

JPET#61465

Title: An antiarrhythmic effect of a chymase inhibitor after myocardial infarction.

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Origin: Department of Pharmacology, Osaka Medical College

Source of support: Grant-in Aid 13670102 (C) for Scientific Research from the
Ministry of Education, Science, Sports and Culture, Japan.

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Running title: Chymase and arrhythmias after myocardial infarction.

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Number of text pages: 15; **Number of tables:** 0; **Number of figures:** 7; **Number of references:** 34

Number of words Abstract: 250; **Introduction:** 485; **Discussion:** 1667

Abbreviations: Ang, angiotensin; MI, myocardial infarction; LAD, left anterior descending coronary artery; VA, ventricular arrhythmias; TY51184, 2-[4-(5-Fluoro-3-methylbenzo[b]thiophen-2-yl)sulfonamide-3-methanesulfonylphenyl]oxazole-4-carboxylic acid; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; +dP/dt and -dP/dt, positive and negative rates of pressure development, LV, left ventricle; RV, right ventricle; NE, norepinephrine; VF, ventricular fibrillation, HR, heart rate, MABP, mean arterial blood pressure, VT, ventricular tachycardia.

Recommended section: Cardiovascular

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Abstract

Chymase plays an important role in the regulation of local angiotensin (Ang) II formation in the cardiac tissue. We recently found that cardiac chymase was activated significantly and survival rate markedly improved by treatment with chymase inhibitors after myocardial infarction (MI) in hamsters. However, the mechanisms for this effect have not been established. Because lethal arrhythmias are generally believed to contribute to sudden cardiac death, we assessed whether inhibition of cardiac chymase would provide an antiarrhythmic effect during the 8-hour ischemic period after TY51184 (a specific chymase inhibitor, 1 mg/kg, i.v.) treatment by ligation of left anterior descending coronary artery (LAD) in dogs. Effects of candesartan (an Ang II type 1 receptor antagonist, 1 mg/kg, i.v.) in this model were also assessed. Total Ang II-forming activity and chymase activity in the infarcted heart were increased significantly 8 hours after LAD ligation. A time-dependent elevation of Ang II in plasma was also observed. A decrease in plasma Ang II levels after TY51184 treatment was occurred concomitantly with suppression of cardiac chymase activity. LAD ligation resulted in a great number of ventricular arrhythmias (VA). TY51184 and candesartan treatments largely suppressed the appearance of VA and the efficacy of the two agents was similar. These findings demonstrate that chymase inhibition can provide an antiarrhythmic effect after MI, and the reduction of Ang II by TY51184 may be mainly responsible for this beneficial effect. An antiarrhythmic effect of chymase inhibitors may contribute to reductions in the mortality rate during the acute phase after MI.

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Recently, chymase, in addition to the traditional angiotensin converting enzyme (ACE), have been shown to play important roles in the regulation of local angiotensin (Ang) II formation in cardiovascular tissues in monkeys, dogs, hamsters and, especially, humans (Miyazaki and Takai, 2000, 2001). Chymase is a chymotrypsin-like serine protease, which is mainly contained in the secretory granules of mast cells. In an extract of human heart tissues, for example, it was reported that chymase accounts for 80% to 90% of Ang II formation (Urata et al, 1990). Therefore, cardiac chymase may play an important role in the pathogenesis of the Ang II related heart diseases in such species. In contrast, rats possess only an ACE-dependent Ang II-forming pathway, because in cardiovascular tissues in rat, chymase acts as an Ang II degrading enzyme rather than an Ang II-generating enzyme (Jin et al, 2000; Takai et al, 2001).

After myocardial infarction (MI), it is well known that all components of the renin-angiotensin system such as ACE activities, Ang II concentration and number of Ang II type 1 (AT1) receptors in the cardiac tissue are increased significantly (Hirsch et al, 1991; Passier et al, 1995; Weber 1997), and blockade of Ang II action with ACE inhibitors or AT1 receptor antagonists can provide prognostic benefits (Pfeffer et al, 1992; Pitt et al, 2000), indicating that increases in Ang II are detrimental in this disease. Considering the importance of cardiac chymase to the generation of Ang II, we had previously performed two experimental studies to define a time-dependent change of cardiac chymase and the functional roles of this enzyme after MI in hamsters (Jin et al, 2001, 2002, 2003), which possesses both chymase and ACE dependent Ang II forming pathways like in humans as mentioned above. In those studies, we found that activation of

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cardiac chymase occurred earlier and lasted longer than that of cardiac ACE after MI. Chymase inhibition by treatment with the specific chymase inhibitors significantly improved cardiac function and survival rate during the acute phase after MI and the extent of this benefit was very similar to that observed for treatment with an AT1 receptor antagonist. On the other hand, there were no obvious prognostic benefits observed as a result of treatment with an ACE inhibitor. These findings indicated that activation of cardiac chymase rather than cardiac ACE plays an important role in the determination of prognosis during the acute phase after MI in such species. However, the detailed mechanisms for this effect of chymase inhibitors after MI have not been established. Because lethal arrhythmias are generally believed to contribute to sudden cardiac death during the acute phase of MI, we determined whether inhibition of cardiac chymase by treatment with 2-[4-(5-Fluoro-3-methylbenzo[b]thiophen-2-yl)sulfonamide-3-methanesulfonylphenyl]oxazole-4-carboxylic acid (TY51184), a specific chymase inhibitor, can reduce the number of ventricular arrhythmias (VA) during cardiac ischemic periods induced by a permanent coronary ligation in dogs.

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Methods

Reagents and Drugs

Candesartan was kind gift from Takeda Chemical Industries (Osaka, Japan). TY51184 was synthesized by Toa Eiyo Ltd (Oomiya, Japan). Pharmacological characteristics for TY51184 were assessed by using the purified human and dog chymase, the methods described as previous (Takai et al, 1996; Sakaguchi et al, 2001).

Animal treatment

Twenty beagle dogs weighing 9 to 12 kg were obtained from Japan SLC (Shizuoka, Japan) and divided into four groups: a sham-operated group (n = 5), an MI group treated with TY51184 (n = 5) or candesartan (n = 4) and a placebo group (n = 6).

TY51184 (1 mg/kg) and candesartan (1 mg/kg) were injected intravenously 10 minutes before left coronary ligation. Placebo-treated dogs were injected with an equal volume of saline. The experimental procedures for animals were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (Animal Research Laboratory, Osaka Medical College).

Experimental protocol

Anesthesia was induced by intravenous injection of pentobarbital sodium (35 mg/kg), and dogs underwent a tracheal intubation for subsequent mechanical ventilation. A left thoracotomy was performed in the fourth intercostal space, the pericardium incised and the heart suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) was then isolated at the bifurcation of the first diagonal branch, and a 3-0 silk

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suture was placed proximally to the LAD. To monitor the changes in left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), as well as positive and negative rates of pressure development (+dP/dt and -dP/dt) during experimental periods, a catheter was inserted into the left ventricle (LV) chamber via the right carotid artery. Catheters were also inserted into the femoral artery and vein to monitor blood pressure and to draw blood and administration of drugs. These catheters were connected to a Polygraph System each via an amplifier (Polygraph System, Nihon kohden, Tokyo, Japan). VA was monitored by using an electrocardiogram (ECG 9120, Nihon kohden, Tokyo, Japan). To analyze VA that occurred during the 8 hour ischemic periods, an electrocardiogram was recorded using Holter ECG throughout the experiments and analyzed by a long term ECG analyzing system (DSC-3200, Nihon kohden, Tokyo, Japan). A premature ventricular contraction (PVC) was defined as a VA in the present study. If an animal died due to severe VT or VF, that animal was excluded from the present analysis. Before ligation of LAD, 5 ml blood was drawn through the femoral vein catheter for later biochemical assays. After these procedures, TY51184 or candesartan or saline were injected as a bolus. Ten minutes later, the suture, which had already been put in place surrounding the LAD, was ligated to induce an MI. At 30, 240 and 480 minutes after LAD ligation, 5 ml blood samples were collected. Eight hours after LAD ligation, the heart was stopped by injection of 2 M KCl and was removed from the chest. To determine infarct size, the heart was cut into 1-cm-thick transverse slices and was incubated in 0.05% nitro blue tetrazolium solution for 15 to 30 minutes. The infarcted zone did not stain by this procedure, while the non-infarcted myocardium

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stained dark blue. Images of these slices were made by a digital camera and were analyzed by a computerized morphometry system (Mac SCOPE Ver 2.2, Mitani Co., Fukui, Japan). Finally, various heart tissues such as right ventricular (RV), non-infarcted left ventricular septum, infarcted LV and border zone of infarcted and non-infarcted were collected for determination of total Ang II-forming activity, ACE and chymase activities.

Biochemical assays

Total Ang II-forming activity, ACE and chymase activities were measured using cardiac tissue slices according to methods described previously (Takai et al 1998; Tsunemi et al, 2002). Plasma norepinephrine (NE), Ang II, CPK-MB and GOT were measured by SRL Inc (Setsu, Japan).

Statistical analysis

All numerical data shown in the text are expressed as the mean \pm standard error of the mean (SEM). Significant differences among the mean values of multiple groups were evaluated by 1 way ANOVA followed by a post-hoc analysis (Fisher's test). $P < 0.05$ was defined as the threshold for significance.

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Results

Figure 1 showed the chemical structure of TY51184. The molecular weight of TY51184 is 510.5. The IC₅₀ value of TY51184 for purified human chymase was 37 nM, however, the IC₅₀ values of TY51184 for the commercial chymotrypsin, elastase and cathepsin G were higher than 10 μM. These findings indicate that TY51184 is highly selective for chymase among serine protease family. To choose the dosage of TY51184 for the treatment of experimental dogs used in the present study, the IC₅₀ value of TY51184 for purified dog chymases was also determined. The IC₅₀ value of TY51184 against the purified dog chymase was 11 μM, therefore, the dosage of TY51184 at 1 mg/kg was used in the present study. If the plasma volume of the dogs was calculated as 10 % of the body weight, the plasma concentration of TY51184 will achieve about two times higher than the IC₅₀ value calculated in the purified dog chymase, when TY51184 at this dosage was intravenously injected.

In the group receiving placebo, 2 dogs died within ten minutes after LAD ligation due to ventricular fibrillation (VF). On the other hand, there was no dog that died in the TY51184 and candesartan treated groups.

As shown in Figure 2, the infarct sizes of surviving dogs did not significantly differ among these three groups 8 hours after LAD ligation. In sham-operated dogs, basal levels of plasma CPK-MB and GOT before LAD ligation were 1.4 ± 0.2 ng/ml and 45 ± 7 IU/L (at 37°C), respectively. In placebo treated dogs, the basal levels of plasma CPK-MB and GOT before LAD ligation were 1.6 ± 0.4 ng/ml and 51 ± 8 IU/L (at 37°C), respectively. When the LAD was ligated to induce MI, a time-dependent elevation of CPK-MB and

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GOT levels in plasma was observed in the placebo-treated group during the 8-hour observation period (Fig. 2). Plasma levels of CPK-MB and GOT were 87 and 8 times higher than basal levels at 8 hours after LAD ligation in this group. The basal levels of plasma CPK-MB before LAD ligation in TY51184 and candesartan treated dogs were 2.5 ± 0.8 and 1.8 ± 0.2 ng/ml, respectively; GOT was 52 ± 5 and 46 ± 6 IU/L (at 37°C), respectively. Neither TY51184 nor candesartan treatment significantly suppressed this elevation (Fig. 2). Basal concentrations of NE in plasma were 79 ± 21 , 106 ± 6 , 53 ± 13 and 88 ± 44 pg/ml in, respectively, sham, placebo, candesartan and TY51184 treated dogs. Except for the value of NE in candesartan treated dogs ($p < 0.05$ vs. placebo), there were no significant differences in basal concentration of NE among groups. Ligation of LAD did not significantly affect plasma concentration of NE in dogs treated with placebo or TY51184 during the 8-hour observation period (Fig. 2). However, the plasma concentration of NE was significantly increased in candesartan treated dogs 30 minutes after LAD ligation and this increase was sustained up to 8 hours (Fig. 2).

Figure 3 shows enzymatic changes in cardiac chymase and ACE as well as total cardiac Ang II-forming activities from RV, septum, infarcted LV and the border zone of infarction 8 hours after LAD ligation. In comparison with the non-ischemic hearts from sham operated dogs, the total Ang II-forming activities in all parts of the myocardium were increased significantly 8 hours after LAD ligation (Fig. 3A). Total Ang II-forming activities tended to decrease in TY51184-treated dogs and were significantly lower in candesartan-treated dogs compared with placebo-treated dogs (Fig. 3A). Cardiac chymase activity in the infarcted LV was also significant increased (Fig. 3B). Cardiac

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chymase activities in RV, septum and border zone of infarction tended to increase (Fig. 3B). In contrast, cardiac ACE activities in all parts of the infarcted myocardium were significantly lower than noninfarcted myocardium from sham-operated dogs (Fig. 3C). TY51184 treatment suppressed cardiac chymase activities to some extent (Fig. 3B) and reversed decreases in cardiac ACE activities (Fig. 3C). Chymase activities in the candesartan-treated dogs were significantly lower in RV and infarcted LV and tended to decrease in septum and at the border of the infarction (Fig. 3B). ACE activities were increased significantly by treatment with candesartan in RV and infarcted LV (Fig. 3C). Basal plasma concentrations of Ang II in sham, placebo, TY51184 and candesartan treated dogs were 58 ± 24 , 62 ± 10 , 123 ± 50 and 140 ± 61 pg/ml, respectively. Differences among groups were not significantly different.

As can be seen in Fig. 4, plasma Ang II concentrations in placebo treated dogs started to increase 30 minutes after LAD ligation. Compared with non-ischemic periods, this increase reached a level that was about 3 times higher than baseline concentrations of Ang II at 8 hours after LAD ligation ($p < 0.01$). TY51184 treatment suppressed this increase in plasma, while candesartan treatment tended to increase the Ang II concentration in plasma compared with baseline (Fig. 4).

Figure 5 shows the total number of heart beats and VA that occurred during the 8 hours ischemic period from placebo, TY51184 and candesartan treated dogs. No significant differences in the total number of heart beats were observed among the three groups. As can be seen in Fig. 5, LAD ligation resulted in a greater number of VA in the placebo treated dogs. TY51184 and candesartan treatment largely suppressed the

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appearance of VA and the efficacy of the two agents was similar (Fig. 5). The incidence of VA during the 8-hour ischemic period is summarized in Figure 6. TY51184 and candesartan treatment markedly prevented the appearance of VA throughout the experimental period and the pattern was similar between TY51184 and candesartan treated dogs (Fig. 6).

Figure 7 summarizes changes in hemodynamic parameters during the experimental period. In placebo treated dogs, ligation of the LDA resulted in a transient reduction in mean arterial blood pressure (MABP), and LVSP as well as $+dP/dt$ and $-dP/dt$, while heart rate (HR) and LVEDP were little affected. A similar pattern was also found in dogs receiving either TY51184 or candesartan treatment (Fig. 7).

Discussion

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There are two main findings of this study. First, the activation of cardiac chymase and the increase in Ang II levels in plasma during ischemic periods induced by a permanent LAD ligation was significantly suppressed by treatment with TY51184, a chymase specific inhibitor. The decrease in plasma Ang II concentration was associated with a reduction in the frequency of VA, suggesting that an elevation in the plasma Ang II concentration accelerates the appearance of VA and that activation of cardiac chymase is responsible for the increase of plasma Ang II.

Second, candesartan treatment reduced the incidence of cardiac VA appearing during the periods of cardiac ischemia. This finding supports the theory that excessive formation of Ang II during cardiac ischemia is arrhythmogenic, probably mediated via AT1 receptors (Harada et al, 1998; Levi et al, 2002; Seyedi et al, 2002).

The mechanism(s) by which treatment with a chymase specific inhibitor can elicit an antiarrhythmic effect during cardiac ischemia appears to be mainly due to a reduction in Ang II generation. It is well known that chymase is usually stored in mast cell secretory granules and cannot exert its enzymatic action without degranulation. In cardiac ischemia in dogs, it was reported that almost all of the mast cells are degranulated (Frangogiannis et al, 1998). This may also happen in the hearts of our experimental dogs after LAD ligation. As indicated by measurements of the enzymatic activity of chymase in cardiac slices from different part of myocardium 8 hours after LAD ligation, the increase in cardiac chymase activity in the infarcted LV is most evident. However, when the enzymatic activity of chymase was measured using extracts obtained from heart homogenates of the same tissue samples, chymase activities from ischemic hearts did not

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significantly differ from tissues from non-ischemic hearts of sham operated dogs (data not shown). This indicates that the total amount of chymase in these cardiac tissues does not increase during the 8 hours of ischemia and the increase in chymase activity measured in ischemic heart slices is mainly due to activation of chymase that results from degranulation of mast cells. Indeed, mast cell numbers identified by toluidine-blue and chymase immunohistochemical staining from infarcted hearts also did not significantly differ from those non-infarcted hearts from sham operated dogs (data not shown). In the present study, the total cardiac Ang II-forming activity and plasma Ang II concentrations were increased significantly in the infarcted dogs and this increase in Ang II producing ability in cardiac tissue and the elevation of Ang II concentration in plasma appears to result from an increase in cardiac chymase activity associated with mast cell degranulation. The elevation of Ang II is unlikely to be due to the activity of ACE, because of cardiac ACE activities in different myocardial regions from infarcted hearts decreased markedly. Furthermore, plasma Ang II concentration was inhibited significantly by treatment with TY51184.

An arrhythmogenic effect of Ang II was predicted as early as about fifteen years ago in a pig MI model, in which it was reported that inducible, sustained ventricular tachycardia (VT) 2 weeks after MI was aggravated by Ang II infusion (de Langen et al, 1989). Thereafter, the antiarrhythmic effects of ACE inhibitors and AT1 receptor antagonists during periods of cardiac ischemia were reported for many different animal species (deGraeff et al, 1988; Zhu et al, 2000; Hiura et al, 2001;), indicating that an excessive production of Ang II may, via stimulating its receptors, promote the appearance of

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arrhythmias. This idea was further supported by the latest findings that if AT1 receptors are over-expressed, the occurrence of reperfusion arrhythmias is increased (de Boer et al, 2002). In contrast, if the AT1 receptors are knocked out, the occurrence of the arrhythmias is diminished (Harada et al, 1998).

Several mechanisms may be involved in the arrhythmogenic effect of AT1 receptors stimulated by Ang II. For example, Ang II via stimulation of AT1 receptors, accelerates NE release from sympathetic nerve endings (Peach, 1997; Levi et al, 2002; Seyedi et al, 2002). Recently, it was reported by Maruyama et al (2000). that chymase-generated Ang II, but not ACE-generated Ang II, in a human model of myocardial ischemia plays a major role in carrier-mediated NE release and they suggested that this chymase-dependent Ang II generation may be important in inducing arrhythmias under ischemic condition, and may have important clinical implications. Their hypothesis is largely supported by our present study because treatment using the chymase specific inhibitor TY51184 as well as the Ang II AT1 receptor antagonist candesartan certainly decreased the incidence of VA appearing during the 8-hour ischemic periods in dogs. However, our results differ somewhat from their findings. They reported that NE release increased significantly when cardiac tissues suffered 70 min of anoxia. However, in our present study, an increase in plasma NE levels was not seen at any point during the 8-hour ischemic period after LAD ligation and further elevation in plasma NE concentration was seen following treatment with candesartan, while the plasma concentration of NE was little affected by treatment with TY51184. Because we could not measure the NE concentration in ischemic cardiac tissues, changes in NE concentrations during local

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ischemia of cardiac tissues in each group are unknown. These issues should be clarified in future studies. After LAD ligation, as can be seen in Figure 7, MABP did not significantly change during the 8-hour ischemic period due to small infarct size except that at 8 hour point in the present study. Therefore, the NA levels in plasma after LAD ligation may be not significantly changed in the placebo-treated dogs. We speculated that candesartan treatment may reduce systemic blood pressure and a decrease in the systemic blood pressure may result in an activation of sympathetic nervous system. In the present study, MABP in the candesartan-treated dogs did not significantly differ from the placebo-treated dogs after LAD ligation. Therefore, the blood pressure lowering effect of candesartan may be masked by the activation of sympathetic nervous system in the present study.

Direct stimulation of AT1 receptors or indirect stimulation of the release of NE due to AT1 receptor stimulation by Ang II appears to be increased in cardiac intracellular Ca^{2+} overload (Timmermans et al, 1993), which is closely related to the delayed after-depolarization. In isolated pairs of cardiac cells from the failing heart of cardiomyopathic hamsters, the intracellular administration of Ang I causes a drastic reduction in the junctional conductance, and this effect was abolished by treatment with an ACE inhibitor or by an AT1 receptor antagonist (De Mello, 1996, 2001).

Furthermore, cardiac refractoriness is reduced by Ang II and spontaneous discharges of action potentials can be elicited by a single stimulus applied during the relative refractory period in ventricular fibers exposed to this peptide (De Mello and Crespo, 1999). These effects are also blocked by treatment with an AT1 receptor antagonist. In this way, a direct stimulation of AT1 receptors may, via an enhancement of heterotopic automaticity and a

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decrease in cell coupling or reduction in the junctional conductance, facilitate the generation of premature ventricular contractions or re-entrant arrhythmias.

Infarct size seemed not to be affected by AT1 receptors, because neither overexpression nor knockout of these receptors altered the infarct size after MI (Zhu et al, 2000; de Boer et al, 2002). In the present study, infarct size and plasma concentrations of CPK-MB and GOT released from ischemic myocardium after 8 hours of LAD ligation was not significantly affected by treatment with candesartan (compared with placebo treated MI dogs). Similar results were also observed in TY51184 treated dogs. This also was observed in a hamster MI model in which both an AT1 receptor antagonist and chymase inhibitors did not significantly affect infarct sizes after MI (Jin et al, 2001, 2002, 2003).

Following development of orally active specific chymase inhibitors, the *in vivo* role of chymase in some heart diseases was gradually defined. For example, when chymase inhibitors were used to treat cardiomyopathic hamsters (Takai et al, 2003a) and the heart failing dogs induced by tachycardia (Matsumoto et al, 2003), it was reported that cardiac fibrosis was suppressed markedly, indicating that chymase plays an important role in the development of chronic heart failure through the promotion of pathological remodeling of the heart in such species. As reported by us previously, chymase inhibitors also provided certain survival and functional benefits during the acute phase of MI in hamsters (Jin et al, 2002, 2003; Hoshino et al, 2003), suggesting that suppression of cardiac chymase is important in the improvement of prognosis after MI. On the other hand, the intima proliferation that occurs after the carotid artery suffers balloon injury (Takai et al,

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2003b) and the external jugular vein was grafted to the ipsilateral carotid artery (Nishimoto et al, 2001) was also significantly suppressed by treatment with chymase inhibitors. These inhibitors, therefore, may be useful in the treatment of coronary artery diseases. These effects of chymase, at least in part, do not depend on Ang II generation. Therefore, an original effect of chymase inhibitors in the treatment of heart diseases may be expected.

In this way, the antiarrhythmic effect of chymase inhibitors found in the present study, taken together with the above beneficial effects of chymase inhibitors, is favorable for the treatment of heart failure that results from either MI or chronic hypertension complicated with arteriosclerosis, and we expect that chymase inhibitors can be tested in the near future in clinical trials.

In conclusion, a specific suppression of activated cardiac chymase after a permanent LAD ligation is associated with a reduction in both plasma Ang II levels and the incidence of ventricular arrhythmias, and the antiarrhythmic effect of TY51184 is similar to that for candesartan treatment. This indicates that excessive production of Ang II induced by activation of cardiac chymase may be involved in the pathogenesis of VA during periods of heart ischemia. An antiarrhythmic effect of chymase inhibitor may contribute to a reduction in mortality rate during the acute phase after MI.

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Figure for legends

Figure 1. The chemical structure of TY51184.

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Figure 2. Bar graphs show infarct sizes 8 hours after LAD ligation among placebo (n = 4), TY51184 (n = 5) and candesartan (n = 4) treated groups. Line graphs show the time-dependent changes in plasma levels of CPK-MB, GOT, NE in the sham-operated (n = 5) groups or LAD ligated dogs treated with placebo (n = 4), TY51184 (n = 5) and candesartan (n = 4) during the 8-hour observation period. Values are means \pm SEM. #p < 0.05, ###p < 0.001 vs. baseline; *p < 0.05, **p < 0.01, ***p < 0.001 vs. sham; \$\$p < 0.01 vs placebo.

Figure 3. A: Bar graphs show change in total cardiac Ang II-forming activities from different parts of the heart in the sham-operated (n = 5) groups or LAD ligated dogs treated with placebo (n = 4), TY51184 (n = 5) or candesartan (n = 4) 8 hours after surgery. B and C: Comparison of cardiac chymase activities (B) and ACE activities (C) from different parts of the heart in sham operated or in LAD ligated dogs treated with placebo, TY51184 and candesartan 8 hours after surgery. Values are means \pm SEM. *p < 0.05, **p < 0.01, ***p < 0.001 vs. sham; #p < 0.05, ##p < 0.01, #p < 0.05, ###p < 0.001, vs. placebo.

Figure 4. Line graphs show the time-dependent changes in plasma levels of Ang II in the sham-operated (n = 5) groups or LAD ligated dogs treated with placebo (n = 4), TY51184 (n = 5) and candesartan (n = 4) during the 8-hour observation period. Values are means \pm SEM. *p < 0.05, **p < 0.01 vs. sham; #p < 0.05 vs. baseline; \$p < 0.05 vs. placebo.

Figure 5. Comparison of total number of heart beats and ventricular arrhythmias (VA) during 8 hours of LAD ligation among placebo (n = 4), TY51184 (n = 5) and

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candesartan (n = 4) treated groups. Values are means \pm SEM.

Figure 6. Line graphs show the incidence of ventricular arrhythmias during 8 hours of LAD ligation among placebo (n = 4), TY51184 (n = 5) and candesartan (n = 4) treated groups. Values are means \pm SEM. *p < 0.05, **p < 0.01 vs. placebo.

Figure 7. Line graphs show time dependent changes of hemodynamic parameters in the sham-operated (n = 5) groups or LAD ligated dogs treated with placebo (n = 4), TY51184 (n = 5) and candesartan (n = 4) during the 8-hour observation period. Values are means \pm SEM. *p < 0.05, **p < 0.01, ***p < 0.001 vs. baseline; \$p < 0.05, \$\$p < 0.01, \$\$\$p < 0.001 vs. sham; #p < 0.05 vs. placebo.

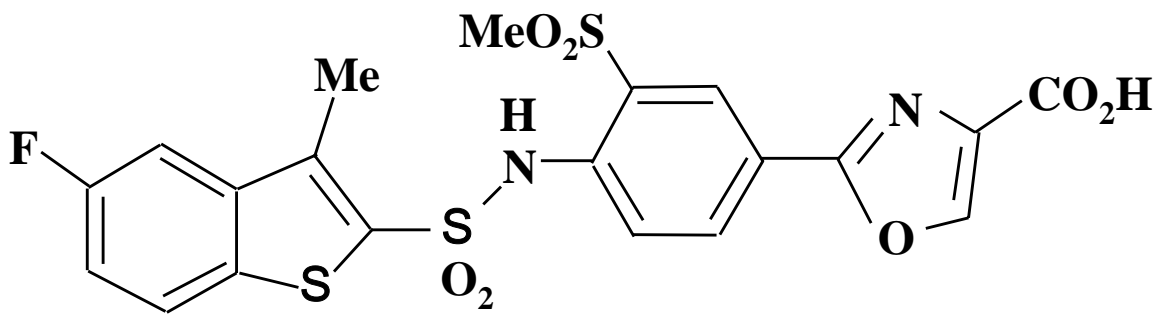


Fig.1

Fig.2

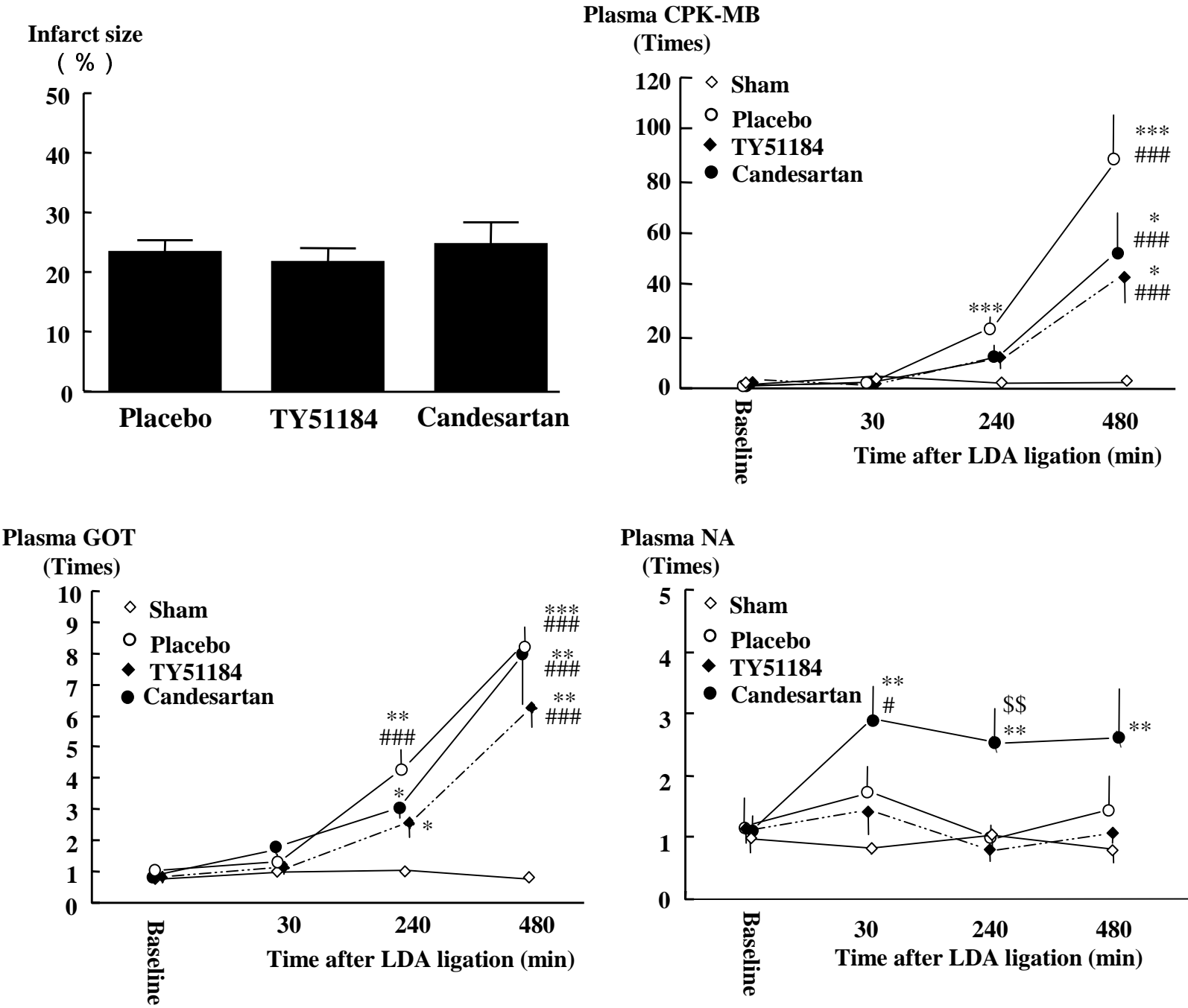
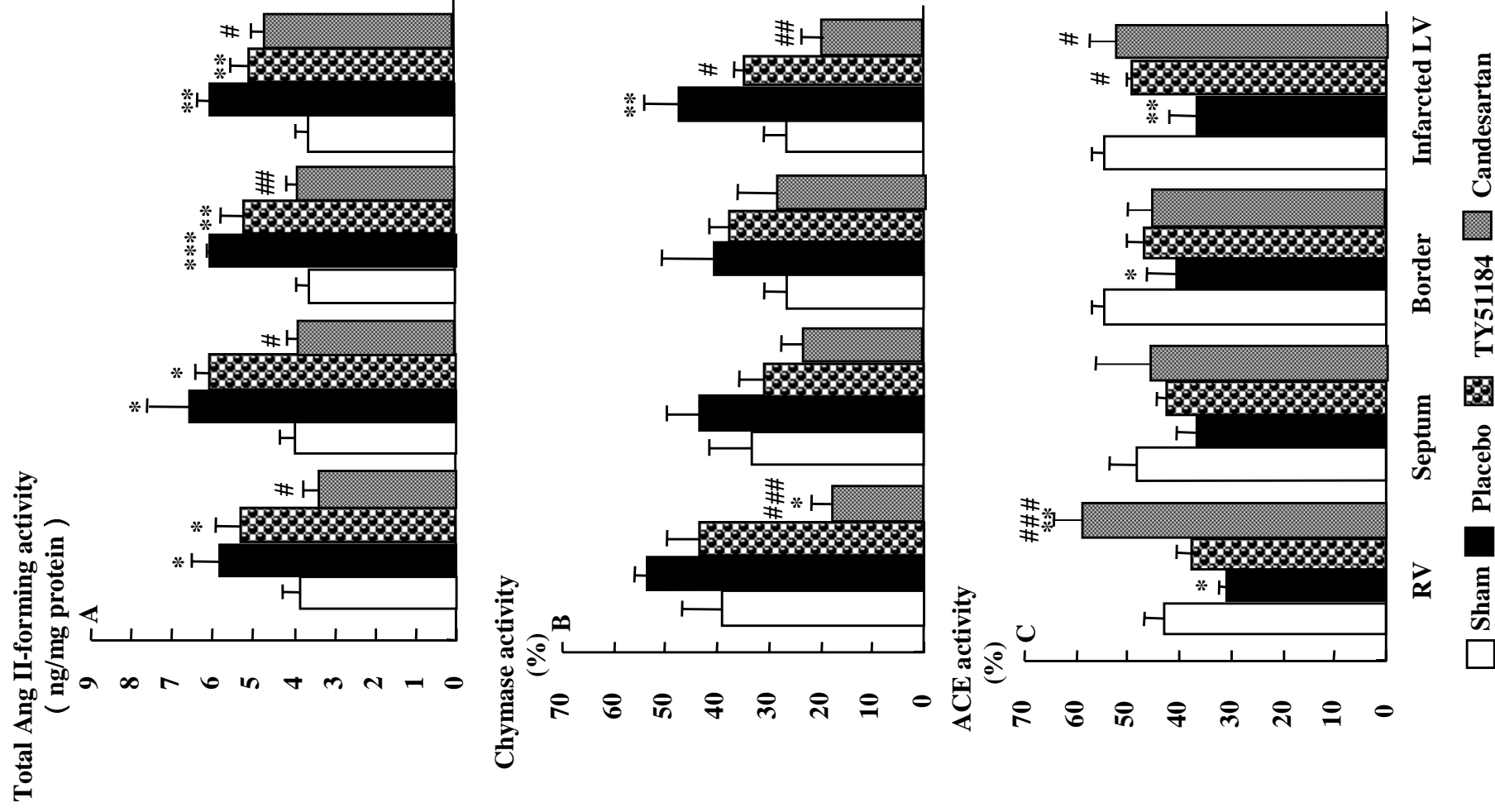


Fig.3



**Plasma Ang II concentration
(Times)**

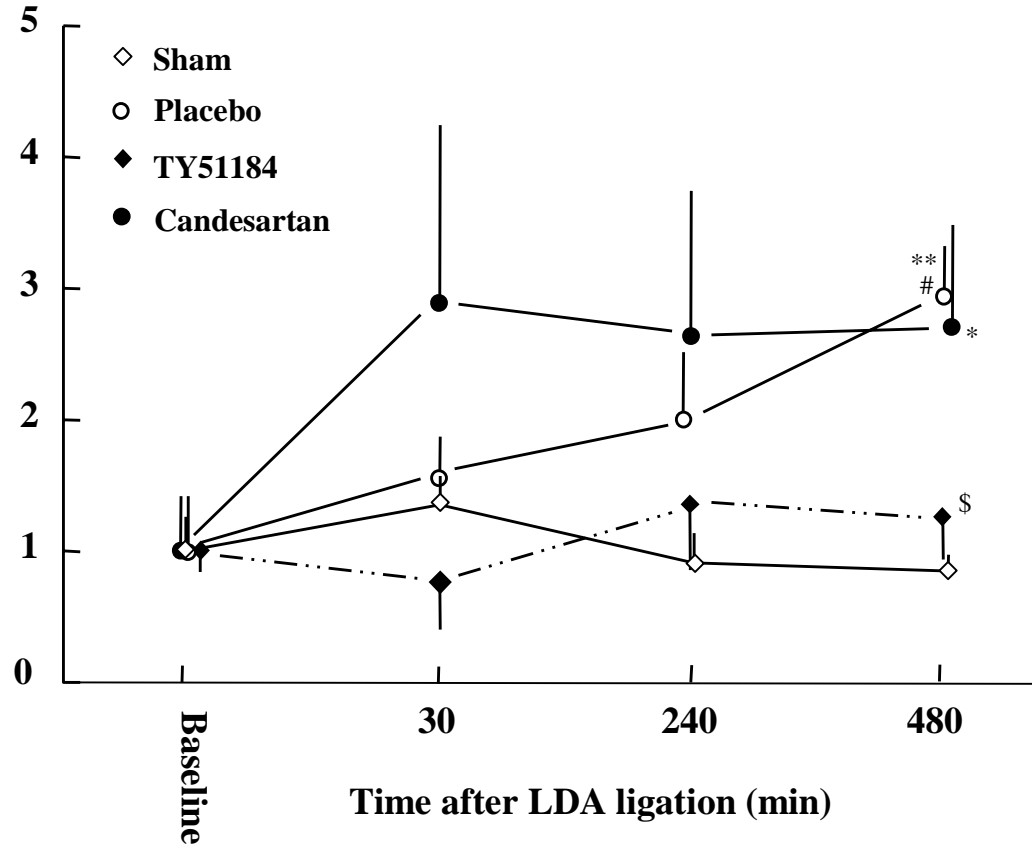
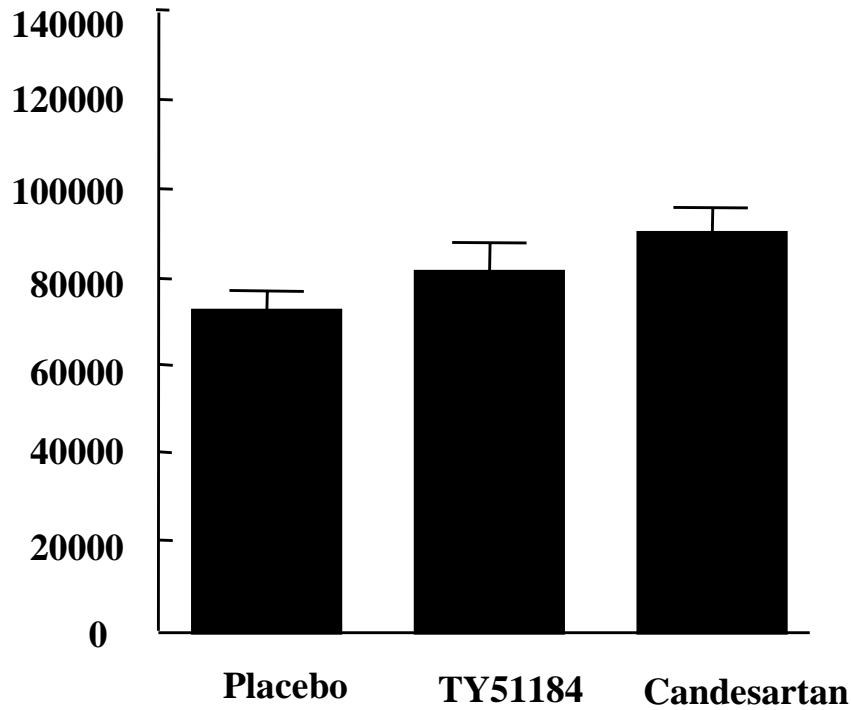


Fig.5

Total number of heart beats



Total number of ventricular arrhythmias

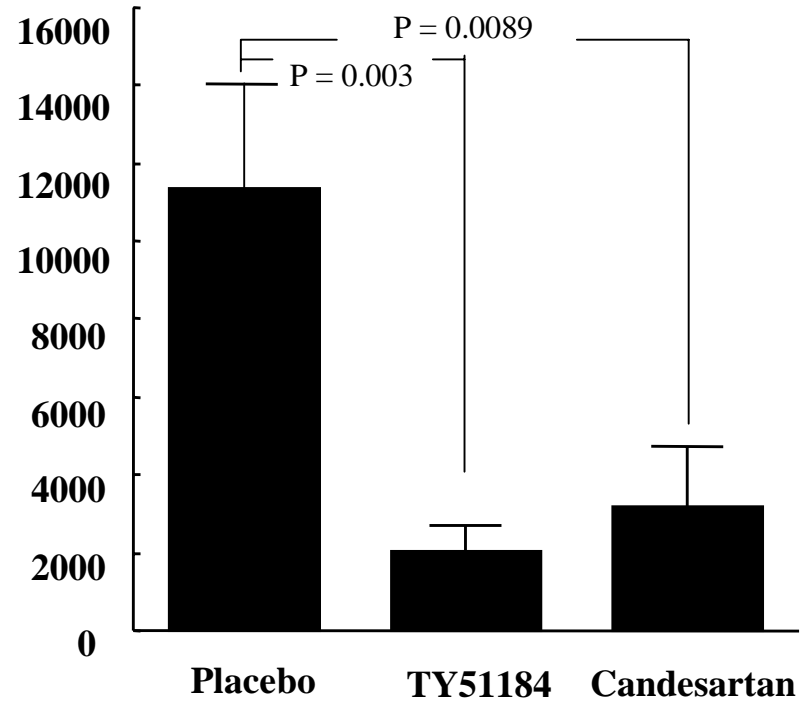


Fig.6

Incidence of arrhythmia

