.7 g and 23.4 ± 1.4 g in the sham-operated and TAC mice without vildagliptin (n = 10 and 17), 25.8 ± 1.7 g and 25.2 ± 2.4 g in the sham-operated and TAC mice with vildagliptin (n =10 and 27). Heart weight-to-body weight ratio (HW/BW) markedly increased in the TAC group compared with the sham-operated group (4.9 ± 0.1 vs. 9.3 ± 0.5 for sham-operated and TAC mice without vildagliptin, respectively; $P < 0.05$), and vildagliptin did not attenuate HW/BW (9.2 ± 0.4 for TAC with vildagliptin; Fig. 4). Volumes of vildagliptin solution or water consumed were similar in all of the groups (5.2 ± 0.2, 4.9 ± 0.5, 4.4 ± 0.9, and 4.5 ± 1.3 ml/day for sham-operated with and without vildagliptin and TAC with and without vildagliptin, respectively; $P \geq 0.05$ for all).

Apoptosis and fibrosis. We performed TUNEL staining to clarify the degree of apoptosis in the murine hearts. Apoptosis in the myocardium of the TAC mice was increased compared with the sham-operated mice, and this increase in apoptotic cell death was largely attenuated by vildagliptin (Fig. 5, *A* and *B*, n = 4 per each group). Next we performed immunoblotting to confirm apoptotic changes in protein levels. We observed increased cleaved caspase-3 protein in pressure-overloaded murine hearts, which was partially ameliorated by vildagliptin

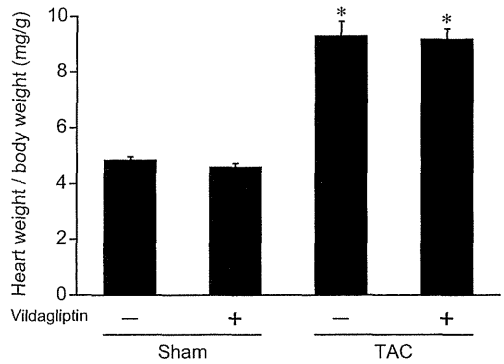

Fig. 4. Heart weight (mg)/body wt (g) ratio 4 wk after the TAC or sham operation. Sham-operated mice (n = 10), sham-operated mice with vildagliptin (n = 10), TAC mice (n = 17), and TAC mice with vildagliptin (n = 27) mice were measured. The values shown are means ± SE. *$P < 0.05$ vs. sham operated.

(Fig. 5, *C* and *D*, n = 4 per each group). These findings indicate that vildagliptin partly reduces myocardial apoptosis in pressure-overloaded murine hearts.

Figure 6*A* shows increases in myocardial collagen in the TAC group, also ameliorated by vildagliptin. Figures 6*B* shows fibrotic areas identified by picrosirius red staining. The total myocardial interstitial collagen area significantly increased in the TAC group compared with the sham-operated groups ($P <$ 0.05 vs. sham operated, n = 5 per each group) but was decreased in the TAC with vildagliptin group ($P < 0.05$ vs. TAC) (sham operated, 1.79 ± 0.22%; sham operated with vildagliptin, 1.77 ± 0.20%; TAC, 12.12 ± 0.27%; TAC with vildagliptin, 8.02 ± 1.84%). We next analyzed expression of *Tgf-1β*, a fibrosis-related gene, using RT-PCR. Myocardial *Tgf-1β* expression significantly increased in the TAC group compared with that in the sham-operated group ($P < 0.05$ vs. sham operated) but significantly decreased in the groups with vildagliptin ($P < 0.05$ vs. TAC; sham operated, 1 ± 0.08; sham operated with vildagliptin, 0.98 ± 0.11; TAC, 1.85 ± 0.12; TAC with vildagliptin, 1.55 ± 0.06; Fig. 6*C*, n = 3 per each group).

Finally, we performed immunoblotting to verify the fibrotic changes that arose through increased levels of TGF-β pathway proteins. We observed increased p-Smad2 and p-Smad3 protein levels in the pressure-overloaded murine hearts, which were partially restored by vildagliptin (Fig. 7, n = 4 per each group). These findings indicate that vildagliptin reverses myocardial fibrosis via the TGF-β pathway in murine pressure-overloaded hearts.

Survival analysis. The number of TAC mice without vildagliptin was 41 and the number of those with vildagliptin was 40. Only 17 (41.5%) TAC mice without vildagliptin survived 28 days, whereas 27 (67.5%) TAC mice with vildagliptin survived 28 days (Fig. 8; $P < 0.05$). These data indicate that vildagliptin treatment is strongly protective. Vildagliptin did not affect the survival rate in the sham-operated mice.

DISCUSSION

This study was the first to demonstrate that a DPP-IV inhibitor improved survival rate in mice with pressure-overloaded HF. We presented the following experimental evidence: *1*) TAC exacerbated the development of impaired glucose

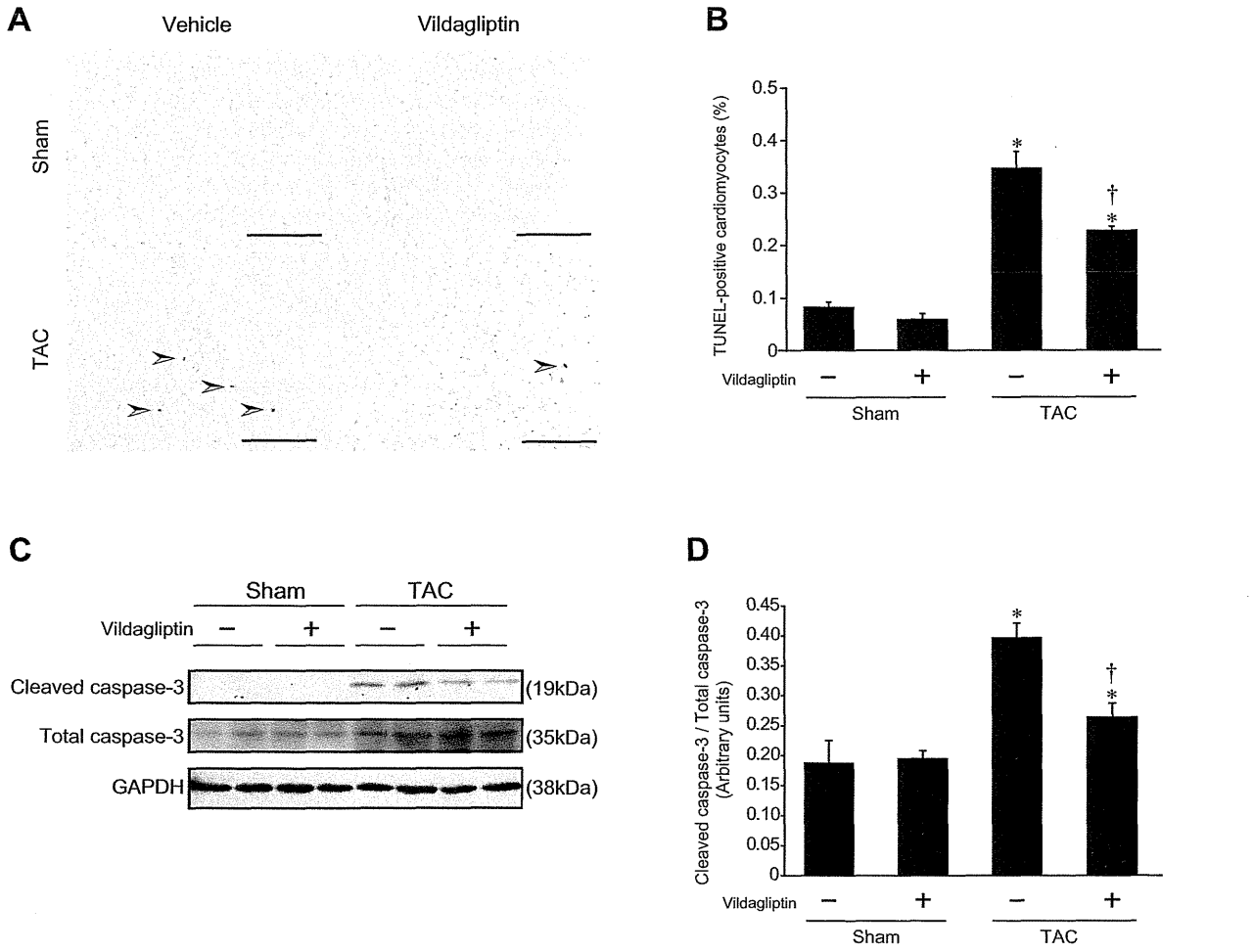

Fig. 5. *A*: representative images of the terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL)-positive cells in murine hearts. Top left, sham operated; top right, sham operated with vildagliptin; bottom left, TAC; bottom right, TAC with vildagliptin. Evidence of apoptosis, including chromatin condensation, is indicated with arrowheads. The proportion of TAC-induced apoptotic cells was decreased by vildagliptin. Bar = 200 μm; original magnification, ×200. *B*: quantitative analysis of murine apoptotic cardiomyocytes. TAC increased the number of apoptotic cells in the myocardium compared with the sham-operated group, and vildagliptin attenuated the increase in apoptosis. $n = 4$ for each group. *C*: representative immunoblotting analysis of cleaved/total caspase-3 and GAPDH in the hearts of sham-operated and TAC mice with or without vildagliptin. *D*: intensity of bands was quantified from four independent experiments by densitometry. Cleaved/total caspase-3 protein levels were increased by pressure overload in the hearts of TAC mice, which were reversed in TAC mice with vildagliptin. $n = 4$ for each group. The values shown are means ± SE. *$P < 0.05$ vs. sham operated, †$P < 0.05$ vs. TAC.

tolerance, which was attenuated by vildagliptin with an attendant increase in total GLP-1 levels; *2*) TAC induced myocardial apoptosis and fibrosis, which were attenuated by vildagliptin; *3*) TAC increased LVDd and LVDs, leading to FS decline, while vildagliptin attenuated increased LVDd and LVDs and increased LVFS. These effects may contribute to the improvement in survival rate generated by vildagliptin in mice with pressure overload-induced HF.

We demonstrated that TAC exacerbated the development of impaired glucose tolerance, which was attenuated by vildagliptin. This result implies that HF causes impaired glucose tolerance and improvement of impaired glucose tolerance may ameliorate HF severity. Glycemic control independently correlates with reduced LV contractile reserve and positivity for HF in diabetic patients (12, 28). We previously reported that HF is associated with impaired glucose tolerance in mice and dogs, and that correction of impaired glucose tolerance with voglibose or metformin reduces HF severity (26, 27, 41). Shimizu et al. (46) reported that systolic dysfunction induced by pressure overload exacerbates plasma glucose and hepatic insulin resistance via Akt and insulin signaling in rodents. In humans, chronic HF is associated with hyperinsulinemia (36, 51). Insulin resistance observed in HF is partly due to the lack of activity and increase in weight gain/fat redistribution. Stolen et al. (49) showed that exercise training improved insulin-stimulated myocardial glucose uptake in patients with dilated cardiomyopathy. Ashrafian et al. (2) proposed the other mechanism of HF-induced insulin resistance. Hyperadrenergic state of HF initiates the elevation of plasma free fatty acids (FFAs). The elevation of plasma FFAs induces insulin resistance due to increased triglycerides, increased cellular FFAs, and increased cytoplasmic fatty acid metabolites in hearts and skeletal muscle (43).

To our knowledge, this is the first study to evaluate an improvement in impaired glucose tolerance in animals with HF in the presence of DPP-IV inhibitors. Indeed, vildagliptin increased the plasma GLP-1 levels in animals with TAC-induced HF, suggesting that HF is attenuated by the correction

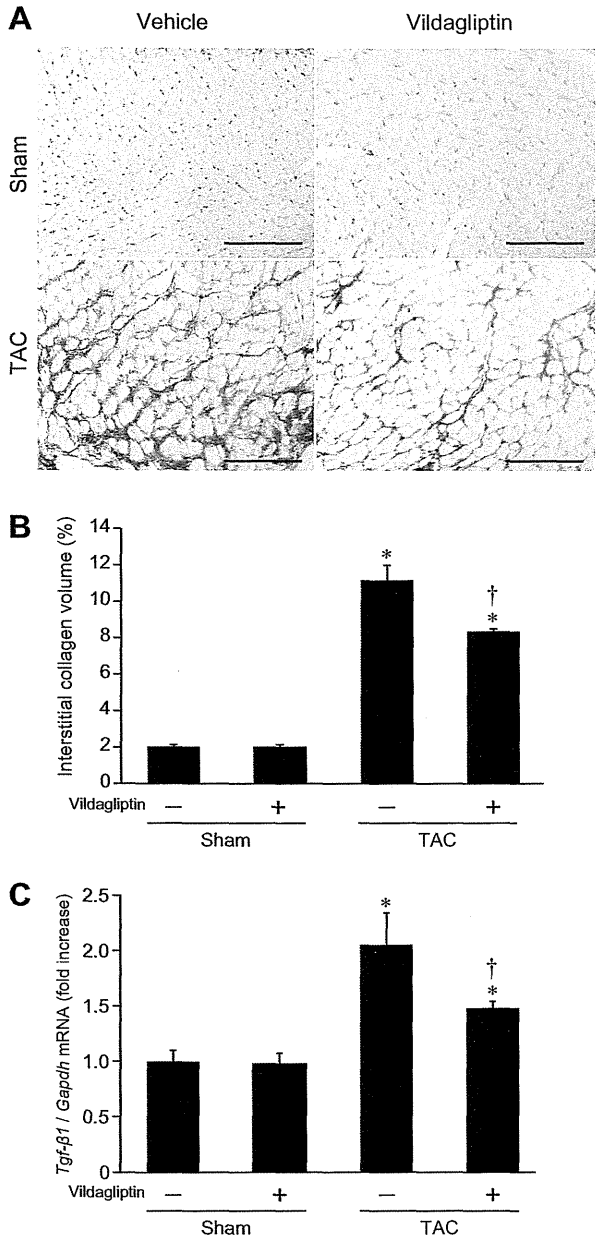

Fig. 6. *A*: representative images of the murine myocardium stained by picrosirius red. Collagen accumulation induced by TAC was regressed with vildagliptin. Top left, sham operated; top right, sham operated with vildagliptin; bottom left, TAC; bottom right, TAC with vildagliptin. Bar = 100 μm; original magnification, ×400. *B*: quantitative analysis shows that vildagliptin ameliorated myocardial collagen deposition resulting from pressure overload. $n = 5$ for each group. *C*: quantitative analysis of *transforming growth factor-1 β (Tgf1β)* in murine hearts: the expression level (normalized to *Gapdh*) in TAC group was increased compared with that in sham operated, which was alleviated in TAC with vildagliptin. $n = 3$ for each group. Data are presented as the relative change vs. sham operated. The values shown are means ± SE. $^*P < 0.05$ vs. sham operated, $\dagger P < 0.05$ vs. TAC.

of glucose intolerance by DPP-IV inhibitors. This hypothesis is supported by our findings that vildagliptin attenuates LV apoptosis and fibrosis in the TAC mice, which may explain the amelioration of LV dilatation and dysfunction. This evidence is consistent with previous studies in which sitagliptin was shown to attenuate HF severity induced by rapid pacing in pigs (14), ameliorate myocardial fibrosis in diabetic ($db/db^{-/-}$) mice (23), and improve diastolic dysfunction without altering ejection fraction in a rat model of uremic cardiomyopathy (9).

Intriguingly, GLP-1 reportedly has cardioprotective properties besides its ability to correct glucose intolerance in HF. GLP-1 receptors are expressed in the heart and activate PI3 kinase and Akt in addition to cyclic AMP (6, 19). Protein kinase A activation via accumulation of cyclic AMP may activate p38 MAP kinase, which may in turn mediate cardio-

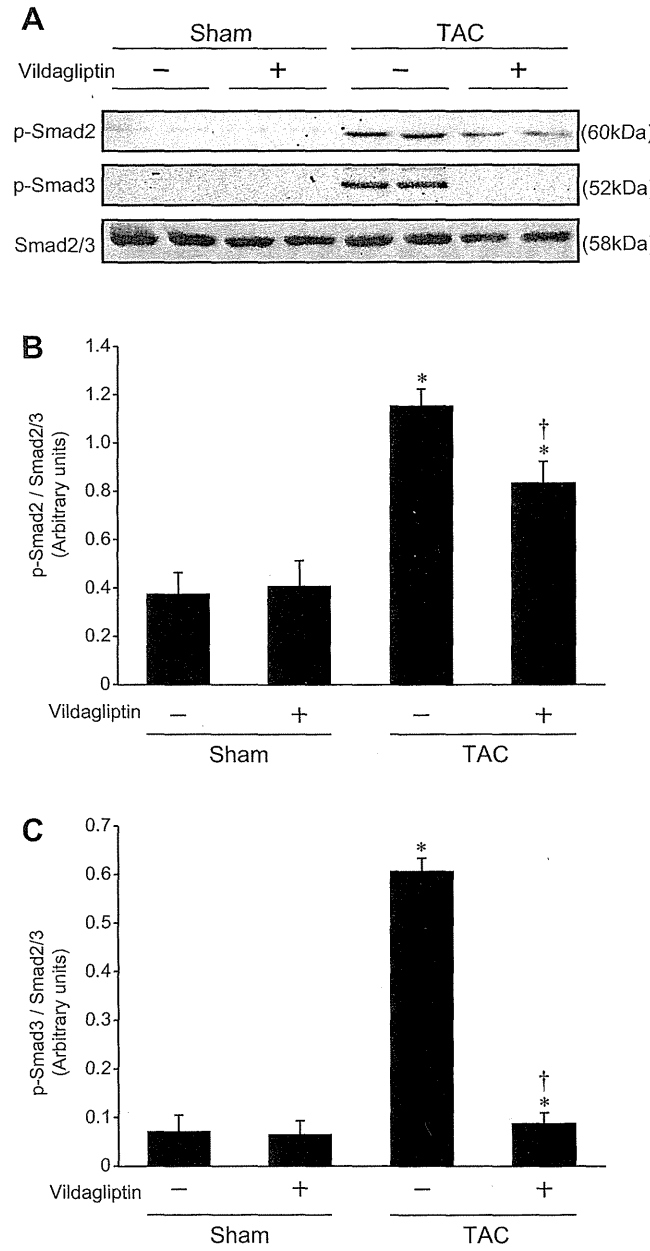

Fig. 7. *A*: representative immunoblotting analysis of phosphorylated Smad2 (p-Smad2), p-Smad3, and Smad2/3 in the hearts of sham-operated and TAC mice with or without vildagliptin. *B*: band intensity quantified by densitometry. p-Smad2/Smad2/3 protein levels increased as a result of pressure overload in the hearts of TAC mice, but recovered in TAC mice with vildagliptin. *C*: band intensity quantified by densitometry. p-Smad3/Smad2/3 protein levels increased as a result of pressure overload in the hearts of the TAC mice, but recovered in the TAC mice with vildagliptin. $n = 4$ for each group. The values shown are means ± SE. $^*P < 0.05$ vs. sham operated, $\dagger P < 0.05$ vs. TAC.

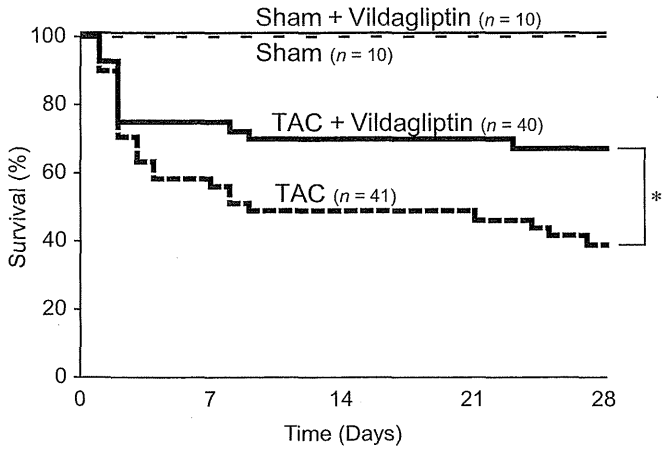

Fig. 8. The Kaplan-Meier curve analysis shows that the TAC group with vildagliptin exhibited improved survival compared with the TAC group without vildagliptin. We enrolled 41 mice in the TAC and 40 mice in the TAC with vildagliptin groups, respectively. The survival rates at 28 days after the TAC operation were 41.5% (17/41) in the TAC and 67.5% (27/40) in the TAC with vildagliptin groups, respectively. *$P < 0.05$ vs. TAC; *n*, number of mice.

protection (40, 59), and activation of PI3 kinase and Akt may further enhance these cardioprotective effects. A recent report (55) demonstrated amelioration of nonalcoholic steatohepatitis in mice by an analog of exenatide, a GLP-1 receptor agonist, supporting the antifibrotic effect of GLP-1 in murine hearts. Indeed, GLP-1 administration in patients with HF decreased the HF severity with or without DM, suggesting that GLP-1 may have cardioprotective properties independent of its effects on blood glucose levels (48). However, GLP-1 levels were elevated ~10-fold (17), significantly higher than the GLP-1 levels observed with DPP-IV inhibitors (4), suggesting that even a 1-pM increase in GLP-1 may be sufficient for cardioprotection. Moreover, in a large meta-analysis, vildagliptin was not associated with an increased risk of adjudicated cardio- and cerebrovascular events relative to all comparators in the patients with type 2 diabetes, including increased cerebrovascular risks (44). Chaykovska et al. (9) showed that increased *Tgf-β1*, *collagen type I α1*, and *collagen type III α1* expression in uremic rat hearts, compared with the sham-operated rat hearts, was significantly reduced by linagliptin, a DPP-IV inhibitor, supporting our observations. Importantly, DPP-IV inhibitors impact cardioprotection independently of GLP-1; DPP-IV also reportedly degrades peptides tyrosine-tyrosine, stromal cell-derived factor-1, and B-type natriuretic peptide (BNP) (7, 32, 45, 47). Taken together, these data suggest that DPP-IV inhibitors are cardioprotective, suggesting that they may also be beneficial for patients with HF. However, this hypothesis is limited because we used 8-wk-old mice with TAC as a model of HF in this study. Although this model is one of established animal models for HF, this model is not the universal model for the patients with HF, or does not mimic the background of the HF patients (e.g., age, dyslipidemia, ischemia, etc.). Further basic and preclinical studies are needed to apply DPP-IV inhibitors to HF patients.

The most important issue in this study was to determine whether DPP-IV inhibitors increase the survival rate because improvements in HF do not necessarily increase the survival rate. Indeed, inotropic agents such as phosphodiesterase III inhibitors (e.g., vesnarinone) improved pathophysiological parameters of HF in basic studies, even improving symptoms and cardiac function in patients with HF in clinical studies, but these drugs actually decreased the patient survival rate in large-scale clinical trials (10). This unexpected finding is attributable to the fact that the effect of these drugs on survival rate was never tested in experimental models of chronic HF. Yin et al. (60) reported the rat models with the administration of vildagliptin 2 days before or 3 wk after acute myocardial infarction surgery. They did not show the cardiac contractility and survival or any change in glucose metabolism with vildagliptin treatment. Compared with their protocol, we administered vildagliptin from 1 day postsurgery of murine TAC, which finally reversed the survival rate. These discrepancies between the study of Yin et al. and our present study may be attributable to the manner of HF induction (e.g., models and species), their glucose levels, and the dosage of vildagliptin. Their echocardiographic data seem to show worse HF than ours, which was too severe to treat with their dose set. In addition, although they did not mention any condition of the feeding (e.g., fasting or ad libitum feeding) during sampling, no difference in their blood glucose levels may suggest that the dosage was not enough to be cardioprotective. We observed that vildagliptin increased survival rate in the context of pressure overload-induced HF in mice, indicating that an adequate dose of DPP-IV inhibitors is ultimately cardioprotective against HF.

This study includes the limitations. Since TAC animals are fragile, especially when using the narrower size of needle (30 gauge) to create severe HF, the procedures of the examination such as glucose tolerance test may worsen the HF of the TAC mice. Indeed, in the preliminary study, we tried to perform oral glucose tolerance test at first, but 2 of 6 died because of the onset of acute severe HF (pulmonary edema shown by dissection). This is the reason that we shifted to the intraperitoneal glucose tolerance test, which did not cause severe HF leading to death. Importantly, the timing and number of procedures were identical in the groups with or without vildagliptin, suggesting that these additional stresses of examination to TAC do not largely affect the present results and conclusions.

In conclusion, vildagliptin, a DPP-IV inhibitor, improved the pathophysiology of HF in pressure-overloaded mice. This effect was mediated partly by improved glucose tolerance and partly by the cardioprotective effects of GLP-1, both of which ultimately improved survival following HF.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.T., M.A., and M.K. conception and design of research; A.T., S.I., Y.Y., and Y.L. performed experiments; A.T., S.Y., and M.K. analyzed data; A.T., S.I., K.-D.M., K.S., and Y.Y. prepared figures; A.T. drafted manuscript; A.T., S.I., K.-D.M., K.S., S.S., Y.A., S.T., T.M., and H.A.

edited and revised manuscript; S.I., K.-D.M., Y.L., H.I.-U., and M.K. interpreted results of experiments; N.M. and M.K. approved final version of manuscript.

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ORIGINAL ARTICLE

Derivation of a mathematical expression for predicting the time to cardiac events in patients with heart failure: a retrospective clinical study

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The prognoses for patients with certain diseases are estimated by averaging the results of clinical trials. To investigate the possibility of deriving a mathematical formula for the estimation of prognosis, we formulated the equation $\tau = f(x_1, \ldots, x_p)$, where $x_1, \ldots, x_p$ are clinical features and τ represents the clinical outcome for heart failure (HF). We attempted to determine the function to mathematically formulate the relationship between clinical features and outcomes for these patients. We followed 151 patients (mean age: 68.6 ± 14.6 years; men: 61.6%) who were consecutively hospitalized and discharged as a result of acute decompensated HF (ADHF) between May 2006 and December 2009. The mathematical analysis was performed through a probabilistic modeling of the relational data by assuming a Poisson process for rehospitalization owing to HF and by linearly approximating the relationship between the clinical factors and the mean elapsed time to rehospitalization. The former assumption was validated by a statistical test of the data, and the contribution of each parameter was assessed based on the coefficients of the linear relation. Using a regularization method to analyze 402 clinical parameters, we identified 252 factors that substantially influenced the elapsed time until rehospitalization. With the probability model based on the Poisson process, the actual (X; 388 ± 377 days) and estimated (Y; 398 ± 381 days) elapsed times to rehospitalization were tightly correlated ($Y = 1.0076X + 6.5531$, $R^2 = 0.9879$, $P < 0.0001$). We established a mathematical formula that closely predicts the clinical outcomes of patients who are hospitalized with ADHF and discharged after appropriate treatment.
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Keywords: heart failure; mathematical model; prognosis; rehospitalization

INTRODUCTION

Studies show that numerous factors, including disease severity, treatment protocols and the environment, independently determine patients' prognoses. For example, in patients with chronic heart failure (CHF), many studies have shown that various independent indices of the severity of CHF, such as plasma B-type natriuretic peptide (BNP) level, left ventricular function, exercise tolerance or New York Heart Association (NYHA) functional class affect the time to hospitalization or cardiac death.[1–5] However, because we could not identify the elapsed time until hospitalization in certain patients with CHF, we estimated this time using knowledge of the pathophysiology of CHF, our experience with previous comparable patients and Kaplan–Meier plots of their hospitalization in the clinical studies; we then explained our estimation to each patient. This procedure led us to conclude that estimating the elapsed time to rehospitalization is a type of problem that is specific to clinical medical science because the results and outcomes of biology or basic medical sciences can be derived from mathematically formulated equations. Furthermore, other fields of basic science, such as physics and mathematics or applied sciences, such as mechanics, thermodynamics and fluid dynamics, are mathematically formulated; the observational phenomena in applied sciences other than medical science can be predicted by mathematical equations, for example, the law of universal gravitation.[6] The most important issue in deriving a mathematical expression for relationships among two or more factors is the prediction of the future value of one variable based on the other factor(s). All phenomena, such as the severity of CHF and the patients' characteristics before the occurrence of clinical events, may therefore provide a mathematical equation for the clinical outcome if we can relate factors in the patient's clinical status to clinical outcomes such as rehospitalization.

To investigate this possibility, we sought to solve the equation $\tau = f(x_1, \ldots, x_p)$, where $x_1, \ldots, x_p$ represent clinical features affecting the clinical outcome for CHF. We attempted to determine the

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function (f) to yield τ, the time to rehospitalization, from the clinical parameters (x_1, ..., x_p) reflecting patient characteristics at the time of discharge.

METHODS

Ethics statement

This study was approved by National Cerebral and Cardiovascular Center Research Ethics Committee. The Committee decided that the acquisition of informed consent from the 151 subjects was not required according to the Japanese Clinical Research Guideline because this was a retrospective observational study. Instead, we made a public announcement in accordance with the request of the Ethics Committee and the Guideline.

Subjects and clinical parameters

A total of 486 patients with acute decompensated heart failure (ADHF) were admitted between May 2006 and December 2009. Because patients who were admitted for ADHF only once were excluded, the remaining 151 patients were included in this study. The oldest hospitalization was adopted regarding repeat patients during this study. The diagnosis of HF was confirmed by an expert team of cardiologists using the Framingham criteria.[7] Careful history-taking, physical examinations, laboratory tests, chest X-rays, electrocardiograms, Doppler echocardiographic studies, coronary angiography and right heart catheterization were performed during the hospitalization. The timing of patient discharge was determined by the expert team of cardiologists in charge of the HF department; discharge was recommended when the patients presented no signs of decompensation, such as NYHA functional class <3, no sign of rales, no galloping rhythm, stable blood pressure and an improvement in renal function due to an optimal treatment that followed international guidelines.[8] Rehospitalization for the enrolled patients was defined as hospitalization for decompensated HF. The primary end point was the first rehospitalization for decompensated HF.

Cardiac catheterization

Left ventricular pressure was recorded with a 5-F pigtail catheter. Left ventricular volume and ejection fraction were determined with left ventriculography with a contrast medium using Kennedy's formula. Right-sided catheterization was performed using a 7 F Swan–Ganz catheter to measure pulmonary capillary wedge pressure, mean pulmonary artery pressure (PAP), right ventricular end-diastolic pressure and mean right atrial pressure. Cardiac output was measured using the estimated Fick principle and the Thermal dilution. Systemic vascular resistance and pulmonary vascular resistance were calculated using the established formulas: systemic vascular resistance = 80 × (mean pulmonary artery pressure − mean right atrial pressure)/cardiac output and pulmonary vascular resistance = 80 × (mean pulmonary artery pressure − pulmonary capillary wedge pressure)/cardiac output.

Echocardiography

Echocardiographic examinations were performed with a Sonos-5500 (Philips Medical System, Andover, MA, USA), Alpha 10 (Hitachi-Aloka Medical, Tokyo, Japan), Vivid 7 Dimension (GE Healthcare, Buckinghamshire, UK), ACUSON Sequoia C256 (Mochida Simens Medical System, Tokyo, Japan) or Aplio XV (Toshiba Medical Systems, Tochigi, Japan) machine with a 2.5-MHz probe. Patients underwent a Doppler echocardiographic study for HF at admission and before discharge. Standard views were recorded, including the parasternal long-axis, short-axis and apical 4- and 2-chamber views, and cardiac chamber sizes and left atrial dimensions were evaluated according to the recommendations of the American Society of Echocardiography.[9] The severity of valve regurgitation was quantified on a semicontinuous scale from none (0) to severe.[4] Pulsed-wave Doppler examination and Doppler tissue imaging of the mitral annulus was performed. The peak mitral early diastolic inflow and atrial filling (E and A) velocities and the E-wave deceleration time were obtained. The sample volumes of the pulsed Doppler tissue imaging were determined at the septal and lateral margins of the mitral annulus. The peak early mitral annular velocities were measured, and then the average values of the septal and lateral velocities were used as E'.

The mathematical model for the rehospitalization process

To construct a model for future rehospitalization using the basic clinical factors for the patients, we adopted two working assumptions for the practical rehospitalization process.

Assumption 1. A mean elapsed time τ_i from discharge to the rehospitalization of patient i depends on some of the given clinical factors $X^i = \left\{x_1^i, \ldots, x_p^i\right\}$ of the patient, that is, a common subset $X_S^i \subseteq X^i$ over all patients. The dependency is primarily approximated by the following inverse linear relation:

$$\tau_i \cong \frac{1}{\sum_{x_j^i \in S^i} \beta_j x_j^i + \gamma} \tag{1}$$

where the denominator represents the expected frequency of cardiovascular rehospitalization per day, X_S^i is a set of values of the factors in X_S for patient i, β_j is the contributing weight of the jth factor to the frequency and γ is the intrinsic frequency for any patient.

Assumption 2. The clinical factors X_S^i of patient i are fairly stable between discharge and rehospitalization. Thus, the expectation value of the mean elapsed time τ_i remains nearly constant for patient i. As any event occurring with a constant frequency in a given time period is generated by a Poisson process,[10] rehospitalization also occurs via this process under Assumption 2. Thus, the probability density $p_i(t)$ for the rehospitalization of patient i at an elapsed time t after discharge is represented by the following exponential formula:

$$p_i(t) = \frac{1}{\tau_i} \exp\left(-\frac{t}{\tau_i}\right) \tag{2}$$

The parameter τ_i is given by Equation (1) according to Assumption 1.

We next describe the assumption test. Assumption 1 is limited to the relationship between the parameter τ_i and the clinical factors X_S^i. If the accuracy of the approximation is insufficient, we can easily extend it to a nonlinear relation such as a higher-order polynomial. Assumption 2 essentially characterizes the process of the occurrence of rehospitalization and defines the formula for its probability density $p_i(t)$. Accordingly, before the modeling of the rehospitalization process based on a given data set, a test should be applied to verify that Assumption 2 actually holds true for the given data set.

With n samples in the data set $D = \{(X^i, \tau_i) | i = 1, \ldots, n\}$, where X^i is the set of clinical factor values for patient i, and τ_i is the elapsed time at rehospitalization after discharge, we first compute a histogram of the rehospitalization occurrences over t, that is, the number of rehospitalization occurrences $\hat{m}_k$ in each elapsed time interval $((k-1)\Delta t, k\Delta t)$ $(k = 1, \ldots, q)$ in the data set. The number of equal-width bins q into which to partition the sample range $[0, q\Delta t]$ is appropriately chosen to be $q = \sqrt{n}$. (Venables and Repley)[11] We also expect a certain value of $\hat{m}_k$ by Equation (2) under Assumption 2. The value $\hat{m}_k$ computed from the data set and its value expected by Equation (2), m_k, should be consistent if Assumption 2 holds for the data set. Consistency with m_k and $\hat{m}_k$ is evaluated by the following G-score:[12]

$$G = 2\sum_{k=1}^{q} \hat{m}_k \ln \frac{\hat{m}_k}{m_k} \tag{3}$$

Because this G-score is known to follow a χ^2 distribution of degree $q-2$, we applied a χ^2-test to the null hypothesis that the histogram of the given data set is consistent with Equation (2), that is, that Assumption 2 holds true for the data set. If the P-value of the test is less than a specific risk level a such as $a = 0.05$, we conclude that Assumption 2 does not hold for the data set. This G-test is known to be more rigorous than the well-known Pearson's χ^2-test.

Thus, our problem was to derive the expectation valuem_k $(k = 1, \ldots, q)$ from Equation (2). We considered that τ_i of the patients in D are sampled from a common population distribution $p_\tau(\tau)$. Therefore, the total probability

distribution of the rehospitalization time $P(t)$ is expected to be a superposition of Equation (2) for various τ sampled from $p_\tau(\tau)$, as follows, where $p(t)$ is $p_i(t)$ in Equation (2) for a general τ:

$$P(t)=\int_0^\infty p_\tau(\tau)p(t)\mathrm{d}\tau=\int_0^\infty p_\tau(\tau)\frac{1}{\tau}\exp\left(-\frac{t}{\tau}\right)\mathrm{d}\tau$$

We use the following natural conjugate prior distribution for the unknown $p_\tau(\tau)$:

$$p_\tau(\tau)=\frac{\tau^{-n}\exp\left(-1/\tau\sum_{i=1}^{n}\tau_i\right)}{\int_0^\infty \tau^{-n}\exp\left(-1/\tau\sum_{i=1}^{n}\tau_i\right)\mathrm{d}\tau}$$

where τ_i is given by the data set D. The selection of this parameter distribution is widely considered to be reasonable in Bayesian statistics because it preserves the exponential shape of the distribution of elapsed times t.[13] After several manipulations, the following $P(t)$ is derived:

$$P(t)=\frac{(n+1)\left(\sum_{i=1}^{n}\tau_i\right)^{n+1}}{\left(\sum_{i=1}^{n}\tau_i+t\right)^{n+2}}$$

Accordingly, the expectation m_k is given by the accumulation of $P(t)$ over $((k-1)\Delta t, k\Delta t]$ as follows:

$$\begin{aligned}m_k&=n\int_{(k-1)\Delta t}^{k\Delta t}P(t)\mathrm{d}t\\&=n\left(\frac{\sum_{i=1}^{n}\tau_i}{\sum_{i=1}^{n}\tau_i+(k-1)\Delta t}\right)^{n+1}-n\left(\frac{\sum_{i=1}^{n}T_i}{\sum_{i=1}^{n}\tau_i+k\Delta t}\right)^{n+1}\end{aligned}\quad(4)$$

Using Equations (3) and (4), we tested the validity of Assumption 2 for the given data set D.

Finally, we describe the modeling algorithm. First, the value of every factor $x_j^i i$ for all patients $i=1, \ldots, n$ in D was normalized to fit into the interval [0,1] using the maximum and minimum values. This normalization to eliminate differences in the factor scales was necessary to allow for the measurement of the essential contribution of each factor's variation to τ_i. Subsequently, we applied Equations (1) and (2) to the normalized data set D_N to model the probabilistic rehospitalization process when Assumption 2 holds for the data set. We determined the model parameters β_j and γ in Equation (1) to maximize the following objective function:

$$\begin{aligned}L\left(\beta_1,\ldots,\beta_p,\gamma\right)=\ln\left[\prod_{i=1}^{n}\left(\sum_{j=1}^{p}\beta_j x_j^i+\gamma\right)\exp\left\{-\left(\sum_{j=1}^{p}\beta_j x_j^i+\gamma\right)\tau_i\right\}\right]\\-\lambda\left(\sum_{j=1}^{p}\left|\beta_j\right|+|\gamma|\right)\end{aligned}\quad(5)$$

The first term is the log-likelihood of the model consisting of Equations (1) and (2) over D_N. The second term is called an $L1$-regularization term, which penalizes the coefficients of negligible factors by setting them equal to zero when the larger hyper-parameter λ eliminates more factors.[13,14] This term avoids the over-fitting of the model to the data set by selecting a set of effective factors X_S^i from a given X^i. In our study, λ is tuned to be 0.02 to maintain the largest value of Equation(5) similarly to the other parameters β_j and γ.

To seek the optimum parameter values of $\beta_1,\ldots,\beta_p$, γ that maximize the objective function $L(\beta_1,\ldots,\beta_p, \gamma)$, we applied a simple greedy hill-climbing algorithm, in which the parameter values are iteratively modified toward their gradient direction $(\partial L/\beta_1,\ldots,\partial L/\beta_p, \partial L/\gamma)$. When the improvement of L becomes nearly negligible, the resulting parameter values are taken as the optima. Because this process depends on the initial values of the parameters, we repeated this optimization 100 times starting with random initial values and selected the result providing the maximum L.

RESULTS

Patients characteristics

Out of the 151 patients, 36 died of cardiovascular events after rehospitalization during the follow-up period. The remaining 115 patients were readmitted to our hospital at a median time of 296 days after discharge (range, 3–1891). Among these patients, the HF etiologies were valvular heart disease ($n=38$), dilated cardiomyopathy ($n=30$), hypertrophic cardiomyopathy ($n=22$), ischemic heart disease ($n=20$), hypertensive heart disease ($n=17$) and others. Their mean age was 68.6 ± 14.6 years (range, 19–93), and 38% of the patients were women. The clinical characteristics of the 151 patients are summarized in Table 1.

Validation of the formula

We hypothesized that the time-to-rehospitalization histogram for all patients (Figure 1) should be distributed exponentially if the mathematically estimated formula for the prognosis of each patient is regarded as a Poisson distribution. We therefore validated the assumptions of the model architecture. The goodness of fit was controlled by a χ^2-test, considering that the incidence rates of rehospitalization or death differ depending on the patients. Thus, the null hypothesis that the observed frequency is a mixed Poisson process was tested, as explained in the Methods section. We chose an elapsed time to rehospitalization of 150 days, which is one-thirteenth of the range of the time interval [1,1,950] according to the measure of $q=\sqrt{n}=\sqrt{151}\cong 13$. As a result, the P-value was 0.29, which was far larger than 0.05, and we confirmed that the null hypothesis was not rejected. Therefore, we concluded that the mathematically derived estimation formula for the rehospitalization of each patient was a mixed Poisson distribution.

Factors in rehospitalization for HF

We collected 402 clinical factors (Figures 2 and 3), and 150 out of 402 factors having small effects on the prognosis were automatically excluded by the regularization method described in the Methods section. Finally, we selected 252 factors for the analysis (Figures 2 and 3). The estimation results for the attribute coefficients are presented in bar graph form and numerically.

Regarding underlying diseases in HF, whereas dilated cardiomyopathy (−4.5), hypertrophic cardiomyopathy (−1.5) and hypertensive heart disease (−1.0) had better outcomes, valvular disease (7.4) and dilated phase hypertrophic cardiomyopathy (2.4) had poor prognoses. Ischemia (4.4) was the worst trigger of HF. Based on laboratory data, whereas elevated inflammatory response values, such as white blood cell counts (−1.6/5.8; at admission/at discharge) or C-reactive protein levels (−2.2/8.1; at admission/at discharge), did not indicate a poor prognosis at admission, these elevated inflammatory response values at discharge were associated with a poor prognosis. Increases in the levels of aspartate aminotransferase (6.6), alanine aminotransferase (3.2), uric acid (6.6) and BNP (4.8) at discharge also indicated a poor prognosis. Patients who received dopamine (11.9), isosorbide dinitrate (5.0) or diuretic (2.0) infusions in the acute management of HF showed worse prognoses. In contrast, the use of dobutamine (−2.5) or nitroglycerin (−2.5) drip infusions resulted in better prognoses.

Regarding oral medications at discharge, the angiotensin-converting enzyme alacepril (−4.2), the β-blocker carvedilol (−7.1, the best response), the angiotensin receptor blocker telmisartan (−1.6), the diuretic furosemide (−4.2), the lipid-lowering drugs pitavastatin

Table 1 Patient characteristics

	Population (n = 151)
Age (years)*	68.6 ± 14.6
Gender, female, *n* (%)	58 (38)
Medical history	
Frequency of heart failure (time)*	3.2 ± 2.5
Hypertension	73 (48)
Diabetes mellitus	55 (36)
Hyperlipidemia	45 (30)
Signs at admission	
Elevated jugular venous pressure	84 (56)
S_3 gallop	85 (56)
Lower extremity edema	76 (50)
NYHA functional class: II/III/IV	54/44/53
Clinical scenario: 1/2/3/4/5	28/77/34/0/12
Nohria—profile A	2 (1)
Nohria—profile B	108 (72)
Nohria—profile C	28 (19)
Nohria—profile L	13 (9)
Baseline characteristics at admission/at discharge	
Heart rate (beats min^{-1})*	84.4 ± 26.7/73.2 ± 58.3
Systolic BP (mm Hg)*	124.4 ± 31.8/ 111.0 ± 15.8
Diastolic BP (mm Hg)*	68.5 ± 17.5/59.4 ± 8.4
Body weight (kg)*	57.3 ± 13.5/52.3 ± 11.9
Δ Body weight (kg)*	4.6 ± 3.8
Laboratory factors at admission/at discharge	
Hemoglobin ($g\,dl^{-1}$)*	12.4 ± 7.7/11.8 ± 2.0
Leukocytes ($10^9\,l^{-1}$)*	6940 ± 2982/ 5968 ± 2464
Blood urea nitrogen ($mg\,dl^{-1}$)*	28.6 ± 20.7/30.0 ± 19.7
Creatinine ($mg\,dl^{-1}$)*	1.27 ± 0.90/1.24 ± 0.69
Sodium ($mEq\,l^{-1}$)*	137.6 ± 3.9/136.8 ± 4.3
Uric acid ($mg\,dl^{-1}$)*	7.5 ± 2.0/7.4 ± 2.1
T-bil ($mg\,dl^{-1}$)*	0.92 ± 0.67/0.71 ± 0.42
C-reactive protein ($mg\,dl^{-1}$)*	1.3 ± 2.8/0.7 ± 1.8
BNP ($pg\,ml^{-1}$)*	920 ± 956/439 ± 548
Δ BNP ($pg\,ml^{-1}$) (1 month after discharge-at discharge)*	78 ± 226
Echocardiographic factors at admission/at discharge	
Left ventricular end-diastolic dimension (mm)*	58.9 ± 13.3/58.3 ± 11.9
Left ventricular end-systolic dimension (mm)*	47.4 ± 15.2/45.8 ± 14.6
Fractional shortening (%)*	21.2 ± 11.5/23.1 ± 11.4
Ventricular septum thickness (mm)*	9.6 ± 2.9/9.6 ± 2.7
Posterior wall thickness (mm)*	9.8 ± 2.5/9.6 ± 2.0
Left atrial diastolic dimension (mm)*	49.9 ± 8.1/47.8 ± 9.3
Pressure across tricuspid valve (mm Hg)*	37.0 ± 16.3/25.4 ± 10.5
Medication at admission	
Use of dopamine, *n* (%)	10 (6)
Use of dobutamine, *n* (%)	33 (22)
Use of phosphodiesterase inhibitor, *n* (%)	13 (9)
Use of carperitide, *n* (%)	32 (21)
Use of nitroglycerin, *n* (%)	22 (15)
Use of diuretics, *n* (%)	60 (40)

Abbreviations: BNP, B-type natriuretic peptide; BP, blood pressure; NYHA, New York Heart Association; T-bil, total bilirubin.
*Plus or minus values are means ± s.d. Clinical profiles were classified as profile A (dry–warm), B (wet–warm), C (wet–cold) or L (dry–cold).

(−3.3), atorvastatin (−2.9) and ezetimibe (−2.2), the coronary dilator isosorbide dinitrate (−3.1), the antiallergic fexofenadine hydrochloride (−5.1), the sedative-hypnotic triazolam (−3.2), proton pump inhibitor lansoprazole (−0.9) and all antiflatulents, except toughmac, led to better prognoses. However, Ca inhibitor nifedipine (9.4) resulted in the worst outcome, and all diabetes drugs, antiarrhythmic drugs, potassium agents, vitamins and purgatives, excluding senna, were associated with worse prognoses.

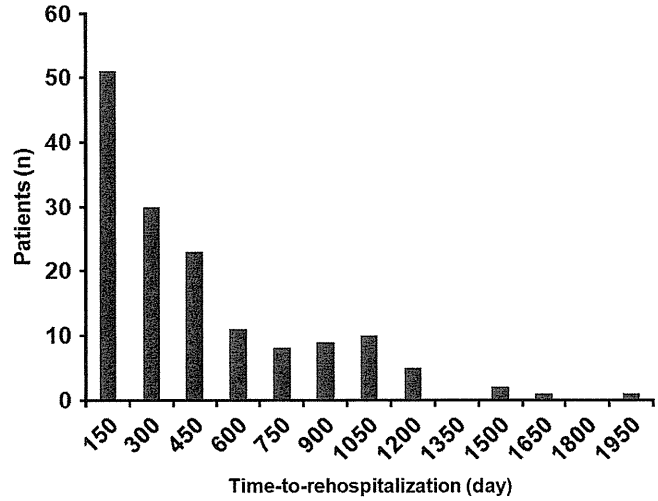


Figure 1 Time-to-rehospitalization histogram for all patients.

Fitting the model to clinical data

The mean actual value for rehospitalization (X) was 388 ± 377 days, whereas the mean estimated value calculated by the probability model based on a Poisson process (Y) was 398 ± 381 days; X and Y were very tightly correlated (Figure 4). The results showed that the mathematical formula for rehospitalization time is the dependent variable, and the clinical and personal factors before rehospitalization are the independent variables.

DISCUSSION

This study provided evidence that the values of numerous factors, including risk factors at one phase of disease, can be used to construct a mathematical equation to predict clinical outcomes. We were able to derive the equation $\tau = f(x_1, \ldots, x_p)$, where τ is the time to a future clinical event and $x_1, \ldots, x_p$ are clinical factors observed before the event. In this case, τ represents the days until rehospitalization after discharge, and $x_1, \ldots, x_p$ are the clinical and personal factors for patients hospitalized for ADHF. This study provides evidence that the clinical outcome of τ in this context is a function of 252 significant factors such as plasma BNP levels at and soon after discharge. This study presents the time to rehospitalization as the dependent variable and the clinical and personal factors before rehospitalization as the independent variables.

This study suggests the novel idea that the time to clinical events, such as rehospitalization or death, can be mathematically formulated from clinical and personal factors, demonstrating that clinical medicine can engage in physical science. The novelty of this study is based on the fact that clinical outcomes have been thought to be determined mainly from medical knowledge and the experience of the physicians. It can be argued that the known effectiveness of drugs may determine the time course of clinical events. Although this is partially true,[15–17] no one knows how one drug or the combination of several drugs affects patients with different degrees of severity of a given disease. It may also be argued that large-scale trials may better depict clinical outcomes; for example, the patients with BNP levels of <170 pg/ml showed a 20% reduction of rehospitalization compared with the patients with BNP levels greater than 170 pg/ml.[18,19] Evaluating such results by Kaplan–Meier analysis is common in clinical medicine; however, this analysis only provides the average tendency of the average patient to undergo rehospitalization and does not

Predictor variables	maximum value	coefficient	graph
Age	93.0	-0.578	
Gender	1.0	-4.455	
Etiology of HF: dilated cardiomyopathy	1.0	-4.471	
Etiology of HF: dilated phase hypertrophic cardiomyopathy	1.0	2.409	
Etiology of HF: hypertensive heart disease	1.0	-1.044	
Etiology of HF: ischemic heart disease (ICM)			
Etiology of HF: hypertrophic cardiomyopathy	1.0	-1.493	
Etiology of HF: cardiac sarcoidosis			
Etiology of HF: myocarditis			
Etiology of HF: valvular heart disease	1.0	7.361	
Etiology of HF: others	1.0	3.789	
Etiology of HF: valvular heart disease + ICM	1.0	0.445	
Endmyocardial biopsy: with or without	1.0	2.475	
Comorbidity: diabetes mellitus			
Comorbidity: Hypertension	1.0	1.968	
Comorbidity: Hyperlipidemia	1.0	-1.886	
Comorbidity: chronic atrial fibrillation	1.0	3.544	
Comorbidity: cerebrovascular disease	1.0	1.172	
Comorbidity: chronic obstructive pulmonary disease	1.0	3.318	
Comorbidity: arteriosclerosis obliterans	1.0	-1.547	
Family history of cardiovascular disease			
Frequency of HF			
Number of living with family	6.0	0.386	
Partner: with or without	1.0	1.599	
Alcohol intake			
Onset type of HF: ADHF (de novo)	1.0	-1.627	
Onset type of HF: acute on chronic			
Onset type of HF: others			
Trigger of ADHF: volume over	1.0	-2.806	
Trigger of ADHF: arrythmia	1.0	-0.271	
Trigger of ADHF: infection			
Trigger of ADHF: anemia	1.0	-3.122	
Trigger of ADHF: others	1.0	1.114	
Trigger of ADHF: afterload mismatch	1.0	2.375	
Trigger of ADHF: ischemia	1.0	4.390	
Trigger of ADHF: missed drug	1.0	2.713	
Trigger of ADHF: chronic change (unclear)			
Nohria: cold	1.0	-2.750	
Nohria: wet			
Nohria: warm	1.0	1.553	
Nohria: dry	1.0	-3.422	
Clinical scenario: 1	1.0	-0.867	
Clinical scenario: 2	1.0	2.704	
Clinical scenario: 3	1.0	2.947	
Clinical scenario: 5	1.0	-3.367	
Findings on admission: NYHA	4.0	-4.070	
Findings on admission: systolic blood pressure			
Findings on admission: diastolic blood pressure			
Findings on admission: heart rate	200.0	0.447	
Findings on admission: body weight			
Findings on admission: body height			
Findings on admission: chest X-ray CTR	88.0	-3.346	
Findings on admission: congestion			
Findings on admission: S_3 gallop	1.0	6.263	
Findings on admission: nocturnal dyspnea	1.0	5.619	
Findings on admission: elevated jugular venous pressure	1.0	0.224	
Findings on admission: lower extremity edema	1.0	-3.961	
Findings on admission: coldness of limbs	1.0	-3.216	
Findings on admission: respiratory rate			
Findings on admission: percutaneous oxygen saturation	100.0	-1.137	
Findings on admission: fraction of inspired oxygen	100.0	-3.858	
ECG (rhythm): sinus rhythm			
ECG (rhythm): atrial fibrillation or tachycardia or flutter	1.0	-0.745	
ECG (rhythm): sick sinous syndrome			
ECG (rhythm): pacemaker	1.0	-5.431	
ECG (rhythm): complete atrioventricular block	1.0	2.702	
ECG (rhythm): others			
ECG: ventricular tachycardia or fibrillation	1.0	-0.404	
ECG: complete left bundle branch block	1.0	3.116	
Laboratory data on admission: leukocytes	26300.0	-1.619	
Laboratory data on admission: neutrophil			-12<-->12
Laboratory data on admission: lymphocyte			
Laboratory data on admission: hemoglobin			

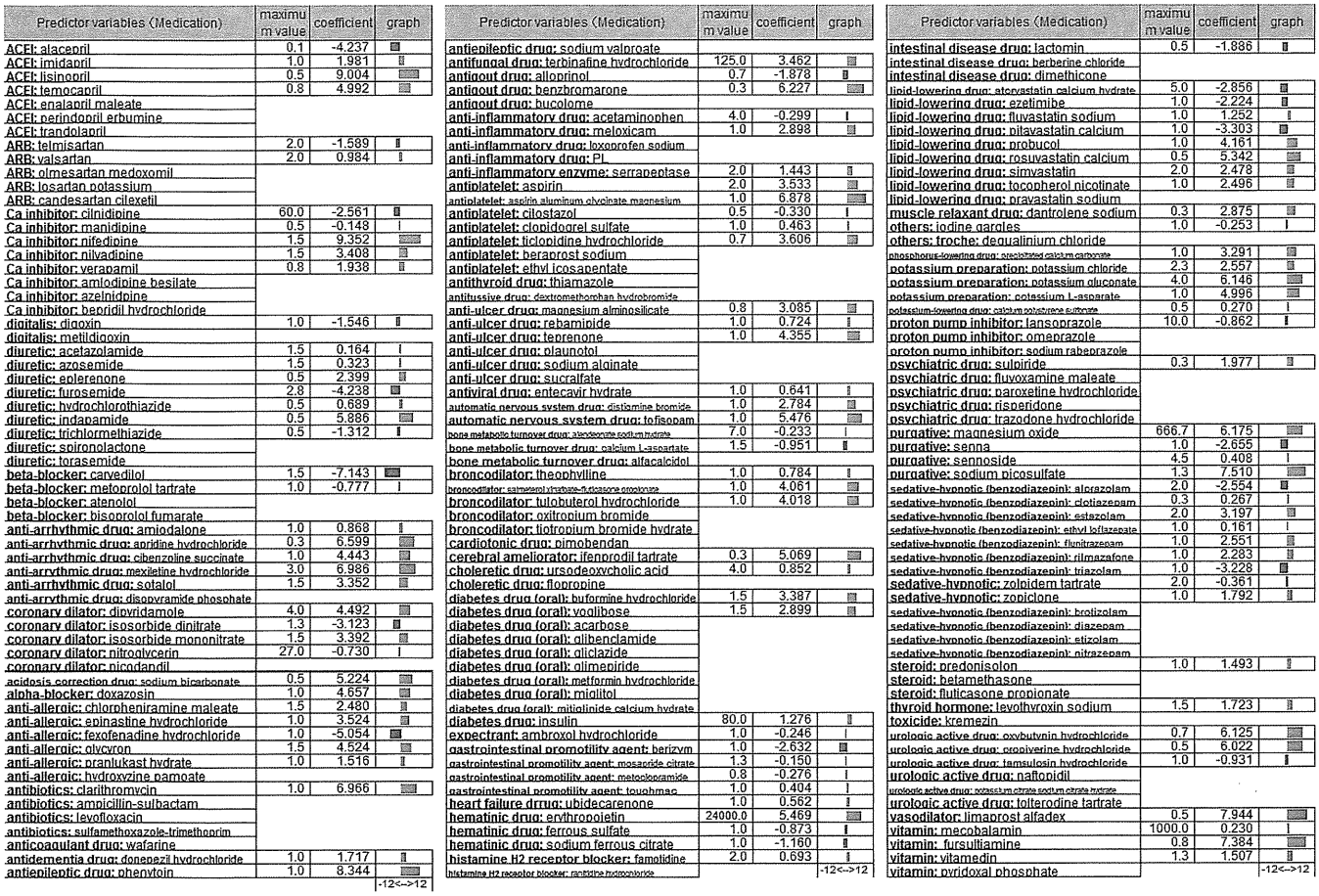
Predictor variables	maximum value	coefficient	graph
Laboratory data on admission: platelet			
Laboratory data on admission: albumin			
Laboratory data on admission: total bilirubin	6.7	-1.697	
Laboratory data on admission: AST	789.0	2.740	
Laboratory data on admission: ALT	653.0	1.359	
Laboratory data on admission: sodium			
Laboratory data on admission: potassium			
Laboratory data on admission: creatinin			
Laboratory data on admission: blood urea nitrogen			
Laboratory data on admission: uric acid			
Laboratory data on admission: C-reactive protein	24.5	-2.160	
Laboratory data on admission: blood sugar			
Laboratory data on admission: hemogrobin A1c			
Laboratory data on admission: BNP			
Laboratory data on admission: iron	421.1	-0.162	
Laboratory data on admission: UIBC	477.0	1.729	
Laboratory data on admission: ferritin			
Laboratory data on admission: free T3	12.6	-1.623	
Laboratory data on admission: free T4			
Laboratory data on admission: hythyroid-stimulating hormone			
Echocardiographic data on admission: LVDd	106.0	-1.205	
Echocardiographic data on admission: LVDs	95.0	-3.233	
Echocardiographic data on admission: %FS	81.0	5.205	
Echocardiographic data on admission: IVS	20.0	2.210	
Echocardiographic data on admission: PW	21.0	3.576	
Echocardiographic data on admission: LAD	98.0	-0.747	
Echocardiographic data on admission: TMF-E	259.0	-1.760	
Echocardiographic data on admission: TMF-A	152.0	-2.120	
Echocardiographic data on admission: TMF-DcT			
Echocardiographic data on admission: TR grade	13.0	-3.414	
Echocardiographic data on admission: TRPG			
Echocardiographic data on admission: PAEDP			
Echocardiographic data on admission: MR grade	4.0	-2.910	
Echocardiographic data on admission: AR grade	4.0	0.344	
Echocardiographic data on admission: AS	1.0	0.936	
Echocardiographic data on admission: MS	1.0	5.126	
Medications on admission: beta-blocker	1.0	-3.031	
Medications on admission: ACEI	1.0	3.098	
Medications on admission: ARB	1.0	-2.150	
Medications on admission: eplerenone	1.0	5.156	
Medications on admission: other diuretics	1.0	8.603	
Medications on admission: spironolactone	1.0	3.804	
Medications on admission: amiodarone	1.0	3.860	
Medications on admission: wafarine	1.0	-0.196	
Medications on admission: statin	1.0	4.241	
Medications on admission: DM (oral drug)	1.0	1.750	
Medications on admission: DM (insulin)			
Medications on admission: digoxin			
Acute phase treatment: carperitide	1.0	1.177	
Acute phase treatment: dopamine	1.0	11.918	
Acute phase treatment: dobutamin	1.0	-2.537	
Acute phase treatment: isosorbide dinitrate	1.0	5.039	
Acute phase treatment: nitroglycerin	1.0	-2.537	
Acute phase treatment: diuretics venoclysis	1.0	1.993	
Acute phase treatment: phosphodiesterase III inhibitor			
Use of biphasic positive airway pressure			
Use of adaptive servo ventilator	1.0	0.228	
Use of assist device: IABP or PCPS	3.0	3.310	
Use of assist device:left ventricle assisting system	1.0	3.993	
Use of blood transfusion			
Right heart catheterization: pulmonary capillary wedge pressure			
Right heart catheterization: right atrium	18.0	-3.104	
Right heart catheterization: systolic right ventricle			
Right heart catheterization: diastolic right ventricle	20.0	-1.569	
Right heart catheterization: systolic pulmonary artery			
Right heart catheterization: diastolic pulmonary artery			
Right heart catheterization: mean pulmonary artery			
Right heart catheterization: cardiac output (c-Fick)	7.6	0.646	
Right heart catheterization: cardiac index (c-Fick)	4.3	1.574	
Right heart catheterization: cardiac output (Thermo)	9.7	3.877	
Right heart catheterization: cardiac index (Thermo)	6.3	4.170	
Right heart catheterization: systemic vascular resitance			-12<-->12
Right heart catheterization: pulmonary vascular resitance			

Predictor variables	maximum value	coefficient	graph
Right heart catheterization: body surface area			
Left heart catheterization: systolic aortic pressure			
Left heart catheterization: diastolic aortic pressure			
Left heart catheterization: aortic pressure mean	136.0	-1.159	
Left heart catheterization (CAG): number of affected vessel	3.0	0.519	
Left heart catheterization: LV ejection fraction			
Left heart catheterization: LVEDVI	477.0	2.252	
Left heart catheterization: LVESVI	432.0	0.772	
Prognosis: left ventricle assisting system	1.0	-3.224	
Cardiac resynchronization therapy: this admission	1.0	-2.286	
Cardiac resynchronization therapy: prior admission	1.0	2.521	
Implantable cardioverter-defibrillator: this admission	1.0	-2.995	
Implantable cardioverter-defibrillator: prior admission	1.0	1.881	
Pacemaker: this admission			
Pacemaker: prior admission	1.0	4.092	
coronary artery bypass graft: this admission	1.0	0.976	
coronary artery bypass graft: prior admission	1.0	-2.455	
Percutaneous coronary intervention: this admission	1.0	-4.455	
Percutaneous coronary intervention: prior admission	1.0	-2.419	
Vascular surgery: this admission	1.0	-0.825	
Vascular surgery: prior admission	1.0	5.661	
Vascular disease: aneurysm	1.0	3.159	
Ablation: this admission			
Ablation: prior admission			
Other surgery: prior admission	1.0	-3.860	
Valvular surgery: this admission			
Valvular surgery: prior admission	1.0	-5.514	
Mitral valve plasty: this admission			
Mitral valve plasty: prior admission	1.0	-2.491	
Tricuspid annuloplasty or valve replacement: this admission			
Tricuspid annuloplasty or valve replacement: prior admission	1.0	2.126	
Aortic valve replacement: this admission			
Aortic valve replacement: prior admission			
Findings at discharge: systolic blood pressure			
Findings at discharge: diastolic blood pressure			
Findings at discharge: heart rate	772.0	-2.456	
Findings at discharge: body weight			
Difference of body weight (on admission - at discharge)			
Laboratory data at discharge: leukocyte	23500.0	5.780	
Laboratory data at discharge: neutrophil			
Laboratory data at discharge: lymphocyte	58.6	-0.270	
Laboratory data at discharge: hemogrobin			
Laboratory data at discharge: platelet			
Laboratory data at discharge: albumin	5.3	-1.356	
Laboratory data at discharge: total bilirubin			
Laboratory data at discharge: AST	575.0	6.585	
Laboratory data at discharge: ALT	511.0	3.184	
Laboratory data at discharge: sodium			
Laboratory data at discharge: potassium	8.5	0.345	
Laboratory data at discharge: creatinin			
Laboratory data at discharge: blood urea nitrogen			
Laboratory data at discharge: uric acid	16.4	6.567	
Laboratory data at discharge: C-reactive protein	17.2	8.109	
Laboratory data at discharge: blood sugar			
Laboratory data at discharge: BNP	3832.6	4.770	
Laboratory data one month after discharge: creatinin			
Laboratory data one month after discharge: BNP	2397.6	-3.767	
Laboratory data: difference of BNP (1 month - at dischrge)	1655.3	1.570	
Echocardiographic data at discharge: LVDd			
Echocardiographic data at discharge: LVDs			
Echocardiographic data at discharge: %FS			
Echocardiographic data at discharge: IVS			
Echocardiographic data at discharge: PW	18.0	0.643	
Echocardiographic data at discharge: LAD	75.0	-6.889	
Echocardiographic data at discharge: AR	3.5	3.091	
Echocardiographic data at discharge: MR	4.0	-0.457	
Echocardiographic data at discharge: TR			
Echocardiographic data at discharge: TRPG	66.0	0.456	
Echocardiographic data at discharge: IVC	1.0	-1.421	
Echocardiographic data at discharge: TMF-E	230.0	0.980	
Echocardiographic data at discharge: TMF-A			
Echocardiographic data at discharge: DcT			
Echocardiographic data at discharge: E/E'	55.0	5.962	
			-12<-->12

Figure 2 Factors influencing the estimation of rehospitalization for HF and the contribution of each parameter. All of the clinical and personal factors for the patients with HF. Predictor variables with coefficient indicate the factors selected after the application of the regularization method. Negative values indicate favorable impact on prognosis, whereas positive values indicate undesirable effect. HF, heart failure; ADHF, acute decompensated heart failure; NYHA, New York Heart Association; CTR, cardiothoracic ratio; ECG, electrocardiogram; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BNP, B-type natriuretic peptide; UIBC, unsaturated iron-binding capacity; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; FS, fractional shortening; IVS, interventricular septal thickness; PW, left ventricular posterior thickness; LAD, left atrial dimension; TMF-E, the peak mitral inflow early diastolic velocity; TMF-A, the peak mitral inflow atrial filling; DcT, deceleration time; TR PG, tricuspid regurgitation pressure gradient; PAEDP, pulmonary artery end-diastolic pressure; MR, mitral regurgitation; AR, aortic regurgitation; AS, aortic stenosis; MS, mitral stenosis; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; DM, diabetes mellitus; IABP, intraaortic balloon pumping; PCPS, percutaneous cardio pulmonary support; EDVI, end-diastolic volume index; ESVI, end-systolic volume index; IVC, inferior vena cava respiratory change; *E*/*E'*, ratio of peak mitral *E*-wave velocity to peak mitral annular velocity.

prospectively provide a future clinical outcome for each patient. Indeed, in the epidemiological study, many biomarkers, such as BNP levels or C-reactive protein levels in addition to the classical risk factors, such as hypertension or diabetes mellitus, are known to be related to cardiovascular events and death. However, Wang *et al.*[20] showed that although multiple biomarkers are associated with a high relative risk of adverse events, even in the combination of these factors they add only moderately to the prediction of risk in an individual person. This suggests that the occurrence of cardiovascular events may not be well predictable or mathematically formulated. On the other hand, using the formula developed in this study, we can identify the day of a clinical event to within a small range, suggesting that we need more clinical data to predict the future outcomes or obtain the mathematical formula for the prediction than we expected.

It would be difficult to strictly prove that this mathematical formula is correct because no gold standard or correct answer is available in the medical literature. However, there are hints as to the correctness of this formula. First, we assume that the probability of rehospitalization follows a Poisson distribution; if this is true, a histogram of the day of rehospitalization after discharge should follow a Poisson distribution. We found that the present data for the actual day of rehospitalization are distributed as a Poisson distribution.



Predictor variables (Medication)	maximum value	coefficient	graph
ACEI: alacepril	0.1	-4.237	
ACEI: imidapril	1.0	1.981	
ACEI: lisinopril	0.5	9.004	
ACEI: temocapril	0.8	4.992	
ACEI: enalapril maleate			
ACEI: perindopril erbumine			
ACEI: trandolapril			
ARB: telmisartan	2.0	-1.589	
ARB: valsartan	2.0	0.984	
ARB: olmesartan medoxomil			
ARB: losartan potassium			
ARB: candesartan cilexetil			
Ca inhibitor: cilnidipine	60.0	-2.561	
Ca inhibitor: manidipine	0.5	-0.148	
Ca inhibitor: nifedipine	1.5	9.352	
Ca inhibitor: nilvadipine	1.5	3.408	
Ca inhibitor: verapamil	0.8	1.938	
Ca inhibitor: amlodipine besilate			
Ca inhibitor: azelnidipine			
Ca inhibitor: benridil hydrochloride			
digitalis: digoxin	1.0	-1.546	
digitalis: metildigoxin			
diuretic: acetazolamide	1.5	0.164	
diuretic: azosemide	1.5	0.323	
diuretic: eplerenone	0.5	2.399	
diuretic: furosemide	2.8	-4.238	
diuretic: hydrochlorothiazide	0.5	0.689	
diuretic: indapamide	0.5	5.886	
diuretic: trichlormethiazide	0.5	-1.312	
diuretic: spironolactone			
diuretic: torasemide			
beta-blocker: carvedilol	1.5	-7.143	
beta-blocker: metoprolol tartrate	1.0	-0.777	
beta-blocker: atenolol			
beta-blocker: bisoprolol fumarate			
anti-arrhythmic drug: amiodalone	1.0	0.868	
anti-arrhythmic drug: aprldine hydrochloride	0.3	6.599	
anti-arrhythmic drug: cibenzoline succinate	1.0	4.443	
anti-arrythmic drug: mexiletine hydrochloride	3.0	6.986	
anti-arrhythmic drug: sotalol	1.5	3.352	
anti-arrythmic drug: disopyramide phosphate			
coronary dilator: dipyridamole	4.0	4.492	
coronary dilator: isosorbide dinitrate	1.3	-3.123	
coronary dilator: isosorbide mononitrate	1.5	3.392	
coronary dilator: nitroglycerin	27.0	-0.730	
coronary dilator: nicorandil			
acidosis correction drug: sodium bicarbonate	0.5	5.224	
alpha-blocker: doxazosin	1.0	4.657	
anti-allergic: chlorpheniramine maleate	1.5	2.480	
anti-allergic: epinastine hydrochloride	1.0	3.524	
anti-allergic: fexofenadine hydrochloride	1.0	-5.054	
anti-allergic: olvcvron	1.5	4.524	
anti-allergic: pranlukast hydrate	1.0	1.516	
anti-allergic: hydroxyzine pamoate			
antibiotics: clarithromycin	1.0	6.966	
antibiotics: ampicillin-sulbactam			
antibiotics: levofloxacin			
antibiotics: sulfamethoxazole-trimethoprim			
anticoagulant drug: wafarine			
antidementia drug: donepezil hydrochloride	1.0	1.717	
antiepileptic drug: phenytoin	1.0	8.344	
			-12<->12

Predictor variables (Medication)	maximum value	coefficient	graph
antiepileptic drug: sodium valproate			
antifungal drug: terbinafine hydrochloride	125.0	3.462	
antigout drug: allopurinol	0.7	-1.878	
antigout drug: benzbromarone	0.3	6.227	
antigout drug: bucolome			
anti-inflammatory drug: acetaminophen	4.0	-0.299	
anti-inflammatory drug: meloxicam	1.0	2.898	
anti-inflammatory drug: loxoprofen sodium			
anti-inflammatory drug: PL			
anti-inflammatory enzyme: serrapeptase	2.0	1.443	
antiplatelet: aspirin	2.0	3.533	
antiplatelet: aspirin aluminum glycinate magnesium	1.0	6.878	
antiplatelet: cilostazol	0.5	-0.330	
antiplatelet: clopidogrel sulfate	1.0	0.463	
antiplatelet: ticlopidine hydrochloride	0.7	3.606	
antiplatelet: beraprost sodium			
antiplatelet: ethyl icosapentate			
antithyroid drug: thiamazole			
antitussive drug: dextromethorphan hydrobromide			
anti-ulcer drug: magnesium aluminosilicate	0.8	3.085	
anti-ulcer drug: rebamipide	1.0	0.724	
anti-ulcer drug: teprenone	1.0	4.355	
anti-ulcer drug: plaunotol			
anti-ulcer drug: sodium alginate			
anti-ulcer drug: sucralfate			
antiviral drug: entecavir hydrate	1.0	0.641	
automatic nervous system drug: distamine bromide	1.0	2.784	
automatic nervous system drug: tofisopam	1.0	5.476	
bone metabolic turnover drug: alendronate sodium hydrate	7.0	-0.233	
bone metabolic turnover drug: calcium L-aspartate	1.5	-0.951	
bone metabolic turnover drug: alfacalcidol			
broncodilator: theophylline	1.0	0.784	
broncodilator: salmeterol xinafoate-fluticasone propionate	1.0	4.061	
broncodilator: tulobuterol hydrochloride	1.0	4.018	
broncodilator: oxitropium bromide			
broncodilator: tiotropium bromide hydrate			
cardiotonic drug: pimobendan			
cerebral ameliorator: ifenprodil tartrate	0.3	5.069	
cholerefic drug: ursodeoxycholic acid	4.0	0.852	
cholerefic drug: flopropine			
diabetes drug (oral): buformine hydrochloride	1.5	3.387	
diabetes drug (oral): voglibose	1.5	2.899	
diabetes drug (oral): acarbose			
diabetes drug (oral): glibenclamide			
diabetes drug (oral): gliclazide			
diabetes drug (oral): glimepiride			
diabetes drug (oral): metformin hydrochloride			
diabetes drug (oral): miglitol			
diabetes drug (oral): mitiglinide calcium hydrate			
diabetes drug: insulin	80.0	1.276	
expectrant: ambroxol hydrochloride	1.0	-0.246	
gastrointestinal promotility agent: berizym	1.0	-2.632	
gastrointestinal promotility agent: mosapride citrate	1.3	-0.150	
gastrointestinal promotility agent: metoclopramide	0.8	-0.276	
gastrointestinal promotility agent: tovohmag	1.0	0.404	
heart failure drug: ubidecarenone	1.0	0.562	
hematinic drug: erythropoietin	24000.0	5.469	
hematinic drug: ferrous sulfate	1.0	-0.873	
hematinic drug: sodium ferrous citrate	1.0	-1.160	
histamine H2 receptor blocker: famotidine	2.0	0.693	
histamine H2 receptor blocker: ranitidine hydrochloride			-12<->12

Predictor variables (Medication)	maximum value	coefficient	graph
intestinal disease drug: lactomin	0.5	-1.886	
intestinal disease drug: berberine chloride			
intestinal disease drug: dimethicone			
lipid-lowering drug: atorvastatin calcium hydrate	5.0	-2.856	
lipid-lowering drug: ezetimibe	1.0	-2.224	
lipid-lowering drug: fluvastatin sodium	1.0	1.252	
lipid-lowering drug: pitavastatin calcium	1.0	-3.303	
lipid-lowering drug: probucol	1.0	4.161	
lipid-lowering drug: rosuvastatin calcium	0.5	5.342	
lipid-lowering drug: simvastatin	2.0	2.478	
lipid-lowering drug: tocopherol nicotinate	1.0	2.496	
lipid-lowering drug: pravastatin sodium			
muscle relaxant drug: dantrolene sodium	0.3	2.875	
others: iodine garoles	1.0	-0.253	
others: troche: dequalinium chloride			
phosphorus-lowering drug: precipitated calcium carbonate	1.0	3.291	
potassium preparation: potassium chloride	2.3	2.557	
potassium preparation: potassium gluconate	4.0	6.146	
potassium preparation: potassium L-aspartate	1.0	4.996	
potassium-lowering drug: calcium polystyrene sulfonate	0.5	0.270	
proton pump inhibitor: lansoprazole	10.0	-0.862	
proton pump inhibitor: omeprazole			
proton pump inhibitor: sodium rabeprazole			
psychiatric drug: sulpiride	0.3	1.977	
psychiatric drug: fluvoxamine maleate			
psychiatric drug: paroxetine hydrochloride			
psychiatric drug: risperidone			
psychiatric drug: trazodone hydrochloride			
purgative: magnesium oxide	666.7	6.175	
purgative: senna	1.0	-2.655	
purgative: sennoside	4.5	0.408	
purgative: sodium picosulfate	1.3	7.510	
sedative-hypnotic (benzodiazepin): alprazolam	2.0	-2.554	
sedative-hypnotic (benzodiazepin): clotiazepam	0.3	0.267	
sedative-hypnotic (benzodiazepin): estazolam	2.0	3.197	
sedative-hypnotic (benzodiazepin): ethyl loflazepate	1.0	0.161	
sedative-hypnotic (benzodiazepin): flunitrazepam	1.0	2.551	
sedative-hypnotic (benzodiazepin): nilmazafone	1.0	2.283	
sedative-hypnotic (benzodiazepin): triazolam	1.0	-3.228	
sedative-hypnotic: zolpidem tartrate	2.0	-0.361	
sedative-hypnotic: zopiclone	1.0	1.792	
sedative-hypnotic (benzodiazepin): brotizolam			
sedative-hypnotic (benzodiazepin): diazepam			
sedative-hypnotic (benzodiazepin): etizolam			
sedative-hypnotic (benzodiazepin): nitrazepam			
steroid: predonisolon	1.0	1.493	
steroid: betamethasone			
steroid: fluticasone propionate			
thyroid hormone: levothyroxin sodium	1.5	1.723	
toxicide: kremezin			
urologic active drug: oxybutynin hydrochloride	0.7	6.125	
urologic active drug: propiverine hydrochloride	0.5	6.022	
urologic active drug: tamsulosin hydrochloride	1.0	-0.931	
urologic active drug: naftopidil			
urologic active drug: potassium citrate sodium citrate hydrate			
urologic active drug: tolterodine tartrate			
vasodilator: limaprost alfadex	0.5	7.944	
vitamin: mecobalamin	1000.0	0.230	
vitamin: fursultiamine	0.8	7.384	
vitamin: vitamedin	1.3	1.507	
vitamin: pyridoxal phosphate			-12<->12

Figure 3 Factors influencing the estimation of rehospitalization for heart failure and the contribution of each parameter. All of the medications at discharge for the patients with heart failure. Medications were calculated as ratios of their recommended doses. All drugs were divided into 55 groups. Predictor variables with coefficient indicate the factors selected after the application of the regularization method. Negative values indicate favorable impact on prognosis, whereas positive values indicate undesirable effect. HF, heart failure; ADHF, acute decompensated heart failure; NYHA, New York Heart Association; CTR, cardiothoracic ratio; ECG, electrocardiogram; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BNP, B-type natriuretic peptide; UIBC, unsaturated iron-binding capacity; LVDd, left ventricular end-diastolic dimension; LVDs, left ventricular end-systolic dimension; FS, fractional shortening; IVS, interventricular septal thickness; PW, left ventricular posterior thickness; LAD, left atrial dimension; TMF-E, the peak mitral inflow early diastolic velocity; TMF-A, the peak mitral inflow atrial filling; DcT, deceleration time; TR PG, tricuspid regurgitation pressure gradient; PAEDP, pulmonary artery end-diastolic pressure; MR, mitral regurgitation; AR, aortic regurgitation; AS, aortic stenosis; MS, mitral stenosis; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; DM, diabetes mellitus; IABP, intraaortic balloon pumping; PCPS, percutaneous cardio pulmonary support; EDVI, end-diastolic volume index; ESVI, end-systolic volume index; IVC, inferior vena cava respiratory change; E/E', ratio of peak mitral E-wave velocity to peak mitral annular velocity.

Second, when we compared the day of rehospitalization in a clinical setting and the calculated day of rehospitalization obtained by the formula, these two data are well fitted, suggesting that the current formula is likely to be correct. Third, we prevented over-fitting of the clinical data using the free variables, indicating the suitability of the present formula.

We do not believe that this equation is the perfect formula to predict the day of rehospitalization from numerous variables. Although we included 402 factors as the free variables, including factors as diverse as echocardiographic data and marital status, we may have neglected to include other unknown but important factors that may determine the day of rehospitalization. We did not include information on patient genetic backgrounds, such as point mutations in the myosin heavy chain, or social status, such as occupation or annual income, private matters, such as hobbies or personal characteristics, and mental health parameters, such as depression. The inclusion of these issues may improve the formula presented in this study; however, the present formula already provides a good fit with an R^2 value of 0.9879. Most importantly, the importance of the possibility of constituting such a mathematical formula in clinical practice is now clear.

In this study, we assumed that a linear function of each parameter contributes to the formation of the formula for the clinical outcome. One might suggest the use of nonlinear functions of all of the factors to provide a more accurate approximation of the rehospitalization time. In fact, we performed a nonlinear analysis using this data, and surprisingly, the nonlinear method using support vectors yielded no improvement over the present formula using the linear functions of the factors.

LIMITATIONS

First of all, the factors in this study may have confounded each other, and we used the regularization method to eliminate automatically the factors that have weak effects on prognosis. Although the remaining

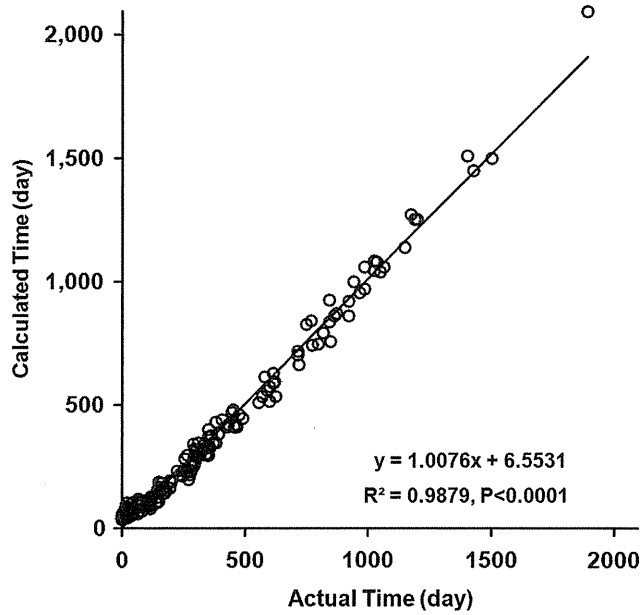


Figure 4 Correlation between the clinical data and the values calculated using the mathematical formula. The clinical data are in excellent agreement with the calculated times.

factors with strong effects on prognosis could have confounded each other, the results of this study are probably not weakened because we obtained a good fitting to the clinical outcome using these factors. When we consider the clinical and pathophysiological meaning of each factor, we need to pay attention to each factor independently.

The other main limitation of this study is that the patient population consists of a retrospective cohort. However, because we enrolled all of the patients who were admitted to our department during the entry period, the selection bias may be small. Furthermore, this is a single-center study, so the formula may be true only in our institute. However, because (1) approximately one-half of the patients who were hospitalized during this time were referred from other hospitals, (2) the nature and treatment of HF did not differ among the hospitals and (3) our hospital sets a high standard for CHF treatment and specializes in receiving CHF patients from all over Japan; we believe that the formula developed in this study may be generalized. We estimated the day of rehospitalization in this study; however, the important issue is the ability to make this prediction, which needs further investigation.

CONCLUSIONS

This study demonstrated that clinical medicine and practice can use a mathematical formula to predict clinical outcomes or events using current data. A prospective study is needed to test whether this formula predicts the day of rehospitalization in CHF patients who are admitted because of ADHF and discharged after treatment. The application of these risk factors to individual CHF patients may distinguish those patients who are at low risk from those who are at high risk and may benefit from closer monitoring and aggressive treatment.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ORIGINAL ARTICLE
Heart Failure

Safety and Tolerability of Once-Daily Controlled-Release Carvedilol 10–80 mg in Japanese Patients With Chronic Heart Failure

Masafumi Kitakaze, MD, PhD; Nobuaki Sarai, MD, PhD; Hidekazu Ando, BSc; Takashi Sakamoto, BSc; Hiromu Nakajima, MD, PhD

Background: The aim of the present study was to assess the safety and tolerability of the controlled-release (CR) formulation of the β-blocker carvedilol in Japanese patients with chronic heart failure (HF).

Methods and Results: In this multicenter, randomized, open-label, phase I/II dose-escalation study, 41 patients receiving standard therapy for chronic HF were randomized in a ratio of 1:1 to carvedilol CR or immediate-release (IR) carvedilol. The primary objective was to evaluate the tolerability and safety of escalating doses of carvedilol CR (10–40 mg/day), with a reference arm of 5–20 mg/day of carvedilol IR. In addition, the tolerability and safety of titration to a carvedilol CR dose up to 80 mg/day were examined, as were plasma concentrations of carvedilol and changes in vital signs. The proportions of patients who completed 40-mg/day carvedilol CR and 20-mg/day carvedilol IR were 42% (8/19) and 50% (11/22), respectively. In the CR group, 7/19 (37%) attained a dose of 80 mg. During the primary evaluation period, 7/19 (37%) and 4/22 (18%) patients experienced drug-related adverse events in the CR and IR groups, respectively, the characteristics of which were similar between groups.

Conclusions: No new safety issues emerged in Japanese chronic HF patients treated with carvedilol CR in contrast to those known in carvedilol IR. (*Circ J* 2012; **76:** 668–674)

Key Words: Adrenergic β-antagonists; Asian continental ancestry group; Pharmacodynamics; Pharmacokinetics

Chronic heart failure (HF) is a common and increasingly frequent condition in developed countries and is associated with very high morbidity and mortality.[1] Because chronic HF is a disease that primarily affects elderly adults, its prevalence is likely to increase in Japan as the population grows older.[2] In addition, lifestyle changes among Japanese people are expected to increase rates of diabetes and/or dyslipidemia, which will result in rising numbers of adults with ischemic heart disease and subsequent ischemic HF. To address the pathophysiologic mechanism of chronic HF, β-blockers, which once had been contraindicated for chronic HF,[3–8] are now commonly used in the clinical setting and are supported by abundant beneficial evidence[9–14] and clinical guidelines.[15–17]

Carvedilol is a competitive antagonist of α_1-, β_1-, and β_2-receptors and has antioxidant effects.[18,19] A number of US clinical studies demonstrated that long-term use of carvedilol improved the vital prognosis of patients with mild to severe chronic HF.[14,20–22] In 2002, an immediate-release (IR) carvedilol formulation was approved in Japan for the treatment of chronic HF, based on the findings of the Multicenter Carvedilol Heart Failure Dose Assessment (MUCHA) trial.[23] The maximum approved daily dose in Japan is 10 mg twice daily (20 mg/day), which is considerably lower than the twice-daily dose of 25 mg (50 mg/day) approved for treatment of chronic HF in Western countries.[24] Ho et al reported, however, that 31 of 70 Taiwanese patients with chronic HF were successfully titrated to a twice-daily dose of 25 mg of carvedilol (50 mg/day) and that outcomes were favorable after a treatment period of 24 months.[25] These findings suggest that the tolerability, safety, and efficacy of carvedilol might be comparable in Asian and Western populations.

A once-daily, controlled-release (CR) carvedilol formulation has been developed and marketed in the USA.[26] Carvedilol CR is supplied as capsules of 10, 20, 40, and 80 mg. These dose levels were designed to give a total daily exposure of carvedilol equivalent to twice-daily carvedilol IR doses of 3.125, 6.25, 12.5, and 25 mg, respectively, as determined from the area under the concentration-time curve from zero (before dosing) to 24 h (trough concentration; AUC_{0-24}).[26] Thus, 80 mg of carvedilol CR is equivalent to 25 mg of carvedilol IR twice daily (50 mg total daily dose).[27] The weight difference between

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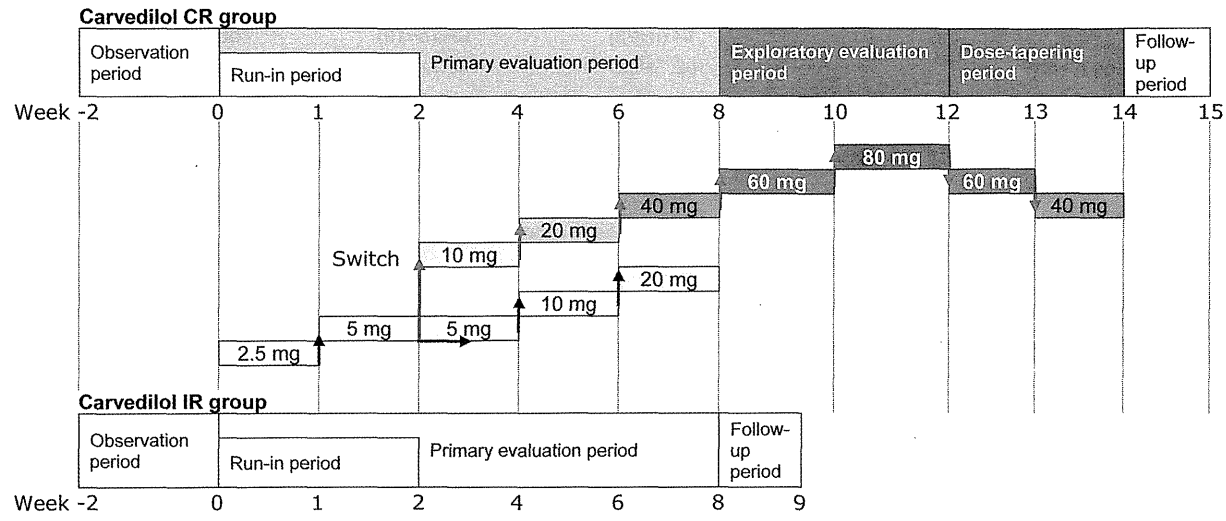

Figure. Study design. CR, controlled release; IR, immediate release.

the formulations (80 vs. 50 mg) is the result of the use of different salt forms of carvedilol (IR, free base; CR, phosphate salt) and the adjustment of the total dose due to minor differences in bioavailability (IR, 50 mg carvedilol; CR, 64.8 mg carvedilol).

Although 1 trial found no difference in adherence between chronic HF patients receiving CR and IR carvedilol formulations,[28] other studies have found that a once-daily regimen generally improves adherence as compared with twice-daily dosing in several disease fields.[29–31] In addition, Bennett et al reported that non-compliance with treatment accounted for 15–40% of hospitalizations for decompensated HF.[32] Taken together, these findings suggest that simplification of complex polypharmacy regimens is not only more convenient, it may also be more efficacious in patients with chronic HF.

The primary objective of this study was to evaluate the safety and tolerability of a once-daily carvedilol CR dose of 10–40 mg in Japanese patients with chronic HF, as well as the pharmacokinetics (PK), pharmacodynamics (PD) and efficacy, with a reference arm of a twice-daily carvedilol IR dose of 2.5–10 mg. During the exploratory period of the trial, we assessed the safety, tolerability, PK, PD, and efficacy of once-daily carvedilol CR doses of 60 and 80 mg.

Methods

Patients

We recruited outpatients who were between the ages of 20 and 80 years, had symptomatically stable chronic HF due to ischemic heart disease or dilated cardiomyopathy, had not received β-blockers for 2 weeks before screening, and were receiving conventional treatment with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers for which the dosage/use had not changed during the period 2 weeks before screening. Additional inclusion criteria were New York Heart Association (NYHA) functional class I–III, left ventricular ejection fraction (LVEF) of 25–45%, a resting heart rate (HR) of ≥60 beats/min, a sitting systolic blood pressure (BP) of ≥90 mmHg, and no signs of fluid retention.

We excluded patients for whom β-blockers were contraindicated and those with a history of obstructive pulmonary disease requiring bronchodilators or steroid therapy. Patients were also excluded if they had a history of bronchospastic disease or acute myocardial infarction within 2 weeks before screening or if they had cardiogenic shock or unstable angina, coronary spastic angina, or angina at rest.

Study Design

This was a randomized, multicenter, open-label, phase I/II dose-escalation study of carvedilol CR. The study design is shown in Figure. Patients who fulfilled all inclusion criteria were randomly assigned in a 1:1 ratio to receive carvedilol IR (Daiichi-Sankyo, Tokyo, Japan) or carvedilol CR, by an independent organization using a permuted block method stratified by NYHA functional class (I, II, or III) at baseline.

During the primary evaluation period, all patients received an initial dose of 1.25 mg of carvedilol IR twice daily (2.5 mg/day), followed by an increase in dose to 2.5 mg twice daily (5 mg/day) in week 1. Patients assigned to the CR formulation were switched to carvedilol CR 10 mg once daily at week 2, which was then increased to 20 mg once daily and to 40 mg once daily at 2-week intervals. Patients assigned to the IR formulation continued on carvedilol IR 2.5 mg twice daily from week 1 through week 3, after which the dose was then increased to 5 mg twice daily and finally to 10 mg twice daily at 2-week intervals.

During the subsequent 4-week exploratory evaluation period, among those patients who had reached a carvedilol CR dose of 40 mg in the 8-week treatment period, the dose was increased to 60 mg and finally to 80 mg at 2-week intervals. After the exploratory evaluation period, the daily dose of carvedilol CR was reduced to 60 mg and then to 40 mg at weekly intervals during the dose-tapering period. Finally, carvedilol CR was switched to 10 mg of carvedilol IR twice daily for routine treatment in the follow-up period. The dose increase and switch to carvedilol CR were conducted after confirming that each dose level was well tolerated, that there were no safety concerns, and that the patient satisfied all dose-escalation criteria defined in the study protocol, that is, HR ≥50 beats/min, a sitting systolic BP ≥85 mmHg, no exacerbation of HF symptoms, and

Table 1. Patient Characteristics

	IR (n=22)	CR (n=19)	Total (n=41)
Age (years)	62.7±8.2	66.7±12.0	64.6±10.2
Sex			
Female	2 (9)	1 (5)	3 (7)
Male	20 (91)	18 (95)	38 (93)
Weight (kg)	63.4±11.7	63.8±10.5	63.6±11.1
Height (cm)	163.5±8.7	162.1±5.8	162.8±7.4
BMI (kg/m²)	23.6±3.3	24.3±3.7	23.9±3.5
Ischemic background*	10 (45)	12 (63)	22 (54)
Dilated cardiomyopathy*	13 (59)	8 (42)	21 (51)
NYHA functional class	n=20	n=19	n=39
I	9 (45)	9 (47)	18 (46)
II	10 (50)	10 (53)	20 (51)
III	1 (5)	0	1 (3)
Concomitant medications			
Enalapril maleate	7 (32)	4 (21)	11 (27)
Candesartan cilexetil	4 (18)	7 (37)	11 (27)
Losartan potassium	4 (18)	4 (21)	8 (20)
Valsartan	3 (14)	1 (5)	4 (10)
Olmesartan	2 (9)	1 (5)	3 (7)
Furosemide	14 (64)	13 (68)	27 (66)
Spironolactone	12 (55)	5 (26)	17 (41)
Digoxin	0	4 (21)	4 (10)
Metildigoxin	3 (14)	0	3 (7)

Data given as n (%) or mean±SD.
*One patient in each IR and CR group had overlapping etiology.
IR, immediate-release carvedilol group; CR, controlled-release carvedilol group; NYHA, New York Heart Association; BMI, body mass index.

no evidence of rapid fluid retention. Patients were withdrawn from the study if they did not satisfy the criteria for dose escalation or if they had a rapid increase in brain natriuretic peptide (BNP) to a concentration >200 pg/ml and greater than double the last measured value.

This study was funded and conducted by GlaxoSmithKline Japan in accordance with good clinical practice, all applicable participant privacy requirements, and the guiding principles of the Declaration of Helsinki (Edinburgh 2000, Washington 2002, and Tokyo 2004). The relevant institutional review boards approved the study protocol. The study objectives and procedures were fully explained to all participants, and written informed consent was obtained from each patient before participation in the study.

Endpoints

The primary endpoint of the study was the safety and tolerability of a once-daily dose of 10–40 mg of carvedilol CR with a reference arm of a twice-daily dose of 2.5–10 mg of carvedilol IR, as indicated by the frequency and severity of adverse events (AEs) and changes from baseline in laboratory values, vital signs, body weight, 12-lead electrocardiogram (ECG), and cardiothoracic ratio (CTR). The secondary endpoints were carvedilol CR PK, PD (ie, 24-h BP profile on ambulatory monitoring and changes in 24-h HR on Holter monitor ECG), and efficacy, as indicated by changes from baseline in NYHA functional class, BNP concentration, and LVEF. During the exploratory period, we assessed the safety, tolerability, PK, PD, and efficacy of once-daily carvedilol CR doses of 60 and 80 mg.

Pharmacokinetics

Plasma concentrations of S(–)-carvedilol were determined using high-performance liquid chromatography-tandem mass spectrometry at the GlaxoSmithKline Department of Drug Metabolism and Pharmacokinetics (King of Prussia, PA, USA). The determination range for S(–)-carvedilol concentrations was 0.20–200 mg/ml. The following PK parameters were assessed: C_{max}, time to maximum observed drug concentration (t_{max}), AUC_{0-24}. PK parameters were calculated using WinNonlin Professional Version 4.1 (Pharsight, Mountain View, CA, USA).

Statistical Analysis

The planned sample size of 60 evaluable patients (30 per treatment group) was not based on statistical considerations, but on clinical feasibility. Because the primary objective of this trial was to evaluate the safety and tolerability of repeated daily doses of 10–40 mg of carvedilol CR, and treatment with carvedilol IR was included only as a reference arm, there were no formal hypotheses to be tested. Therefore, statistical power was not evaluated, but all data were descriptively summarized by group. All patients who received at least 1 dose of the study drug were included in the safety evaluation. AEs were coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 12.0. The dose proportionality of S(–)-carvedilol was assessed using a power model. The slope parameters in the regression of log-transformed AUC_{0-24} and C_{max} were calculated with associated 90% confidence intervals.[33]

Table 2. Patient Disposition

	IR	CR	
	Primary period	Primary period	Exploratory period*
No. patients planned	30	30	–
No. patients randomized	22	19	8
Completed study	11 (50)	8 (42)	7 (37)
No. patients prematurely withdrawn	11 (50)	11 (58)	1 (5)
Primary reason for withdrawal			
Adverse event	2 (9)	4 (21)	0
Protocol deviation	1 (5)	0	0
Fulfilled protocol-defined stopping criteria	8 (36)	5 (26)	1 (5)**
Withdrew consent	0	2 (11)	0

Data given as n (%).
*The 8 patients who completed the primary evaluation period entered the exploratory evaluation period.
**Patient fulfilled protocol-defined stopping criteria just after completing dosing with carvedilol CR 40 mg.
Abbreviations see in Table 1.

Results

Patient Characteristics

A total of 41 patients were enrolled: 38 men (93%) and 3 women (7%). Although the planned sample size was 60 patients, enrollment was prematurely terminated when more than 40 patients were enrolled, because the minimum requirement of the study, which was to evaluate the safety and tolerability of carvedilol CR in Japanese patients with chronic HF, could be fulfilled with this sample size.

Table 1 lists demographic and clinical patient characteristics. Age ranged from 26 to 79 years; mean age±SD was 64.6±10.2 years. All patients were of Japanese ethnicity, and all were receiving concomitant cardiovascular medications.

Safety and Tolerability of Carvedilol CR

Twenty-two patients received carvedilol IR, and 19 received carvedilol CR. Dose escalation criteria defined in the study design were applied at each titration step. Consequently, the proportions of patients who completed 40 mg/day of carvedilol CR and 20 mg/day of carvedilol IR were 42% (8/19) and 50% (11/22), respectively. Seven of 19 patients receiving carvedilol CR were titrated to a daily dose of 80 mg. Twenty-three patients did not complete the study: 6 because of AEs, 1 because of deviation from the study protocol, 2 due to withdrawal of consent, and 14 due to fulfilling protocol-defined stopping criteria (Table 2). The protocol-defined stopping criteria accounted for study withdrawal in 8 of 22 (36%) patients in the carvedilol IR group and in 6 of 19 (32%) patients in the carvedilol CR group. In the CR group, 5 withdrawals occurred during the primary evaluation period and 1 during the exploratory evaluation period, before dosing with 60 mg of carvedilol CR.

The AEs are summarized in Table 3. During the primary evaluation period, a higher overall incidence of AEs and of drug-related AEs was observed in the carvedilol CR group, but the nature of the AEs in the carvedilol CR group was similar to those reported in the IR group, and most of these events were of mild or moderate intensity. AEs led to withdrawal in 6 patients: 2 (9%) in the IR group and 4 (21%) in the CR group. There were no withdrawals in the CR group after escalation to once-daily doses of 60 and 80 mg.

A total of 4 serious AEs (SAEs) were reported: 1 in the carvedilol IR group (cardiac failure) and 3 in the carvedilol CR group (bradycardia, edema, and bronchitis). The 1 SAE

Table 3. AEs Reported in Primary Evaluation Period (Excluding Run-in Period)

	n (%)	
	IR (n=22)	CR (n=19)
Any AE	9 (41)	12 (63)
Any drug-related AE	4 (18)	7 (37)
Cardiac disorders	1 (5)	3 (16)
Bradycardia	0	1 (5)*
Cardiac failure	1 (5)*	0
Cardiac failure congestive	0	1 (5)*
Nodal rhythm	0	1 (5)*
Ventricular extrasystoles	0	1 (5)*
Investigations	1 (5)	1 (5)
Blood alkaline phosphatase increased	0	1 (5)
Blood calcium decreased	1 (5)	0
Blood creatine phosphokinase increased	0	1 (5)
Brain natriuretic peptide increased	0	1 (5)
Platelet count decreased	1 (5)	0
Nervous system disorders	1 (5)	1 (5)
Dizziness	1 (5)	1 (5)
Respiratory, thoracic, and mediastinal disorders	1 (5)	1 (5)
Dyspnea	0	1 (5)*
Pleural effusion	1 (5)*	0
General disorders and drug site conditions	0	1 (5)
Edema	0	1 (5)*
Vascular disorders	0	1 (5)
Hypotension	0	1 (5)
Any serious AE	1 (5)	3 (16)
Any serious drug-related AE	1 (5)	2 (11)
Death	0	0

Presented by Preferred Term and System Organ Class of Medical Dictionary for Regulatory Activities ver. 12.0
*AEs resulting in withdrawal. Dyspnea, nodal rhythm, ventricular extrasystoles occurred in the same patient.
AE, adverse event. Other abbreviations see in Table 1.

Table 4. Pharmacokinetic Parameters for S(–)-Carvedilol in Plasma

Dosage	n	Visit (week)	AUC_{0-24} (ng·h/ml)	C_{max} (ng/ml)	t_{max} (h)
5mg IR	6	2	21.14 (92.0)	1.59 (77.9)	3.98 (1.92–4.05)
10mg CR	6	4	47.05 (89.4)	4.27 (74.6)	4.01 (1.98–6.20)
20mg CR	5	6	102.81 (57.8)	9.99 (52.2)	4.12 (3.88–6.17)
40mg CR	4	8	155.11 (61.1)	12.82 (92.7)	5.96 (4.00–6.08)
80mg CR	5	12	341.10 (42.2)	25.59 (65.1)*	5.83 (3.80–6.00)*
Slope (90%CI)**			0.967 (0.849–1.085)	0.914 (0.756–1.072)	NC

Data given as geometric mean (CVb%) or median (range).
*n=6. **Estimated by power model (dose proportionality analysis).
AUC_{0-24}, area under the concentration-time curve from zero (before dosing) to 24h; C_{max}, maximum plasma concentration; t_{max}, time to maximum observed drug concentration; CI, confidence interval; NC, not calculated. Other abbreviations see in Table 1.

reported in the carvedilol IR group and 2 of the 3 SAEs (bradycardia and edema) reported in the carvedilol CR group were judged to be related to the study drug. All observed SAEs resolved during the study period. There were no deaths.

No clinically important changes were observed in laboratory values, vital signs, body weight, 12-lead ECG, or CTR.

PK and PD

The results of PK analysis are given in Table 4. In general, AUC_{0-24} and C_{max} for S(–)-carvedilol increased in an approximately dose-proportional manner with increasing doses of carvedilol CR. AUC_{0-24} and C_{max} estimates for plasma S(–)-carvedilol from the power model were close to unity. Thus, dose proportionality was confirmed in the CR group.

The mean half-hourly systolic BP, diastolic BP, and mean HR did not substantially differ between the IR and CR groups. With increasing concentrations of carvedilol IR and carvedilol CR, exposure to S(–)-carvedilol had no specific pattern with respect to systolic and diastolic BP. There was, however, a trend toward a slight decrease in HR with increasing plasma S(–)-carvedilol (data not shown).

Efficacy

No patient in either treatment group showed deterioration from baseline in NYHA functional class during the period of the study. Mean±SD plasma BNP concentration was 108.93±112.192pg/ml (n=20) and 226.46±339.640pg/ml (n=19) at baseline, and change from baseline was 14.75±61.290pg/ml (n=11) and 55.10±64.585pg/ml (n=8) at week 8 in the IR and CR groups, respectively, and 15.90±41.044pg/ml (n=7) at week 12 in the CR group. LVEF±SD was 34.0±8.43% (n=20) and 32.2±10.09% (n=19) at baseline, and change from baseline was 8.9±5.38% (n=11) and 4.8±12.80% (n=8) at week 8 in the IR and CR groups, respectively, and 7.0±13.77% (n=7) at week 12 in the CR group.

Discussion

This is the first study to investigate the safety and tolerability of carvedilol CR in Japanese chronic HF patients. It is also the first study to show that Japanese chronic HF patients receiving conventional treatment with angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers were successfully titrated to a carvedilol CR dose comparable to that used in Western populations, that is, a daily dose of 80mg. In this study, the proportions of patients who reached 40mg/day of carvedilol CR (42%) and 20mg/day of carvedilol IR (50%) were generally comparable, and the type of observed AEs in patients receiving carvedilol CR did not substantially differ from those in patients receiving carvedilol IR. Thus, the absence of new safety issues is confirmed. These proportions are numerically lower than that in the MUCHA study,[23] in which approximately 68% of enrolled patients achieved the target dose of 20mg/day of carvedilol IR, partly because 36% and 31% of patients in the present IR and CR groups were withdrawn due to protocol-defined stopping criteria (Table 2). Accordingly, the proportion of patients who reached 80mg/day of carvedilol CR (37%) in the present study was lower than the 73% and 66% who achieved 50mg/day of carvedilol IR in the US carvedilol and COPERNICUS studies, respectively.[20,22] It should be stressed that, during the exploratory evaluation period, there were no withdrawals among patients in the CR group after escalation to once-daily doses of 60 and 80mg.

In Japan, the approved maximum doses of angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and β-blockers are less than half of those in Western countries, but there is little evidence of the tolerability of daily carvedilol doses higher than 20mg with regard to Japanese chronic HF patients. In a 1989 study comparing differences in response to propranolol between US white men and men of Chinese ethnicity, Zhou et al observed that the latter group showed greater sensitivity to the study drug, as indicated by significantly greater reductions in both HR and mean arterial BP.[34] The period of use, however, was limited to 1 day for each of the 4 dose levels. In the present study of the pharmacokinetics of S(–)-carvedilol after repetitive administration of daily doses of 40 and 80mg of carvedilol CR, exposures observed in the present Japanese patients were similar to those reported in a study of US chronic HF patients,[26] and 37% of patients in CR group completed the dose of 80mg/day of carvedilol CR.

There is some evidence that the mortality benefits of carvedilol and other β-blockers are dose dependent.[35–37] The prospective Multicenter Oral Carvedilol Heart failure Assessment (MOCHA) trial found a dose-dependent reduction in mortality after 6 months of carvedilol treatment in chronic HF patients.[38] Also, a recent commentary on a meta-analysis of prospective studies indicated that, although higher doses of β-blocker could increase the rate of withdrawal, such dose levels might be associated with reduced mortality.[39] In Japan, the MUCHA trial found a dose-dependent decrease in mortality and cardiovascular hospitalization in chronic HF patients receiving 5- and 20-mg/day carvedilol as compared with placebo.[23] The Japanese Cardiac Registry of Heart Failure in Cardiology (JCARE-CARD) study noted that the risk of cardiac death was significantly lower in the patient subgroup receiving a carvedilol dose of ≥10mg/day as compared with those receiving