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# T-1032, a novel phosphodiesterase type 5 inhibitor, increases the survival of cardiomyopathic hamsters

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**Abstract**

To evaluate the influence of T-1032 (methyl2-(4-aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinoline carboxylate sulfate), a potent and relatively selective phosphodiesterase 5 inhibitor, on chronic heart failure, we examined the acute hemodynamic profile of T-1032 and its chronic effect on the survival of Bio 14.6 cardiomyopathic hamsters. In the acute study, T-1032 (1, 10, 100 μg/kg) was administered intravenously by means of a dose-escalating procedure in 55-week-old hamsters. T-1032 significantly reduced both the right and left ventricular end-diastolic pressure in a dose-dependent manner. T-1032 modestly reduced the systemic arterial pressure at the highest dose (100 μg/kg i.v.). T-1032 did not change the heart rate or left ventricular $dp/dt_{max}$. In the survival study, chronic administration of T-1032 (50 and 500 ppm in a diet) increased survival, and the survival rate was 24.2%, 45.4% and 48.5% in the control, 50 and 500 ppm-treated groups, respectively. The median survival was 55, 58 and 58 weeks in control, 50 and 500 ppm-treated groups, respectively. Analysis of the survival curves revealed that T-1032 (500 ppm) significantly increased the survival of these hamsters ($P < 0.05$ vs. control). It was concluded that T-1032 had beneficial hemodynamic effects on heart failure in Bio 14.6 cardiomyopathic hamsters, and the favorable hemodynamic changes induced by T-1032 were partly related to the increase in the survival of these hamsters. Phosphodiesterase type 5 inhibitors may have therapeutic potential for the treatment of chronic heart failure. 

*Keywords:* Phosphodiesterase type 5; Heart failure; Cardiomyopathic hamster; Survival

## 1. Introduction

Heart failure is a lethal syndrome characterized by myocardial dysfunction. This syndrome is initiated and/or progresses by a variety of pathologic conditions including myocardial infarction, hypertrophy, viral infection, and hereditary cardiomyopathy. In patients with heart failure, abnormal vasoconstriction is a typical pathophysiologic manifestation (Drexler et al., 1992, 1994; Maguire et al., 1998) and appears to be a crucial component of the deterioration of heart failure. To date, the effectiveness of several vasodilators has been evaluated in several clinical trials (Cohn, 1988; Hash and Prisant, 1997; Young, 1991).

The nitric oxide (NO)/guanosine 3′ 5′ -cyclic monophosphate (cGMP) signaling pathway plays an important role in the regulation of vascular tone. Recent evidence suggested that the NO/cGMP signaling pathway is impaired in human and experimental animals with heart failure (Abassi et al., 1997; Kubo et al., 1991; Lindsay et al., 1992). Therefore, a potentiation of the NO/cGMP signaling pathway is considered to be a therapeutic strategy to improve the vascular responsiveness in heart failure (Rector et al., 1996). In the regulation of cGMP levels in vessels, phosphodiesterase type 5 is a key enzyme for the hydrolysis of cGMP (Beavo, 1995). Recently, selective inhibitors of phosphodiesterase type 5 such as sildenafil and T-1032 (methyl2-(4-aminophenyl)-1,2-dihydro-1-oxo-7-(2-pyridylmethoxy)-4-(3,4,5-trimethoxyphenyl)-3-isoquinoline carboxylate sulfate) (Ukita et al., 1999) have been reported to cause vasodilatation together with the accumulation of cGMP levels in isolated vessels (Takagi et al., 2001; Wallis et al., 1999). Interestingly, phosphodiesterase type 5 inhibitors cause venodilation without causing potent hypotension or tachycardia in anesthetized animals (Inoue et al., 2001; Ng and Pang, 1998). These results suggested that vasodilator therapy with phosphodiesterase type 5 inhibitors is a suitable strategy for the treatment of heart failure.

In order to examine this hypothesis, we first examined the hemodynamic effects of T-1032 in hereditary cardio-

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myopathic Bio 14.6 hamsters, an established model of heart failure (Factor et al., 1982; Gertz, 1972), and next evaluated the effect of chronic treatment with T-1032 on heart failure-related death in these hamsters.

## 2. Methods

This study was approved by the Animal Research Committee of Tanabe Seiyaku.

### 2.1. Animals

Male hamsters of Bio 14.6 strain were purchased from Charles River Japan (Yokohama, Japan). All hamsters were individually housed in our animal room for 2–3 weeks at a temperature of 24 °C and under a 12-h light/dark cycle (0600–1800 h). The hamsters received a powdered diet (CE-7, Nihon Clea, Tokyo, Japan) and tap water ad libitum. Throughout the chronic study, T-1032 was mixed in the daily diet at a concentration of 50 or 500 ppm.

### 2.2. Experimental protocol

#### 2.2.1. Hemodynamic study

Male Bio 14.6 hamsters ($N=6$) aged 55 weeks were anesthetized with thiobutabarbital sodium (100 mg/kg, i.p.). Cardiac pressure measurements were performed as described previously (Yamauchi-Kohno et al., 1999). A catheter (SP-10, Natume, Tokyo, Japan) was inserted into the right carotid artery to measure the left ventricular $dp/dt_{max}$ and left end-diastolic pressure. Subsequently, another catheter (SP-10, Natume) was inserted into the right jugular vein to measure the right ventricular end-diastolic pressure. Mean arterial pressure was measured by means of a catheter inserted into femoral artery (SP-10, Natume), and heart rate was measured by facilitating arterial pulsation. T-1032 was administered through a catheter in the left femoral vein (SP-10, Natume). After a stabilization period, T-1032 (1, 10, 100 μg/kg, i.v.) was administered by a dose escalating procedure at 5-min intervals.

#### 2.2.2. Survival study

Male Bio 14.6 hamsters ($N=101$) were randomly divided into three groups at 30 weeks of age: control ($N=33$), T-1032 50 ppm ($N=34$)-, and T-1032 500 ppm ($N=34$)-treated groups. This study was terminated when 75% of the control hamsters died. Throughout the experiment, the food intake in Bio 14.6 hamsters was 7.2–11.4 g/day. The doses of T-1032 were approximately 2.5–3.5 and 29–37 mg/kg/day in the 50 and 500 ppm-treated groups, respectively, when calculated from the food consumption and body weight. At the end of the experiment, all surviving hamsters treated with T-1032 were killed under ether anesthesia and blood samples were taken to measure plasma concentrations of T-1032. The plasma concentrations were

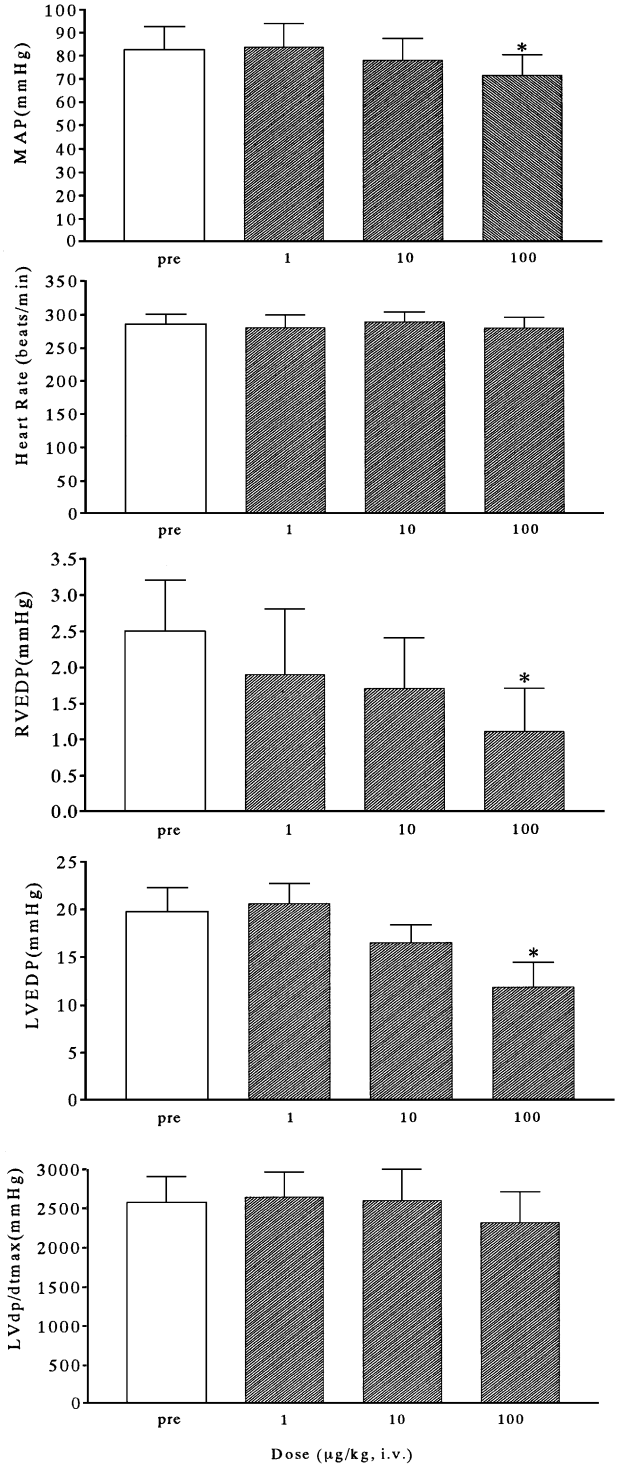


Fig. 1. Dose–response relationships of intravenous administration of T-1032 on mean arterial pressure (MAP), heart rate (HR), left ventricular end-diastolic pressure (LVEDP), right ventricular end-diastolic pressure (RVEDP), and left ventricular $dp/dt_{max}$ (LVdp/dtmax) in anesthetized Bio 14.6 hamsters. Data are presented as percent changes from baseline. Statistically significant differences were observed from baseline values ($P<0.05$ by Dunnett's test).

$0.05 \pm 0.02$ and $1.1 \pm 0.5$ (μg/ml, mean ± S.D.) in the 50 and 500 ppm-treated groups, respectively.

*2.2.3. Statistical analysis*

Changes in cardiovascular parameters are given as means ± S.E.M. Analysis of cardiovascular changes was performed using a randomized completely block design followed by Dunnett's test for comparison with the baseline value. Analysis of survival curves was performed by log-rank test with Kaplan–Meier survival curves. A $P$ value of less than 0.05 was considered to be statistically significant. All data were analyzed with Stat View ver. 5.0 (SAS Institute, Japan).

## 3. Results

Fig. 1 shows the acute effect of intravenously administered T-1032 (1, 10, 100 μg/kg) on mean arterial pressure, heart rate, right ventricular end-diastolic pressure, left ventricular end-diastolic pressure, and left ventricular $dp/dt_{max}$ in anesthetized Bio 14.6 hamsters. Baseline values of mean arterial pressure, heart rate, right ventricular end-diastolic pressure, left ventricular end-diastolic pressure and left ventricular $dp/dt_{max}$ were $82.7 \pm 10$ mm Hg, $285.7 \pm 14.9$ beats/min, $2.4 \pm 0.7$ mm Hg, $19.8 \pm 2.5$ mm Hg, and $2575 \pm 333$ mm Hg/s, respectively. The elevation of the right ventricular end-diastolic pressure and left ventricular end-diastolic pressure suggests that these hamsters were in a diastolic failure. T-1032 (100 μg/kg) modestly but significantly reduced the mean arterial pressure ($P < 0.05$, Dunnett's test). T-1032 reduced both the right- and left ventricular end-diastolic pressure in a dose-dependent manner. The maximum reduction in the right- and left ventricular end-diastolic pressure was $-70.7 \pm 28.7\%$ and $-40.9 \pm 9.1\%$, respectively. T-1032 did not affect the heart rate or the left ventricular $dp/dt_{max}$ at any dose ($P > 0.05$, Dunnett's test).

In the next study, we examined the effect of chronic administration of T-1032 (50, 500 ppm) on the survival of Bio 14.6 hamsters. Time courses of changes in food consumption and in body weight were not different among the three groups (data not shown). The survival parameters are summarized in Table 1. The first death was observed at 35, 38 and 37 weeks of age in control, T-1032 (50 ppm)- and T-1032 (500 ppm)-treated groups, respectively. The median survival time was 55, 58 and 58 weeks in control, T-1032 (50 ppm)- and T-1032 (500 ppm)-treated groups, respectively. The final survival rates were 24.2% (8/33), 45.4% (15/33) and 48.5% (16/33) in control, T-1032 (50 ppm)- and T-1032 (500 ppm)-treated groups, respectively. The survival curves are shown in Fig. 2. There was a significant difference between control and T-1032 (500 ppm)-treated groups ($P < 0.05$ by log-rank test), but not between control and T-1032 (50 ppm)-treated groups ($P = 0.12$ by log-rank test).

Table 1
Effect of chronic administration of T-1032 on the survival of Bio 14.6 hamsters

| Treatment group | First death (weeks age) | Median survival time (weeks age) | Final survival |
|---|---|---|---|
| Control (Biol 14.6) | 35 | 55 | 8/33(24.2%) |
| T-1032 (50 ppm) | 38 | 58 | 15/33(45.4%) |
| T-1032 (500 ppm) | 37 | 58 | 16/33(48.5%) |

The survival parameters are expressed as first death, median survival time, and final survival. Final survival was determined at the time when 75% of the control group had died. N.D. was not determined.

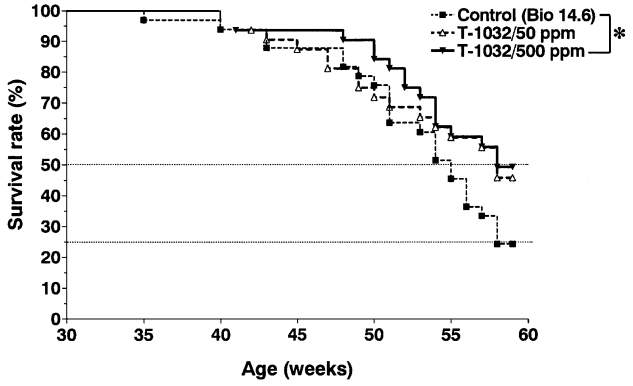


Fig. 2. Survival curves of Bio 14.6 hamsters with or without treatment with T-1032. Statistically significant difference was observed between control and T-1032/500 ppm group ($P < 0.05$ by log-rank test).

## 4. Discussion

In the present study, we examined the acute and chronic effects of T-1032, a potent and relatively selective phosphodiesterase type 5 inhibitor, on heart failure in Bio 14.6 cardiomyopathic hamsters. In the acute study, intravenously administered T-1032 reduced the right and left ventricular end-diastolic pressure and caused mild hypotension, but did not influence the heart rate or the left ventricular $dp/dt_{max}$. The chronic study clearly indicated that oral treatment with T-1032 increased survival. To our knowledge, this is the first report to show the effectiveness of chronic treatment with a phosphodiesterase type 5 inhibitor on the survival of Bio 14.6 cardiomyopathic hamsters. These results suggest that selective phosphodiesterase type 5 inhibitors have a beneficial effect on fatal chronic heart failure.

In patients with chronic heart failure, vasoconstriction is a typical pathophysiologic manifestation (Drexler et al., 1992, 1994; Maguire et al., 1998), and the long-term vasoconstriction appears to be a crucial component of the deterioration seen in this syndrome. Vasodilator therapy is one of the pharmacodynamic interventions for the treatment of heart failure, and this therapy is considered to improve cardiac performance by causing arterial and/or venous dilatation, which decreases resistance against left ventricular

afterload and/or increases venous capacitance. However, the use of vasodilators, such as nifedipine, has deleterious effects because they cause a negative inotropic effect and subsequent neurohormonal activation (Fifer et al., 1985; Packer, 1989). In contrast, a balanced vasodilator such as nitroprusside does not cause neurohormonal activation in heart failure (Fifer et al., 1985).

Because phosphodiesterase type 5 inhibitors potentiate the NO/cGMP pathway, the hemodynamic action is thought to be similar to that of nitrovasodilators. Zaprinast, a relatively selective inhibitor of phosphodiesterase type 5, has been reported to have unique hemodynamic characteristics (Dundore et al., 1992; Fujita et al., 1998; Trapani et al., 1991). Zaprinast causes pulmonary vasodilation in rats and dogs (McMahon et al., 1993; Nagamine et al., 2000; Thusu et al., 1995). Zaprinast also has venodilator properties in anesthetized rats (Ng and Pang, 1998). Similar to zaprinast, T-1032 caused venodilation through an increase in venous compliance in anesthetized rats (Inoue et al., 2001). In addition, our recent findings suggested that intravenous administration (3–300 μg/kg) of T-1032 selectively decreased pulmonary vascular resistance compared with peripheral vascular resistance, such as renal vascular resistance or total peripheral resistance, in anesthetized dogs. Interestingly, T-1032 has been reported to have a more selective action on pulmonary vascular resistance than nitroglycerin (Yano et al., 2000). Thus, phosphodiesterase type 5 inhibitors appear to have a more selective action in both the venous and the pulmonary circulation, and thus would be suitable for vasodilator therapy for heart failure. Therefore, we aimed to investigate the effect of T-1032 on heart failure in Bio 14.6 hamsters.

In the acute study, we used aged hamsters (55 weeks old), in which both the right and left ventricular end-diastolic pressures were elevated abnormally, suggesting the hamsters were in diastolic failure. The doses we used in this study were based on the results of our previous study. We showed that intravenous administration of T-1032 at doses of 3–300 μg/kg significantly decreased pulmonary vascular resistance (Yano et al., 2000). Therefore, this dose level of T-1032 was considered to be appropriate for investigating the hemodynamic action of selective phosphodiesterase type 5 inhibitor.

In Bio 14.6 hamsters, intravenously administered T-1032 (1–100 μg/kg) reduced both ventricular end-diastolic pressures and caused mild hypotension, but neither influenced the heart rate nor the left ventricular $dp/dt_{max}$ (Fig. 1). These results suggest that T-1032 reduced both the right and left ventricular preload without changing cardiac contraction in cardiomyopathic hamsters. The reduction in the right ventricular end-diastolic pressure was greater than that in the left ventricular end-diastolic pressure. As mentioned above, T-1032 has a potent venodilator property. A possible explanation for the relatively potent action on the right ventricular preload is that T-1032 decreases resistance to venous return.

In order to confirm the usefulness of T-1032 for the treatment of fatal heart failure, we further examined the influence of chronic treatment with T-1032 on survival in Bio 14.6 cardiomyopathic hamsters. The survival study indicated that the high dose of T-1032 (500 ppm) significantly increased survival (Fig. 2). Other survival parameters, such as the time to first death and the median survival time, also showed the usefulness of T-1032 (Table 1). However, in the present study, a statistically significant difference in the survival curve was obtained with only the high dose of T-1032, although the final survival rate was similar between the two doses. It is possible that statistical power was not sufficient to detect a significant effect of T-1032 with such a small increase in survival rate. A larger scale study should be done to clarify the minimum effective dose of T-1032 and the dose-related response.

At the end of the experiment, plasma T-1032 concentrations were $0.05 \pm 0.02$ μg/ml ($7.5 \times 10^{-8}$ M) and $1.1 \pm 0.5$ μg/ml ($1.65 \times 10^{-6}$ M) in 50 and 500 ppm-treated groups, respectively (mean ± S.D.). As the plasma protein binding of T-1032 is approximately 98% (unpublished observations), unbound T-1032 concentrations in plasma are expected to be approximately $1.50 \times 10^{-9}$ and $3.30 \times 10^{-8}$ M in the corresponding groups. It has been reported that T-1032 at $10^{-8}$ and $10^{-7}$ M produces vasorelaxation with an increase in cGMP levels in the isolated rat aorta, probably through blockade of phosphodiesterase type 5 (Takagi et al., 2001). However, because plasma concentrations of T-1032 had a large S.D. in this study, it is possible that the concentrations overlapped in animals that survived or died. Therefore, although the plasma T-1032 concentrations in the 500 ppm-treated group appeared to be sufficient to induce hemodynamic changes, it is inappropriate to explain the mechanism of the higher survival rate on the basis of a single measurement of plasma concentrations at the end of the study.

It has been reported that the cause of cardiac death in these hamsters is due to pump failure and/or cardiac arrhythmia (Hano et al., 1991). In this study, however, it was not clear whether T-1032 suppressed the arrhythmia. Moreover, an antiarrhythmic action of selective phosphodiesterase inhibitors has not been reported. We have no explanation for the dose-dependent increase in survival followed by the similar survival of T-1032-treated hamsters at the end of the study. As we did not measure hemodynamics in surviving hamsters, further investigations will be necessary to answer these questions.

The effects of other cardiovascular agents on the survival of cardiomyopathic hamsters have been reported previously. Imidapril, an angiotensin-converting enzyme inhibitor, was reported to increase survival concomitant with an improvement of cardiac function (Narita et al., 1996). Other agents, such as endothelin $ET_A$ receptor antagonist (Yamauchi-Kohno et al., 1999), diuretics (Hanton et al., 1993) and cardiotonic agent (Kawasumi et al., 1999), also increased survival in cardiomyopathic hamsters.

Recent large-scale clinical trials have indicated that angiotensin-converting enzyme inhibitors, β-adrenoceptor antagonist, and the combination with isosorbide dinitrate and hydralazine are effective in reducing mortality among patients with heart failure (Cohn, 1988; Hash and Prisant, 1997; Young, 1991). Although angiotensin-converting enzyme inhibitors and β-adrenoceptor antagonists are accepted as the preferred drug therapies for the treatment of heart failure, other vasodilators could be useful in the occasional patient with intolerance to angiotensin-converting enzyme inhibitors and β-adrenoceptor antagonists. The usefulness of balanced vasodilator therapy lies in an improvement of cardiac performance through both arterial vasodilation by hydralazine and venodilation by nitrates. As phosphodiesterase type 5 inhibitors dilate both the arterial and venous vessels without changes in heart rate and cardiac contractility, the vasodilator effect is similar to that exerted by a combination of isosorbide dinitrate and hydralazine. Therefore, the use of selective phosphodiesterase type 5 inhibitors may be valuable as an alternative vasodilator therapy for the treatment of chronic heart failure.

In conclusion, our present study demonstrated for the first time that chronic administration of phosphodiesterase type 5 inhibitors increases the survival of Bio 14.6 hamsters, probably by improving cardiac performance. Phosphodiesterase type 5 inhibitors have therapeutic potential for the treatment of heart failure with cardiomyopathy.

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