Acute Effect of Human Cardiotrophin-1 on Hemodynamic Parameters in Spontaneously Hypertensive Rats and Wistar Kyoto Rats

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There is considerable evidence to indicate that humoral factors play an important role in the development of left ventricular hypertrophy. Cardiotrophin-1 (CT-1) is a cytokine that has been shown to induce cardiac hypertrophy in a dose-dependent manner. The aim of the present study was to investigate the acute effect of CT-1 on hemodynamic parameters in spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) and to study the relationship between the plasma concentration of CT-1 and its hemodynamic effect. Ten-week-old SHR and age-matched WKY were used. Blood pressure (BP), heart rate (HR) and plasma concentration of CT-1 were measured both before and for 60 min after intravenous bolus injection of human CT-1 (10 μg/kg). CT-1 injection significantly decreased BP and significantly increased HR in SHR and WKY. There were significant differences in BP and HR between the two groups at all time points after injection. The lowest BP, highest HR and maximal plasma concentrations of CT-1 were observed in both groups within 10 min after injection. However, after converting the values into the percentage change from their respective baselines, there were no significant differences between the two groups in BP or HR at any time point. There was also no significant difference between the two groups at any time point in the plasma concentration of CT-1. This study indicates that CT-1 decreases BP and increases HR in both SHR and WKY. The most obvious change occurred within 10 min after injection. However, there was no significant difference in the hypertensive effect of CT-1 on 10-week-old SHR and WKY. (Hypertens Res 2001; 24: 717–721)

Key Words: cardiotrophin-1, hemodynamic parameters, plasma concentration, spontaneously hypertensive rats

Introduction

Cardiac hypertrophy is an independent risk factor for cardiac morbidity and mortality, and causes congestive heart failure, myocardial infarction, arrhythmia, and sudden death (1–3). It is induced by mechanical load and humoral factors, such as angiotensin II, endothelin-1, phenylephrine and peptide growth factors. Recently, accumulating evidence indicates that humoral factors play an important role in the development of left ventricular hypertrophy. Cardiotrophin-1 (CT-1), a 21.5-kDa cytokine, was originally reported by Pennica et al. in 1995 (4). It is a member of the interleukin-6 interleukin-1/leukemia inhibitory factor/ciliary neurotrophic factor/Oncostatin-M family of cytokines. CT-1 affects cells through interacting with leukemia inhibitory factor receptor and the gp130 signaling subunit (5). CT-1 has been shown to induce cardiac hypertrophy in a dose-dependent manner in vitro (4, 5) and in vivo (6). It is involved in cardiac myocyte-nomyocyte interactions during hypertrophy of rat cardiac myocytes in vitro (7). In various pathophysiological conditions such as hypoxic stress (8), mechanical stress (9), and...
experimental congestive heart failure (10), the expression of CT-1 mRNA in cardiac myocytes was significantly augmented. Ishikawa et al. reported a heart-specific increase in CT-1 gene expression preceding the establishment of ventricular hypertrophy in genetically hypertensive rats as well as an augmented expression of CT-1 gene in the ventricles of genetically hypertensive rats (11, 12). However, the acute effect of CT-1 on hemodynamic parameters in vivo in spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) is unknown. The aim of the present study was to investigate the acute effect of CT-1 on hemodynamic parameters in SHR and WKY and to study the relationship between the plasma concentration of CT-1 and its hemodynamic effect.

Methods

Reconstitution of Recombinant Human CT-1
Recombinant human CT-1 was purchased from PreproTech EC Ltd., London, UK and reconstituted as instructed by product information. In brief, this process included a quick spin followed by reconstitution in 20 mM Tris pH 8.0 to a concentration of < 1.0 mg/mL. This solution then was diluted with phosphate-buffered saline (PBS) into working aliquots and stored at -80 °C for use in the study.

Animals and Protocol of the Experiment
Male SHR and age-matched male WKY were purchased (Charles River Japan, Inc., Yokohama, Japan) and were given normal rat chow and free access to tap water. Rats were treated according to the guidelines of the Kagawa Medical University Animal Committee. After being maintained for 3 weeks in an animal center, 10-week-old male SHR (n = 11) and age-matched male WKY (n = 11), weighing 250–300g, were used in the study. After being anesthetized with pentobarbital sodium (50 mg/kg, i.p.), all rats were implanted with PE 50 catheters in both the right internal carotid artery and the right external jugular vein. Blood pressure (BP) was measured at the right external carotid artery. After the BP and heart rate (HR) became stable, CT-1 (10 μg/kg) in 0.1 ml PBS was bolus-injected from the implanted catheter into the right external jugular vein in both SHR and WKY (n = 4 in each group). As vehicle, 0.1 ml PBS was bolus-injected from the implanted catheter into the right external jugular vein in both SHR (n = 3) and WKY (n = 3) to serve as controls. Arterial BP and HR were recorded with an instrument named Omnicare II (RA 1200, NEC San-ei Instruments Ltd., Tokyo, Japan) both before and after 60 min after the injection. Blood samples for measuring the plasma concentration of CT-1 were obtained before injection and at 5, 10, 15, 30 and 60 min after injection from the catheter implanted in the right internal carotid artery in both SHR and WKY (n = 4 in each group). The blood samples were withdrawn into plastic syringes and quickly transferred to chilled polypropylene tubes containing EDTA (1.5 mg/ml) and aprotinin (50 IU/ml). After the blood samples were spun in a prerelatter centrifuge (4°C) at 3,000 rpm for 10 min, the plasma was separated and immediately frozen at -80 °C until the CT-1 concentration was measured.

Measurement of Plasma Concentration of CT-1 by Sandwich ELISA
The rabbit anti-CT-1 antibody (Prepro Tech EC Ltd., London, UK) was labeled with peroxidase by the peroxidase method (13). All reactions were performed at room temperature unless otherwise stated.

Each well of a microtiter plate (Maxisorp, Nalgé Nunc International Co., Tokyo, Japan) was coated with 0.05 ml of rabbit anti-CT-1 antibody (5 μg/ml, Antigenix America Inc., New York, USA), dissolved in PBS (pH 7.4) and incubated for 2 h. After the plate was blocked with 0.1 ml of 1% bovine serum albumin-PBS containing 0.05% Tween 20 (abbreviated as 1% BSA-PBST) for 1 h, CT-1 standard and plasma samples diluted with 1% BSA-PBST were added to the wells and incubated for 2 h. After the plate was washed 3 times with PBST, 0.1 ml of diluted peroxidase-labeled anti-CT-1 antibody prepared as above was added, and the plate was incubated for 2 h. After the plate was washed 3 times, 0.1 ml of o-phenylenediamine dihydrochloride (2 mg/ml) dissolved in 1/10 M citrate-1/5 M Na2HPO4 buffer (pH 5.0) containing 0.03% H2O2 was then added to each well. After 30 min of incubation, the reaction was terminated by the addition of 0.1 ml of 2 N H2SO4, and absorbance was read at 492 nm with a microplate reader. The plasma concentration of CT-1 was determined by reference to a standard curve constructed with recombinant CT-1 (Chemicon International, Inc., Temecula, USA) (Fig. 1).
**Fig. 2.** Mean blood pressures and mean blood pressure percentage changes of anesthetized spontaneously hypertensive rats and age-matched Wistar Kyoto rats at baseline and after intravenous bolus injection of recombinant human cardiotrophin-1 (10 μg/kg) (n=4 in SHR ■, n=4 in WKY ●) or 0.1 ml phosphate-buffered saline only (n=3 in SHR □, n=3 in WKY ○). *p < 0.05 vs. vehicle control, * p<0.01 vs. vehicle control, * p<0.05, SHR vs. WKY.

**Fig. 3.** Heart rates and heart rate percentage changes of anesthetized spontaneously hypertensive rats (SHR) and age-matched Wistar Kyoto rats (WKY) at baseline and after intravenous bolus injection of recombinant human cardiotrophin-1 (10 μg/kg) (n=4 in SHR ■, n=4 in WKY ●) or 0.1 ml phosphate-buffered saline only (n=3 in SHR □, n=3 in WKY ○). *p<0.05 vs. vehicle control, * p<0.01 vs. vehicle control, * p<0.05, SHR vs. WKY.

**Statistical Analysis**

Data were expressed as mean±SD for each group. Comparisons between groups were completed with the use of the unpaired Student’s t-test. The results were considered significantly different at p<0.05.

**Results**

**Blood Pressure**

Mean BP values at baseline and at 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 min after injection were used for analysis. At 6, 8, 10, 20, 30 and 40 min after the CT-1 injection, the mean BP of SHR significantly decreased in comparison with the controls. At 4, 6, 8, 10, 20, 30 and 40 min after the CT-1 injection, the mean BP of WKY significantly decreased in comparison with controls. There were significant differences between SHR and WKY in absolute mean BP at baseline and at all other time points after the CT-1 injection. After converting the mean BP into percentage change from the baseline, significant differences between SHR and the controls were observed at 6, 8, 10, 20 and 30 min after injection. After the percentage conversion, there were also significant differences between WKY and the controls at 2, 4, 6, 8, 10, 20, 30 and 40 min. However, there were no significant differences between SHR and WKY in the percentage change of BP at any time point after the CT-1 injection (Fig. 2).

**Heart Rate**

Data for HR were obtained at the same time points as the data for mean BP. At 6, 8 and 10 min after the CT-1 injection, the HR of SHR significantly increased in comparison with that of the controls. At 6, 8, 10, 20 and 30 min after the CT-1 injection, the HR of WKY significantly increased in compar-
Fig. 4. Plasma concentrations of recombinant human cardiothrin-1 in anesthetized spontaneously hypertensive rats (n=4, SHR) and age-matched Wistar Kyoto rats (n=4, WKY) before and after intravenous bolus injection of recombinant human cardiothrin-1 (10 μg/kg). There is no significant difference (NS) between SHR and WKY at any time point.

ison with controls. There were significant differences between SHR and WKY in absolute HR at baseline and at all other time points after CT-1 injection. After converting absolute HR into a percentage change from baseline, there were significant differences between SHR and the controls in HR at 2, 4, 6, 8 and 10 min. There were also significant differences between WKY and the controls at 2, 4, 6, 8, 10, 20, 30, 40, 50 and 60 min. However, there were no significant differences between SHR and WKY in percentage change of HR at any time point after the CT-1 injection (Fig. 3).

Plasma CT-1 Concentrations

Before the injection of CT-1, there were no significant differences between SHR and WKY in plasma CT-1 concentration. Although there was a tendency for WKY to have higher average plasma concentrations of CT-1, there were no significant differences between WKY and SHR in plasma CT-1 concentration up to 60 min after injection (Fig. 4). There was a positive correlation between the plasma concentration of CT-1 and its hemodynamic effects in both rat groups (Figs. 2–4). The maximal plasma concentrations of CT-1 and the maximal changes in hemodynamic parameters occurred within 10 min after CT-1 injection in both SHR and WKY (Figs. 2–4).

Discussion

First, in the present study, the acute effect of CT-1 on both SHR and WKY after intravenous bolus injection was observed. After injection of CT-1, mean BP was significantly decreased and HR significantly increased in both SHR and WKY in comparison with their respective vehicle controls. The lowest mean BP and highest HR were observed in both SHR and WKY within 10 min after CT-1 injection. The peak plasma concentrations in both rat groups were also observed within 10 min after CT-1 injection. The changes in mean BP and HR in both WKY and SHR were closely related to their respective exogenous plasma CT-1 concentrations. These changes lasted at least 60 min. Our findings of the effect of CT-1 on BP and HR are similar to the findings of previous reports (14, 15), although these other studies used mouse or rat CT-1.

Although there were no significant differences between the two groups in the percentage change of BP and HR, there was a tendency for CT-1 to have a relatively blunted effect on hemodynamic parameters in SHR. In the present study, only young SHR were used. Because the physiological features of young and adult SHR are different in many aspects (16–18), further study is needed to compare the effect of CT-1 on the hemodynamics of young and adult SHR. Investigation of the response of normal and renin-insufficient rats to CT-1 injection would also be useful to clarify whether renal function plays a role under such circumstances.

As of now, the exact mechanism of CT-1 on BP and HR is still open for discussion. We speculate that there are at least two possible mechanisms. One may be related to nitric oxide. The depressor and tachycardiac responses to CT-1 were blocked after nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME) intravenous injection prior to CT-1 administration (14, 15). This blockage confirms that these responses, at least in part, are nitric-oxide-mediated. The second possible mechanism may be related to atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP). These natriuretic peptides decrease BP by vasodilation and natriuresis. They are rapidly up-regulated in the heart in response to hypertrophic signals (19).

CT-1 stimulates ANP and BNP synthesis and secretion in cultured ventricular myocytes (7, 20). Expression of BNP mRNA was significantly augmented 1 h after the injection of CT-1. It reached a maximum at 2 h after injection and returned to the baseline levels within 24 h. Interestingly, ANP mRNA did not change significantly until 24 h after the injection, but it showed an increase thereafter (14). If a chronic increase of endogenous CT-1 secretion is present, circulating CT-1 may increase ANP and BNP expression and secretion. However, this mechanism, at least, may not be a major mechanism in the present acute in vivo study involving exogenous administration of CT-1.

Second, in the present study we developed a method to measure the concentration of recombinant human CT-1 in SHR and WKY. This method does not use any isotope and is different from the methods reported in the past (21, 22). However, one limitation of this method is that endogenous plasma CT-1 might be included because of the cross-reaction of endogenous CT-1 with antibodies. Using this method, plasma concentrations of CT-1 in both SHR and WKY be-
fore the injection of CT-1 were very low and without any significant difference between the two groups. If we subtract the pre-injection values from the data at 5, 10, 15, 30 and 60 min in SHR and WKY, there is almost no effect on the data. Although it is not likely, if the injected CT-1 could induce a significant increase of endogenous secretion of CT-1, our data would be much higher than the exact plasma human CT-1 values. In such a case, if standard recombinant rat CT-1 is available, this bias could be excluded.

However, until now there is no standard rat CT-1 available. Therefore, our method cannot exclude the possibility of the presence of rat endogenous CT-1 in our data. On the other hand, if our method is used for measuring CT-1 in human blood samples, this cross-reaction will not occur. Talwar and colleagues provided new data showing that plasma concentrations of CT-1 may join the ranks of candidate circulating markers of cardiac function and prognosis (23). A recent report has demonstrated that endogenous CT-1 in whole blood has prolonged stability (24). Thus, there is a possibility that the method we have developed in the present study can become a practical and much easier option for measuring CT-1 in large numbers of human blood samples in clinical medicine.

References