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ORIGINAL ARTICLE

A novel soluble guanylate cyclase activator with reduced risk of hypotension by short-acting vasodilation

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Abstract

Cinaciguat, a soluble guanylate cyclase (sGC) activator, was under clinical development for use in acute decompensated heart failure (ADHF), but was discontinued due to occurrence of hypotension. We hypothesized that short-term activation of sGC in ADHF patients would exert a vasodilative effect without hypotension irrespective of disease state, using a novel short-acting sGC activator, TY-55002. The objective of this study was to investigate the vasodilation and hemodynamic effects of TY-55002 in comparison with those of cinaciguat. TY-55002 and cinaciguat activated both normal and heme-oxidized sGC in a dose-dependent manner and caused rapid relaxation of phenylephrine-contracted rat aorta. However, TY-55002 had a milder effect than cinaciguat in enhancing the dose-activity response between normal and oxidized sGC. Therefore, we suggest that the pharmacological effect of TY-55002 is less subject than cinaciguat to oxidative stress associated with complications such as cardiovascular disease or diabetes. In normal dogs, the effects of intravenous TY-55002 or cinaciguat on blood pressure were evaluated in conjunction with the plasma concentrations of the compounds, and pharmacokinetic (PK)-pharmacodynamic (PD) analyses were carried out. The plasma-to-effect-site transfer rate constant (Ke_0) for TY-55002 was three times greater than for cinaciguat. On the other hand, there was a small difference in blood half-life ($T_{1/2}$) between the compounds. It is possible that the rapid fall in blood pressure after the initial administration of TY-55002 and the quick recovery after cessation were due to the pharmacodynamic property of the compound. In heart failure-model dogs, TY-55002 and cinaciguat improved the condition to the same degree, and the short-term action of TY-55002 was replicated. In conclusion, TY-55002 is a novel short-acting sGC activator, which offers the possibility of easy dose management without excessive hypotension. It therefore holds potential to serve as an innovative drug in the pharmacotherapy of ADHF.

Abbreviations: ADHF, acute decompensate heart failure; CHF, chronic heart failure; Emax, maximal effective concentration; HF, heart failure; Ke_0, equilibration rate-constant; LC/MS/MS, liquid chromatography-tandem mass spectrometry; LVEDP, left ventricular end diastolic pressure; ODQ, 1H-[1,2,4]oxadiazolo [3,4-a]quinoxalin-1-one; PCWP, pulmonary capillary wedge pressure; PK-PD, pharmacokinetic-pharmacodynamic; SBP, systolic blood pressure; sGC, soluble guanylate cyclase.

KEYWORDS
acute decompensate heart failure, pharmacokinetic-pharmacodynamic, soluble guanylate cyclase activator, TY-55002

1 | INTRODUCTION

More than one million patients in both Europe and the United States are hospitalized annually with heart failure (HF).[1] In the United States, the yearly total cost of HF treatment is estimated to exceed $30 billion, with more than half spent on the cost of hospitalization.[2] Patients with HF remain at a substantial risk of acute decompensated heart failure (ADHF), which is associated with a poor clinical outcome. In-hospital mortality remains at ~4%, while re-hospitalization or death occurs in ~40% of patients within 12 months after discharge.[3]

ADHF is a clinical syndrome caused by insufficient cardiac output, arising from any cardiac structural or cardiovascular functional disorder and resulting in volume overload on the lungs to cause pulmonary congestion and edema, followed by dyspnea.[4,5] Nitrates are commonly used for the treatment of patients with ADHF. These activate the nitric oxide receptor, soluble guanylate cyclase (sGC). The activation of sGC facilitates the conversion of GTP into an intracellular second messenger, cyclic GMP, resulting in a reduction of cardiac preload and afterload by venous and arterial vasodilation.[6] ADHF is complicated with several cardiovascular diseases as well as other comorbidities with oxidative stress, including diabetes, hyperlipidemia, and renal disease. Oxidative stress, a risk factor for cardiovascular disorders, changes the conformation of sGC by oxidation of heme at the catalysis site of the enzyme to the NO-insensitive form.[7,8] Therefore, the above-mentioned NO/sGC/cGMP signaling pathway can be disrupted in the patients with ADHF.[6,9-11]

Cinaciguat is the first of a new class of sGC activators that act on NO-insensitive sGC and induces vasodilation in ADHF-related disease vessels. In healthy male volunteers, the intravenous administration of 50-250 μg/h cinaciguat for 4 hours yielded a rapid on-off effect, where the plasma levels of the compound reached a near-maximum within the first 30 minutes of infusion and declined after the cessation of the infusion.[12] In patients with ADHF, however, time delays between plasma cinaciguat concentration and the hemodynamic effect were observed when it was administered intravenously with doses ranging from 50 to 400 μg/h, and 3-4 hours were required for complete hemodynamic recovery after the cessation of infusion.[13] Cinaciguat led to a dose-dependent decrease in pulmonary capillary wedge pressure (PCWP) in patients with ADHF, but the majority of patients developed hypotension at doses less than 200 μg/h.[14] An excessive decrease in blood pressure can cause outcomes such as kidney failure,[15] and on this basis, the clinical development of cinaciguat was terminated prematurely.

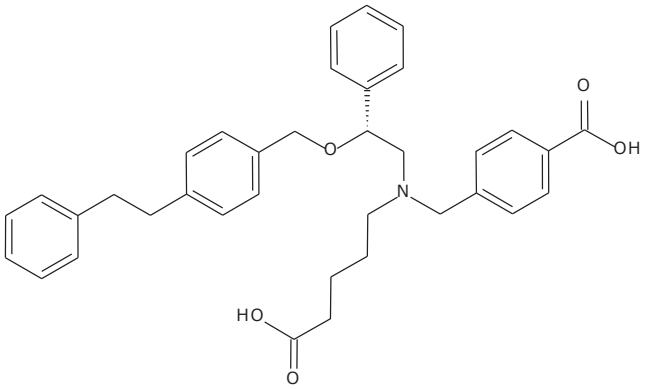
Cinaciguat has a more potent effect on oxidized/heme-free sGC than its normal form, and this is considered a reason behind excessive hypotension. Therefore, it is worthwhile to develop a novel sGC activator that exerts clinical effects regardless of the heme status of the enzyme. Furthermore, the rapid on-off effect is another key factor for the prevention of excessive hypotension and the safe management of vasodilation therapy.

A novel sGC activator, (-)-4-[[(4-Carboxybutyl)[(2*R*)-2-phenyl-2-[[4-(2-phenylethyl) phenyl]methoxy]ethyl]amino]methyl]benzoic acid (TY-55002, Figure 1), was designed for conserved vasodilation activity with reduced hypotensive effect regardless the condition of the patients, so as to make dose adjustment straightforward. The objective of this study was to clarify the pharmacological profile of TY-55002 and to confirm whether it is superior to cinaciguat from the standpoint of dose adjustment without excessive hypotension. We evaluated the potency of TY-55002 and cinaciguat in sGC activation and vasorelaxation profiles in rat aorta with and without 1H-[1,2,4] oxadiazolo [3,4-a]quinoxalin-1-one (ODQ) that oxidizes heme in sGC.[16] We also evaluated the effects of intravenously-infused TY-55002 and cinaciguat on hemodynamics in normal dogs in conjunction with plasma concentrations of the compounds, with PK-PD relationship analyses conducted based on the effect-compartment model, and finally examined the effects of intravenous TY-55002 in chronic heart failure (CHF) dogs to confirm the short-acting character of the compound under pathophysiological condition.

2 | MATERIALS AND METHODS

2.1 | Animals

All animal experiments were reviewed and approved by the Experimental Animal Committee of R&D DEPT. TOA EIYO LTD.



TY-55002

FIGURE 1 Heme-independent soluble guanylate cyclase activator, TY-55002

(Fukushima, Japan). Sprague-Dawley rats (9-10 weeks old) were obtained from Japan SLC (Shizuoka, Japan) and housed at a 12-h light/dark cycle with a standard chow diet (F-2, Funabashi Farms, Chiba, Japan) with water ad libitum. Beagle dogs (KITAYAMA LABS CO., LTD., Ina, Japan) were housed individually in cages under regulated temperature (23 ± 3°C) and humidity (50 ± 20%), and a 12-hr light/dark cycle before the study. Each dog received a standard laboratory diet (DM-2, Funabashi Farms, Chiba, Japan) once daily and had free access to water, in accordance with the Guide for the Care and Use of Laboratory Animals, as issued by the National Research Council.

2.2 | Test compounds and Reagents

TY-55002 and cinaciguat were prepared by TOA EIYO LTD, Tokyo Research Laboratories (Saitama, Japan). Recombinant human soluble guanylate cyclase (α1β1), ODQ, acetylcholine chloride, and phenylephrine hydrochloride were purchased from Enzo Life Sciences (Farmingdale, NY), Sigma-Aldrich (St Louis, MO), Daiichi-Sankyo (Tokyo, Japan), and Wako Pure Chemicals (Osaka, Japan), respectively. All other reagents were of the highest quality available (Sigma-Aldrich or Wako Pure Chemical Industries Ltd, Osaka, Japan). cGMP HTRF Kit was purchased from Cisbio Bioassays (Bedford, MA).

2.3 | Measurement of sGC activity

Recombinant human soluble guanylate cyclase (0.01 ng) was incubated in assay buffer (50 mmol/L Triethanolamine, 100 μmol/L DTT, 0.005% Brij35, 2 mmol/L $MgCl_2$, 0.2 μg/μL BSA, 100 μmol/L GTP, 100 μmol/L ODQ, pH 7.4) with TY-55002 or cinaciguat at 37°C for 20 minutes. Assays were performed at 8-point concentrations of 1:10 serial dilution starting at 3000 nmol/L (n = 7).

CHO-K1 cells stably expressing human sGC α/β subunit were obtained by transfection of the expression vector (pcDNA3.1-Zeo, Thermo Fisher Scientific, Waltham, MA) into human sGC α/β subunits gene and selection by zeocin. Human sGC α/β subunit expressed CHO-K1 cells were washed with Hanks' Balanced Salt Solution, then incubated at 37°C for 90 minutes in Hanks' Balanced Salt Solution containing experimental compounds, 250 μmol/L IBMX, and 10 μmol/L ODQ (in the presence of ODQ) or no ODQ (in the absence of ODQ). Assays were performed at 7-point concentrations of 1:10 serial dilution starting at a 3000 nmol/L (n = 5 for TY-55002 or n = 4 for cinaciguat).

After incubation with compounds, cGMP concentration in the well was measured by cGMP HTRF Kit using EnVision Multilabel Reader.

2.4 | Measurement of vasorelaxing profiles

Experiments were conducted using aorta ring preparations isolated from male Sprague-Dawley rats (350-400 g). Rats were anesthetized by the intraperitoneal injection of 30 mg/kg pentobarbital sodium (Kyoritsu Seiyaku, Japan). Aortas were dissected and cut into rings approximately 1.2 mm in length. Each ring was mounted for isometric force recording (UFER, Iwashiya Kishimoto Medical Instruments, Kyoto, Japan). Tissues were maintained at 37°C in a Krebs-Henseleit solution of the following composition (in millimoles): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4 \cdot 7H_2O$, 1.2; $NaHCO_3$, 25; and, glucose, 10. The bath solution was continuously aerated with 95% O_2 and 5% CO_2. All rings were placed under an optimal resting tension of 1 g and were allowed to equilibrate for 1 hour before the addition of test compounds.

For testing compounds in tissues, phenylephrine hydrochloride and acetylcholine chloride were dissolved in distilled water. Experimental compounds and ODQ were dissolved in dimethyl sulfoxide (DMSO). Tissues were contracted sub-maximally with 1 μmol/L phenylephrine (PE). The endothelial integrity of each ring was routinely checked by reaching >70% relaxation in response to the addition of 10 μmol/L acetylcholine (ACh). Changes in isometric force were recorded using a chart recorder or PowerLab 16/35 data acquisition system (ADInstruments, Bella Vista, NSW, Australia). In the concentration-response study, tissues were contracted with 1 μmol/L PE, and relaxation responses were evaluated according to cumulative addition of each test compound to the bath. Experimental values were obtained in the absence and presence of 10 μmol/L ODQ. ODQ was applied 10 minutes before PE administration (n = 6).

In the time-course-exploration study, tissues were contracted with 1 μmol/L PE, and relaxation responses due to the addition of fixed concentrations of cinaciguat or TY-55002 (at IC_{50} values) to the bath were evaluated (n = 9). Relaxation responses were analyzed statistically between 5 and 40 minutes (5, 10, 20, or 40 minutes) after the addition of the test compounds, which were determined based on preliminary experiments.

2.5 | PK-PD study in anesthetized normal dogs

In the pharmacodynamics study, five male beagle dogs (9-11 kg body weight) were used. Dogs were lightly anesthetized with intravenous sodium pentobarbital at 10 mg/kg. A chronic indwelling catheter (BCOEX-T22 tubing; Instech Soloman) was placed into the abdominal aorta via the femoral artery and tunneled subcutaneously to the cephalad portion of the dog's back, where it was exteriorized. Dogs were allowed to recover for at least 2 days before the experiment. To evaluate the effect of the test compounds on hemodynamics, TY-55002 (0.25 μg/kg/min) or cinaciguat (0.075 μg/kg/min) was administered intravenously for 180 minutes to the dogs, under anesthetic conditions. Measurements of aortic pressure were carried out from 30 minutes prior to 60+ minutes after administration, until observable effects disappeared. Aortic pressure was measured in the implanted catheter using a pressure transducer (AD Instruments) and was recorded using a PowerLab 16/35 data acquisition system, which calculated a mean value each minute. Change in systolic blood pressure (SBP) from baseline was defined as ΔSBP, with a moving average calculated every 5 minutes.

One week after the pharmacodynamics study, a pharmacokinetic study was conducted using the same animals. An infusion catheter

was placed in the cephalic vein of one foreleg and an indwelling needle was inserted into the cephalic vein of the other foreleg for blood sampling. Administration to all dogs was performed in the same manner as described above. Blood samples were collected via the cephalic vein using a heparinized syringe at 0 (pre-infusion), 2, 5, 10, 20, 30, 45, 60, 90, 120, 180 (end of infusion), 182, 185, 190, 200, 210, 240, 270, 300, and 360 minutes after the onset of infusion. Blood samples were kept on ice prior to centrifugation at 14 000*g* for 10 minutes at 4°C. Total blood volume collected did not exceed 1% body weight of any dog.

TY-55002 and cinaciguat were extracted from plasma samples using acetonitrile-protein-precipitation and quantitated using liquid chromatography-tandem mass spectrometry. A calibration curve was established over the range of 0.02-20 ng/mL for TY-55002 and 0.02-10 ng/mL for cinaciguat. The lower limit of the quantification method was 0.02 ng/mL for both TY-55002 and cinaciguat. The calibration curve and measurement of quality control samples were examined for the assurance of quality of each assay batch, based on the 2013 MHLW guideline for industry regarding bioanalytical method validation. Half-life ($T_{1/2}$) was derived using a non-compartmental analysis (WinNonlin, ver. 2.1, Pharsight).

The relationship between ΔSBP and plasma concentrations was analyzed using a two-compartment-linked sigmoid Emax PK-PD model.[17] The individual PK-PD parameters were estimated using nonlinear regression analysis (WinNonlin, ver. 2.1, Pharsight).

The pharmacodynamic model consisted of the Hill equation:

$$E = Emax \sim Ce^{\gamma}/(ECe_{50}{}^{\gamma} + Ce^{\gamma})$$

where E is ΔSBP, Emax is maximal ΔSBP, ECe_{50} is the drug concentration at the effect site yielding 50% of the maximum effect in ΔSBP, Ce is the drug concentration at the effect site, and γ is the shape factor. The estimated PK-PD parameters included Emax, ECe_{50}, γ, and Ke_0 as the first order transfer rate constant of the test compound from plasma to and elimination from the effect compartment.

2.6 | Hemodynamic effect in CHF dogs

CHF was prepared experimentally in 10 male beagle dogs (8-11 kg body weight). CHF was induced by high-frequency ventricular pacing to achieve a pathophysiological profile considered to be similar to that of severe CHF in humans.[18,19] Ventricular dysfunction was induced by rapid ventricular pacing.[20,21] Intact dogs were anesthetized using intravenous ketamine hydrochloride (15 mg/kg) and xylazine (1.5 mg/kg), and a pacing lead (Beflex RF46D, Japan Lifeline, Tokyo, Japan) was inserted into the free wall of the right ventricle via the right external jugular vein. The pacing lead was tunneled subcutaneously to the back for electrical pacing by an external pacemaker (PACE 101H, OSYPKA Medical, Berlin, Germany). CHF was induced by rapid ventricular pacing at 280 bpm and terminated after 21 days of pacing. Animals were then anesthetized using intravenous ketamine hydrochloride (15 mg/kg), maintained by an infusion of sodium pentobarbital (4.5 mg/kg/h), and subsequently intubated using a cuffed endotracheal tube and mechanically ventilated with room air. Left ventricular pressure was measured using a multisegment pressure-volume conductance catheter (Ventri-Cath 507S; Millar Instruments Inc, Houston, TX), and aortic pressure was measured using a catheter inserted via the femoral artery. Hemodynamics was monitored using a multi-channel amplifier (MEG-6108, Nihon Kohden, Tokyo, Japan). We confirmed the CHF condition of each animal throughout the experimental period by monitoring left ventricular end diastolic pressure (LVEDP; around 30 mmHg) before each dosing. After the baseline values of cardiovascular variables were obtained, TY-55002 or cinaciguat was infused intravenously via the femoral vein for 180 minutes, at 0.3 μg/kg/min (n = 5) and 0.015 μg/kg/min (n = 5), respectively.

2.7 | Statistics

To measure activity on sGC, the value of half maximal effective concentration (EC_{50}) and maximum effect (Emax) based on the amount of cGMP was calculated using a four-parameter logistic model in Assay Explore software (BIOVIA, San Diego, CA). To measure vasorelaxing profiles, IC_{50} values were calculated using a four-parameter equation to describe a sigmoidal curve, using the Assay Explorer software. With the exception of the PK-PD study, each value represents the mean ± standard error. Statistical analyses were performed using a statistics package (EXSUS; CAC EXICARE, Osaka, Japan). Data from the vasorelaxation study were analyzed using two-way repeated measures ANOVA, and the difference between the profiles of the compounds was considered to exist when the interaction was statistically significant. Following ANOVA analyses, Student's *t* tests between the compounds were conducted at each time point, where the significance level of each comparison was adjusted using Bonferroni's correction so that the familywise error rate was set to 0.05. The data of blood pressure and LVDEP were analyzed separately during and after administration. Each analysis was performed in the same manner as the vasorelaxation study data, and the post hoc comparisons were conducted at the end of each period. Other data were analyzed using Student's *t* test. Differences were considered to be statistically significant at $P < 0.05$, and when p-values were lower than 0.01, they are presented as "$P < 0.01$".

3 | RESULTS

3.1 | Activation of soluble guanylate cyclase (sGC)

We found that TY-55002 activated heme-oxidized sGC in a dose-dependent manner (EC_{50} = 7.6 ± 3.3 nmol/L, Emax = 156.1 ± 38.8 nmol/L cGMP). This potency was somewhat less than that of cinaciguat (EC_{50} = 1.3 ± 0.6 nmol/L, Emax = 114.0 ± 23.3 nmol/L cGMP). When evaluating the activity of TY-55002 or cinaciguat were evaluated in the presence or absence of ODQ, we found that in human sGC α/β subunits expressed in CHO-K1 cells, the activity of TY-55002 was enhanced in the presence of ODQ (Table 1). These

TABLE 1 Potency of TY-55002 compared with cinaciguat in the presence or absence of ODQ

	EC_{50} (nmol/L)	Emax (nmol/L cGMP)
TY-55002		
ODQ–	19.7 ± 2.5	127.2 ± 23.2
ODQ+	5.9 ± 1.1	378.2 ± 69.8
Cinaciguat		
ODQ–	7.2 ± 1.7	55.9 ± 14.7
ODQ+	5.0 ± 1.6	277.3 ± 62.9

ODQ+, presence of ODQ; ODQ–, absence of ODQ.
Data shown give the mean ± SE for each group (n = 5 for TY-55002 or n = 4 for cinaciguat).

findings for TY-55002 are similar to those of cinaciguat in the presence or absence of ODQ, suggesting that TY-55002 is a heme-independent sGC activator.

3.2 | Effects on aortic relaxation with and without ODQ

Cinaciguat and TY-55002 caused relaxations of PE-contracted aorta rings in a concentration-dependent manner with IC_{50} values of 1.22 ± 0.46 and 3.92 ± 0.83 nmol/L, respectively. The addition of 10 μmol/L ODQ, a highly selective and irreversible sGC heme iron oxidant, shifted the concentration-response curves leftwards (IC_{50} values: cinaciguat; 0.0648 ± 0.0188 nmol/L; TY-55002; 0.338 ± 0.0544 nmol/L, Figure 2). This suggests an enhancement of the effects of cinaciguat and TY-55002 in the presence of ODQ. However, the vasorelaxation enhancement induced by ODQ for cinaciguat (18.8 ± 1.49-fold) was more potent than that of TY-55002 (11.6 ± 0.51-fold) ($P < 0.01$). These results suggest that TY-55002 may activate oxidized/heme-free sGC mildly compared with cinaciguat.

3.3 | Time-course of changes in vasorelaxation

The vasorelaxation induced by cinaciguat at 1.22 nmol/L (IC_{50} in the previous experiments) was −2.8 ± 0.6, −11.8 ± 1.5, −34.4 ± 4.2, and −56.6 ± 5.5% at 5, 10, 20, and 40 minutes after administration, respectively, while that induced by TY-55002 at 3.92 nmol/L (IC_{50} in the previous experiments) was −12.6 ± 2.9, −33.7 ± 5.7, −52.8 ± 6.2, and −57.1 ± 5.5%, respectively (Figure 3A). A difference in the profiles between the compounds was statistically significant according to two-way ANOVA ($P < 0.01$). At each time point, significant differences between the compounds were observed at 10 and 5 minutes ($P < 0.05$) after the addition. However, there was no significant difference at 20 or 40 minutes (Figure 3B). These results suggest that TY-55002 exerts vasorelaxation more rapidly than cinaciguat does, and the effect dissipates similarly quickly.

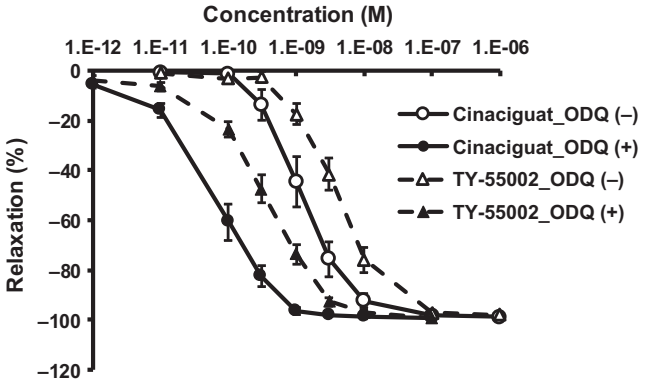


FIGURE 2 Effects of cinaciguat and TY-55002 on the relaxation of aortic rings contracted by PE (1 μmol/L) with and without ODQ (10 μmol/L). Experimental values were obtained in the absence (○, △) and presence (●, ▲) of ODQ. Data were calculated as relative changes as relaxation from the contraction produced by PE in each ring, which was taken as 100% contraction. Data are expressed as mean ± SEM (n = 6 for cinaciguat, n = 6 for TY-55002)

3.4 | Effect on blood pressure and PK-PD analysis in normal dogs

The dose of each test compound was selected based on the separate dose-finding studies, and the dose ratio of TY-55002 to cinaciguat for an equivalent reduction in blood pressure was roughly three-fold. There was no difference in SBP before administration between the TY-55002 group (188.3 ± 7.0 mmHg) and the cinaciguat group (189.3 ± 7.9 mmHg). TY-55002 at 0.25 μg/kg/min or cinaciguat at 0.075 μg/kg/min infused for 180 minutes reduced SBP with equal maximal response in normal dogs. TY-55002 more rapidly reduced blood pressure than cinaciguat after commencing infusion (Figure 4), and a significant interaction between group and time during the infusion were seen ($P < 0.01$) as the result of the ANOVA analysis of these data. However, at the end of the infusion, there was no difference in SBP reduction between the groups. Furthermore, in the TY-55002 group, the reduced blood pressure began to recover 30 minutes after the cessation, whereas cinaciguat, by contrast, reduced blood pressure slowly, with the decrease sustained after infusion ceased. An interaction between group and time by the ANOVA analysis ($P < 0.01$) and a difference in ΔSBP 30 minutes after the cessation ($P < 0.05$) were both significant.

There was no significant difference in $T_{1/2}$ between TY-55002 (55.2 ± 14.2 minutes) and cinaciguat (79.2 ± 8.26 minutes). The effect vs concentration plot showed a counter-clockwise hysteresis loop, indicating a time delay between concentration and ΔSBP (Figure 5). The time to onset of the antihypertensive effect for TY-55002 and cinaciguat was 20 and 30 minutes after the beginning of infusion, respectively. Although both compounds were similarly rapidly eliminated from plasma, the antihypertensive effect of cinaciguat lasted longer than that of TY-55002. According to the effect-compartment model, the Ke_0 of cinaciguat and TY-55002 was 0.00712/min and 0.0212/min (n = 5), respectively (Table 2). These

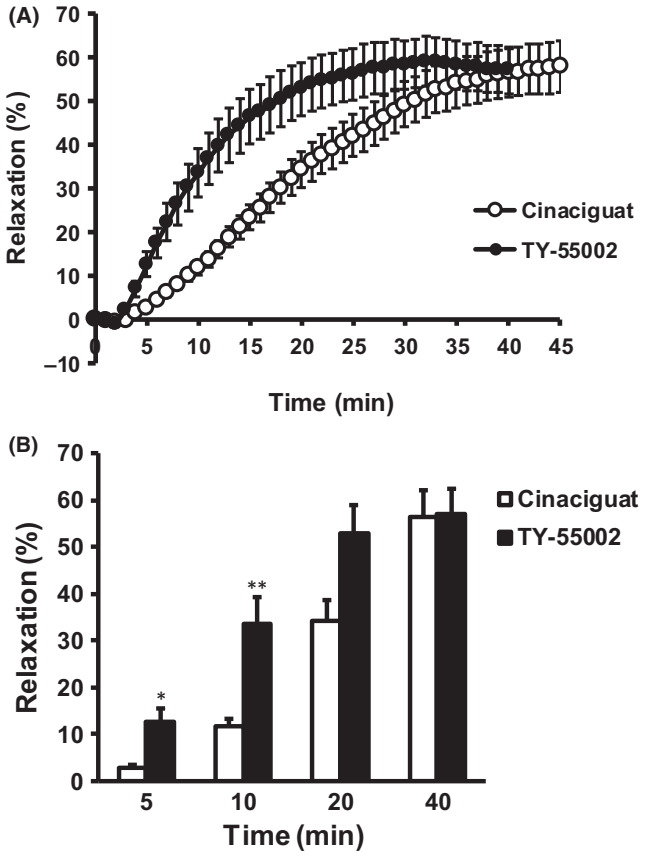


FIGURE 3 Time-course of changes in vasorelaxation induced by fixed concentrations of cinaciguat (○) and TY-55002 (●) in aortic rings contracted by PE (1 μmol/L). Relaxation responses were evaluated for time points between 5 and 40 minutes (5, 10, 20, and 40 minutes) after the addition of test compounds. Data were calculated as relative changes, as relaxation from the contraction induced by PE in each ring, which was taken as 100% contraction. Changes are shown every minute (A) and every ten minutes (B). Data are expressed as mean ± SEM (n = 9 for cinaciguat, n = 9 for TY-55002). * indicates $P < 0.0125$ and ** $P < 0.0025$ vs cinaciguat according to Student's *t* test with Bonferroni-adjustment

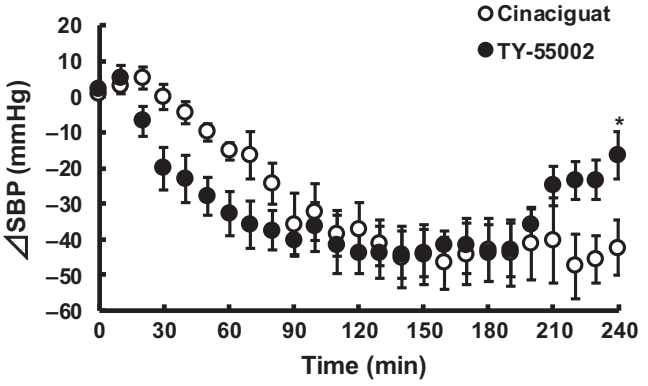


FIGURE 4 Effects of cinaciguat and TY-55002 on blood pressure in normal dogs. Cinaciguat 0.075 μg/kg/min or TY-55002 0.25 μg/kg/min was infused for 180 minutes. Data are expressed as the change in SBP from pretreatment, as the mean ± SEM for five dogs. * indicates $P < 0.05$ and vs cinaciguat according to Student's *t* test

results suggest that the faster onset and the shorter duration of the antihypertensive effect of TY-55002 were due to faster distribution to and elimination from the effect compartment.

3.5 | Effect on hemodynamics in CHF dogs

The dose of each test compound was selected based on our preliminary study using CHF dogs to obtain a decrease in LVEDP, which was more likely to be affected than the SBP, by 10-20 mmHg. There were no significant differences in SBP (149.5 ± 8.4 vs 156.8 ± 5.6 mmHg) or LVEDP (31.1 ± 1.5 vs 33.4 ± 3.3 mmHg) before test compound infusion between the TY-55002 and cinaciguat groups. Figure 6 summarizes the effects of intravenous administration of TY-55002 and cinaciguat on changes in SBP and LVEDP. Both TY-55002 and cinaciguat reduced cardiac load by decreasing SBP and LVEDP in CHF dogs. In LVEDP data, significant interactions between group and time were seen ($P < 0.01$) as the results of ANOVA analyses for both during and after the infusion, however, there was no difference between groups both at the end of infusion and evaluation. These results indicated that time courses of the action of the compounds were somewhat different but that overall improvement effects on heart failure were comparable. In ΔSBP data during the infusion, a significant interaction between group and time were seen ($P < 0.01$) as the result of the ANOVA analysis, and no difference between groups was observed at the end of infusion. These results indicated the difference in time course of the action between the compounds; however, there were few implications in contrast to the observations in the normal dogs because they crossed each other. After the end of drug administration, decreased SBP recovered in the TY-55002 group, by contrast, recovery of these parameters was not observed in the cinaciguat group. Cinaciguat reduced SBP to a greater degree than TY-55002 at the end of the evaluation, and the difference between the groups in addition to the interaction between group and time after the infusion by ANOVA were both statistically significant. In CHF dogs, the differences in time courses of SBP after the infusion between TY-55002 and cinaciguat were seen to correspond to the observations in normal dogs.

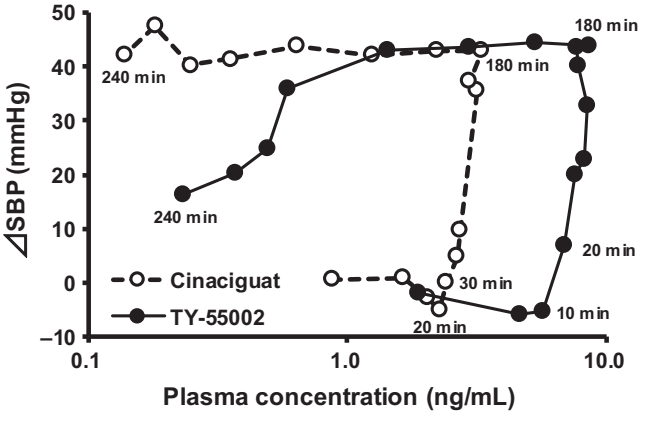


FIGURE 5 Relationship between the mean ΔSBP vs mean plasma concentrations of TY-55002 and cinaciguat in normal dogs (n = 5)

4 | DISCUSSION

sGC exists under physiological conditions in equilibrium between its reduced and oxidized state, and oxidative stress shifts this equilibrium towards the NO-insensitive ferric/heme-free form.[8] Cinaciguat is a novel sGC activator that activates the oxidation-impaired/heme-free form of sGC, causing dilation in diseased blood vessels.[8,22] Indeed, it has been reported that cinaciguat more potently dilated PE-contracted vessels in diseased animal models such as hypertensive rats and hyperlipidemic rabbits compared to normal animals. These effects are enhanced in the presence of ODQ, which oxidizes sGC.[8] These data suggest that cinaciguat can more strongly activate oxidized sGC than reduced sGC.

TABLE 2 Average $T_{1/2}$ and Ke_0 of TY-55002 and cinaciguat

	TY-55002	Cinaciguat
$T_{1/2}$	55.2 ± 31.7 (min)	79.2 ± 18.5 (min)
Ke_0	0.0212 ± 0.00460 (min^{-1})	0.00712 ± 0.00425** (min^{-1})

Ke_0 were obtained by fitting a two-compartment, linked sigmoid Emax PK-PD model following TY-55002 and cinaciguat infusion in normal dogs (n = 5). Data are expressed as mean ± SD.
**$P < 0.01$ compared with values for TY-55002

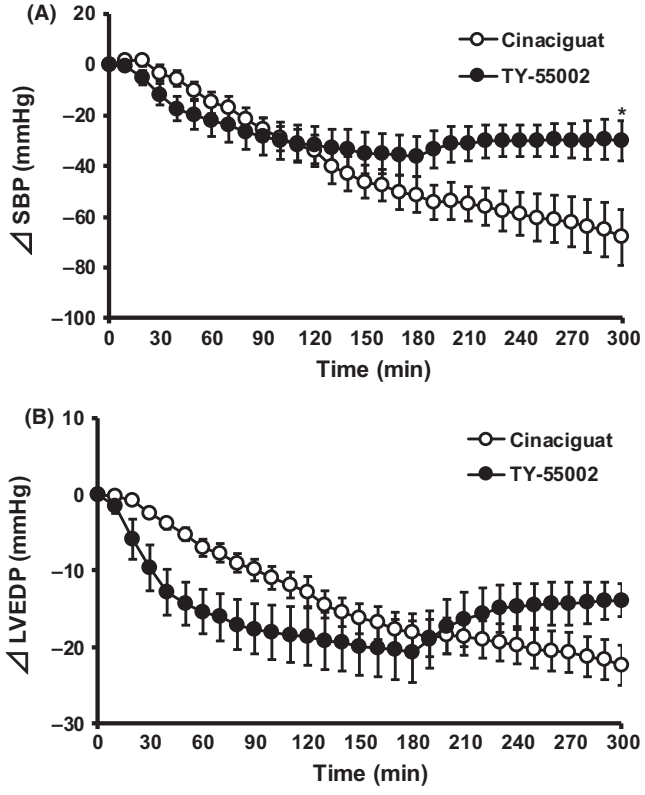


FIGURE 6 Effects of cinaciguat and TY-55002 on changes in (A) SBP and (B) LVEDP in CHF dogs. Cinaciguat 0.015 μg/kg/min or TY-55002 0.3 μg/kg/min was infused for 180 minutes. Data represent changes from pretreatment and are expressed as mean ± SEM for five dogs. * indicates $P < 0.05$ and vs cinaciguat according to Student's *t* test

In our study, cinaciguat inhibited the PE-induced contraction of aorta isolated from rats in a concentration-dependent manner (IC_{50}: 1.22 nmol/L), and its inhibitory response was enhanced in the presence of ODQ (IC_{50}: 0.0648 nmol/L), as reported previously.[16] Furthermore, the IC_{50} values of cinaciguat obtained in this study were similar to those reported in previous studies (IC_{50} of 0.15 and 1.23 nmol/L with and without ODQ, respectively;.[8] TY-55002 (EC_{50}: 7.6 nmol/L) had a potency of approximately 1/5.8-fold in terms of activation of oxidized/heme-free recombinant sGC, compared with cinaciguat. TY-55002 also counteracted PE-induced contraction of rat aorta in a concentration-dependent manner, with or without ODQ. Similarly, TY-55002 activated sGC in sGC-expressing cells regardless of the presence of ODQ. Therefore, we suggest that TY-55002 is a heme-independent sGC activator. The IC_{50} values of TY-55002 were 3.92 and 0.338 nmol/L without and with ODQ, respectively. The vasorelaxation effect in rat aorta induced by cinaciguat (18.8-fold) was more enhanced in the presence of ODQ than TY-55002 (11.6-fold). These data suggest that the activation effect of cinaciguat on sGC is more heterogeneous than that of TY-55002; the former depends more on the oxidation state of sGC of the patients. In the COMPOSE study, hypotension occurred unexpectedly under low dose of cinaciguat,[14,23] and oxidative stress in patients with ADHF was considered to be one of the reasons. Therefore, in order to avoid excessive hypotension, the desired sGC activator may be one that activates oxidized/heme-free sGC mildly to make the adjustment of the dose easy for treatment of patients with ADHF, irrespective of their disease states.

Hoffmann et al[24] reported that cinaciguat at 5 μg/kg/h reduced SBP by 14% at the end of a 60-minute infusion in conscious dogs. Our experiments were also conducted under unanesthetic conditions, and the data obtained with cinaciguat at 0.25 μg/kg/min (4.5 μg/kg/h) produced an SBP reduction at 60 minutes after the onset of infusion of 8%, nearly equal to their results. We also examined the effects of TY-55002 at 0.25 μg/kg/min (15 μg/kg/h), and showed that the compound requires about three times as much as cinaciguat to reduce blood pressure to the same extent. Cinaciguat reduced the blood pressure slowly, and more than 150 minutes was necessary to reach a steady-state. Further, the reduced blood pressure did not recover even at 60 minutes after the end of the infusion. On the other hand, TY-55002 reduced blood pressure more rapidly in the early phase of infusion than cinaciguat did, and the reduced blood pressure began to recover 30 minutes after the end of the infusion. These results suggested that the effects of TY-55002 are more short-acting compared with cinaciguat. The small difference in $T_{1/2}$ between the compounds suggests that this is insufficient to explain the mechanism of pharmacodynamic differences and that the short-acting character of TY-55002 is mainly due to the pharmacodynamic property of the compound.

The hemodynamic results for both test compounds were analyzed using a PK-PD model to investigate the difference in action in detail. A hysteresis loop between ΔSBP and the plasma concentrations was observed for both TY-55002 and cinaciguat. We found that Ke_0 of TY-55002 was significantly greater than that of

cinaciguat, and the difference was larger than in the pharmacokinetic elimination rate. These results suggest that the slower onset and the longer duration of the antihypertensive effect of cinaciguat treatment were due to the slower distribution to and elimination from the effect compartment. The mechanism responsible for the difference in Ke_0 between these test compounds remains unclear, but is likely due to the difference in molecular kinetic properties such as binding to and dissociation from sGC enzyme.

Considering these PK-PD characteristics of cinaciguat, it may be possible that blood pressure-based dose titration of cinaciguat will be difficult, leading to the induction of excessive hypotension in patients with ADHF. TY-55002 can decrease SBP depending more on PK than can cinaciguat, and dose adjustment can be easier with TY-55002. Presently, clevidipine is used for treating patients with acute severe hypertension. The effect has a rapid onset/offset of action and easy titratability with predictable blood pressure.[25] Clevidipine, indeed, has been shown to have a lower occurrence of hypotension than long-acting calcium channel blockers.[26] Therefore, in terms of the short-acting character, TY-55002 and clevidipine are considered conceptually comparable, and we expect that TY-55002 can similarly regulate blood pressure without excessive hypotension.

The vasodilatory properties of cinaciguat are particularly enhanced in diseased vessels.[8] No serious adverse event was reported when cinaciguat was administered intravenously to healthy male volunteers at 50-250 μg/h for 4 hours.[12] However, in cases involving patients with ADHF, the majority of patients developed excessive hypotension at doses less than 200 μg/h. In these instances, cinaciguat shows a dose-dependent decrease in PCWP, as mentioned above.[23] Therefore, we examined the effects of TY-55002 in a canine model of CHF and compared it to those of cinaciguat. This model closely mimics severe CHF in humans.[20] In our canine CHF model, the right ventricle was paced at 280 bpm for 21 days, and the dose of each test compound was selected based on our preliminary study using CHF dogs, where LVEDP was reduced by 10-20 mmHg. The effects of TY-55002 and cinaciguat in LVEDP reduction during the experiments were nearly equal. On the other hand, both TY-55002 and cinaciguat similarly decreased SBP during the infusion, however, the decrease in SBP by cinaciguat continued even after the end of infusion, in contrast to the attenuation of the effect in the TY-55002 group. Thus, we suggest that the recovery of SBP after the administration of TY-55002 can also be exerted under heart failure conditions. The dose ratio of TY-55002 vs cinaciguat for reducing SBP to the same extent was roughly threefold in normal dogs; however, it was more than 20-fold in the pacing-induced canine CHF model. These results suggest that the effect of TY-55002 is less affected by disease status than cinaciguat. Therefore, it may be easier to select the therapeutic dose of TY-55002.

Recent studies have implicated organ damage and persistent or recurrent congestion after admission as contributors to the poor prognosis of ADHF.[27,28] Injury or end-organ dysfunction, including myocardial damage and worsening renal function, is an independent predictor of increased mortality in patients with AHF.[29,30] A cGMP-producing sGC activator has cardioprotective effects with improving progressive cardiac remodeling and renal-protective effects independent of hemodynamics.[31-33] Therefore, it can be expected that prognosis may be improved through the organ protection provided by sGC activators.

The pathogenesis of acute heart failure has been previously theorized to be body fluid accumulation to cause congestion; accordingly, the main constituent of treatment has been to control body fluid using diuretics.[34] However, the improper use of diuretics causes renal dysfunction and electrolyte abnormalities during clinical acute heart failure.[35,36] In recent years, the contribution of congestion due to a redistribution of circulating blood has been shown to contribute to clinical pulmonary edema, and initial treatment using a vasodilator is recommended for cases of adaptation.[37,38] In clinical heart failure, vascular endothelial dysfunction is often seen, and the bioavailability of NO is decreased.[39,40] Use of a sGC activator, which is an endothelial-function-improving agent that is independent of NO, is considered reasonable in treating ADHF, if hypotension can be avoided by appropriate use.

In this study, we show that TY-55002 is short-acting irrespective of disease severities such as CHF in dogs, and suggest that the effect of TY-55002 is less affected by the oxidation/reduction state of sGC compared to cinaciguat. Therefore, TY-55002 can be expected to improve heart failure without excessive blood pressure reduction. Whether or not the TY-55002 characteristics obtained in this basic research study remain in humans is a question for future research. In conclusion, TY-55002 is a novel short-acting sGC activator having the possibility of easy dose management without excess hypotension, and thus expected to be an innovative drug for patients with ADHF.

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DISCLOSURE

None declared.

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