

Effects and mechanisms of curcumin on the hemodynamic variables of isolated perfused rat hearts

Erkan KILINÇ^{1*}, Ziya KAYGISIZ², Bedri Selim BENEK¹, Kenan GÜMÜŞTEKİN¹

¹Department of Physiology, Faculty of Medicine, Abant İzzet Baysal University, Bolu, Turkey

²Department of Physiology, Faculty of Medicine, Eskişehir Osmangazi University, Eskişehir, Turkey

Received: 30.10.2014 • Accepted/Published Online: 14.02.2015 • Final Version: 05.01.2016

Background/aim: There is no information on the dose–response relationship of curcumin on the hemodynamic variables of the heart at the organ level in isolated perfused rat hearts. We aimed to investigate the effects and mechanisms of curcumin on the hemodynamic variables of isolated perfused rat hearts.

Materials and methods: Rats were randomly divided into 9 groups. The isolated rat heart was retrogradely perfused with modified Krebs–Henseleit solution. After the stabilization period, each group was administered one of the following treatments for 25 min: saline, dimethyl sulfoxide, and curcumin (0.2 μ M, 1 μ M, and 5 μ M); atropine (1 μ M); atropine (1 μ M) + curcumin (1 μ M); L-NAME (100 μ M); or L-NAME (100 μ M) + curcumin (1 μ M). Hemodynamic variables of the heart were measured.

Results: Curcumin at dose of 1 μ M decreased the heart rate (from 271 ± 11.1 to 200.4 ± 14.3 beats/min, $P = 0.011$) but increased end-diastolic pressure (from 7.0 ± 0.4 to 54.6 ± 7.9 mmHg, $P = 0.0008$). A dose of 5 μ M curcumin caused a decrease in the developed pressure (from 87.58 ± 9.0 to 65.40 ± 7.0 mmHg, $P = 0.047$) but an increase in the end-diastolic pressure (from 6.8 ± 0.6 to 48.9 ± 7.7 mmHg, $P = 0.005$). Atropine (1 μ M) reversed the effects of curcumin on the heart.

Conclusion: Our results suggest that curcumin produces dose-dependent negative chronotropic and inotropic effects in isolated perfused rat hearts.

Key words: +dP/dtmax, cardiac contractility, curcumin, heart rate, isolated perfused heart

1. Introduction

Curcumin is a polyphenol extracted from turmeric (*Curcuma longa* L.), responsible for the yellow color of turmeric (1,2). In traditional Chinese and Indian medicine, curcumin has been used to treat a variety of diseases, and recent scientific research has demonstrated its antiinflammatory, antioxidant, anticarcinogenic, and antithrombotic effects (3).

Earlier preclinical studies have showed that curcumin has properties such as anticancer, antihypertrophic, cardioprotective, antithrombotic, and antiatherosclerotic qualities (4). Swamy et al. (4) revealed the cardioprotective effect of curcumin against doxorubicin-induced cardiotoxicity in rats. It has been reported that curcumin protects the heart from ischemia/reperfusion (I/R) injury (5). Additionally, it protects animals against decreases in the heart rate and blood pressure following ischemia (6).

In an in vitro study, it was shown that curcumin blocked phenylephrine-induced cardiac hypertrophy (7).

Furthermore, earlier studies reported that curcumin had a protective role against isoproterenol-induced myocardial infarction (8) and also decreased acute adriamycin-induced myocardial toxicity in rats (9). It has also been suggested that the antiinflammatory effects of curcumin may prevent atrial arrhythmias (10). Curcumin attenuates the development of aortic fatty streaks in rabbits fed a high cholesterol diet (11). Thereby, it can exhibit a potential effect against atherosclerosis. Moreover, it has also been proposed in the treatment of idiopathic pulmonary arterial hypertension (12).

In phase I clinical trials of oral curcumin in patients with advanced colorectal cancer, diarrhea and nausea were reported in relationship with doses of curcumin (13). However, the adverse effects and dose–response relationship of the effects of curcumin when used as a drug need to be studied further. In the past decade, many studies were carried out to research the cardiac therapeutic effects of curcumin, but in the preliminary studies, there

* Correspondence: e_kilinc_27@hotmail.com

is no information about the dose–response relationship of the effects of curcumin on the hemodynamic variables of the heart (2).

Therefore, in the present study, we aimed to investigate the effects and mechanisms of curcumin on the hemodynamic variables of isolated perfused rat hearts.

2. Materials and methods

2.1. Isolated heart preparation and perfusion

All procedures applied were approved by the local research ethics committee (Eskişehir Osmangazi University Ethical Animal Care and Use Committee). Sprague Dawley male rats were fed a standard diet and were housed in their cages with a 12-h light/dark cycle at a temperature of 20–25 °C. A total of 63 male rats weighing 300–400 g were used. The animals (n = 63) were divided into 9 groups. Rat hearts in group 1 (n = 7) were treated with only curcumin at a dose of 0.2 µM. Rat hearts in group 2 (n = 7) were treated with only curcumin at a dose of 1 µM. Rat hearts in group 3 (n = 7) were treated with only curcumin at a dose of 5 µM. Rat hearts in group 4 (n = 7) were treated with only atropine (an antagonist for the muscarinic acetylcholine receptors) at a dose of 1 µM. Rat hearts in group 5 (n = 7) were treated with atropine (at a dose of 1 µM) and curcumin (at a dose of 1 µM). Rat hearts in group 6 (n = 7) were treated with only L-NAME (N^G-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor) at a dose of 100 µM. Rat hearts in group 7 (n = 7) were treated with L-NAME (at a dose of 100 µM) and curcumin (at a dose of 1 µM). Rat hearts in group 8 (n = 7) were treated with only saline and rat hearts in group 9 (n = 7) were treated with dimethyl sulfoxide (DMSO with working concentration of 0.05%), for the control.

One hour after the intraperitoneal administration of 1000 IU heparin, the thorax of the rat was opened under sodium pentothal anesthesia (50 mg/kg i.p.). The heart was rapidly excised and was placed into ice-cold modified Krebs–Henseleit solution until contractions ceased. After the surrounding tissue was removed, the aorta was rapidly secured to a stainless steel cannula of the perfusion system, and the heart was retrogradely perfused using a noncirculating Langendorff technique. The pulmonary artery was incised to facilitate complete coronary drainage in the ventricles. The modified Krebs–Henseleit solution used for perfusion was prepared daily with the following composition (mM): NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, and glucose 11. The solution was continuously oxygenated with 95% O₂ and 5% CO₂ (pH 7.4) and was maintained at 37 °C. The hearts were perfused under a constant pressure (60 mmHg) and under constant flow conditions (16 mL/min) using a peristaltic pump (Ismatec Reglo, Hugo Sachs Electronic, Germany).

2.2. Measurement of hemodynamic variables

Myocardial contractile force was determined by using the method described by Kaygısız et al. (14). To measure the left ventricular peak systolic and end diastolic pressure, a liquid-filled latex balloon was connected to a pressure transducer (Isotec, Germany) and was inserted into the left ventricle of the heart via the mitral valve. Diastolic balloon pressure was maintained at 7 mmHg. Left ventricular developed pressure (LVDP; an index of cardiac contractility) was calculated as the difference between the systolic and diastolic pressures and was accepted as the contractile force. Left ventricular end-diastolic pressure (LVEDP) was also measured by data acquisition software (Isoheart Software, Version 1.5 for Microsoft Windows NT/2000/XP, Hugo Sachs Electronic) via the liquid-filled latex balloon.

Heart rate (HR) was calculated from the signals of the left ventricular pressure. The left ventricular pressure was processed with data acquisition software and then $+dP/dt_{\max}$ (maximum rate of increase in the left ventricular pressure, used as another index of cardiac contractility) and $-dP/dt_{\min}$ (a valuable index) were determined. $+dP/dt_{\max}$ was used as an additional contractility index. On the other hand, $-dP/dt_{\min}$, which is the maximum rate of pressure decrease in the left ventricle, served as an index of relaxation in the evaluation of diastolic function of the left ventricle. Rate pressure product (RPP) was calculated by using the formula $RPP = HR \times \text{systolic blood pressure (SBP)}$.

The coronary flow, which is an index of the coronary vascular tone, was measured via the timed collection of the coronary effluent in a graduated cylinder. Thereby, the hemodynamic parameters were analyzed by a data acquisition and analysis system (Isoheart Software). The hearts were equilibrated for 30 min to establish a stable baseline. The criteria for determining stability were LVDP > 60 mmHg, $+dP/dt_{\max} > 2800 \text{ mmHg s}^{-1}$, heart rate > 200 beats/min, and normal sinus rhythm. After stabilization, drugs were administered to the hearts for 25 min.

2.3. Infusion of drugs and experimental protocols

Curcumin, DMSO, atropine, and L-NAME were purchased from Sigma-Aldrich. Curcumin was dissolved in a saline solution containing 0.1% DMSO to obtain a stock solution of 10 mM; it was then diluted with saline. After the stabilization period, the curcumin doses (0.2 µM, 1 µM, and 5 µM) were administered using an infusion pump (Model 3400, Graseby Medical, UK) for 25 min, and readings were obtained every 5 min. The infusion rate was 0.2 mL/min for all of the experiments. Each heart group was treated with a different dose. Then atropine at 1 µM (15,16), atropine at 1 µM + curcumin at 1 µM, L-NAME at 100 µM (17), and L-NAME at 100 µM + curcumin at 1 µM were administered to the hearts for 25 min and readings

were obtained every 5 min. For 20 min after the final reading, the vitality of the hearts was observed.

2.4. Statistical analysis

In addition to the control group, the values obtained prior to the addition of the drugs were considered as control values. The values are given as mean \pm SEM. Statistical analysis was performed using SPSS 17.0 for Windows (SPSS Inc., USA). Data were analyzed by two-way analysis of variance (ANOVA) followed by Tukey HSD multiple comparisons test. $P < 0.05$ was considered to be statistically significant.

3. Results

Saline and DMSO (0.05%) did not affect any hemodynamic variables of the heart. On the other hand, 25 min after infusion, the 3 doses of curcumin, 0.2 μM ($n = 7$), 1 μM ($n = 7$), and 5 μM ($n = 7$), decreased the heart rate from 271.8 ± 19.4 to 257.6 ± 19.9 beats/min, from 271 ± 11.1 to 200.4 ± 14.3 beats/min ($P < 0.05$), and from 250 ± 21.5 to 196.6 ± 22.3 beats/min, respectively (Figure 1). Although an infusion of curcumin at doses of 0.2 and 5 μM induced decreases in heart rate, they were not found statistically significant. On the other hand, curcumin at a dose of 1 μM induced statistically significant decreases in heart rate ($P < 0.05$) (Figure 1). None of the doses (0.2, 1, and 5 μM) of curcumin caused significant alterations in the RPP or coronary flow. Neither the 0.2 dose nor the 1 μM dose of curcumin altered the LVDP; however, the 5 μM dose of curcumin caused a decrease in the LVDP (from 87.58 ± 9.0 to 65.40 ± 7.0 mmHg, $P < 0.05$) (Figure 2).

Curcumin at a dose of 0.2 μM decreased $-dP/dt_{\min}$ (from 2559 ± 124 to 2239 ± 145 mmHg, $P < 0.05$), but it did not affect the other hemodynamic variables of the

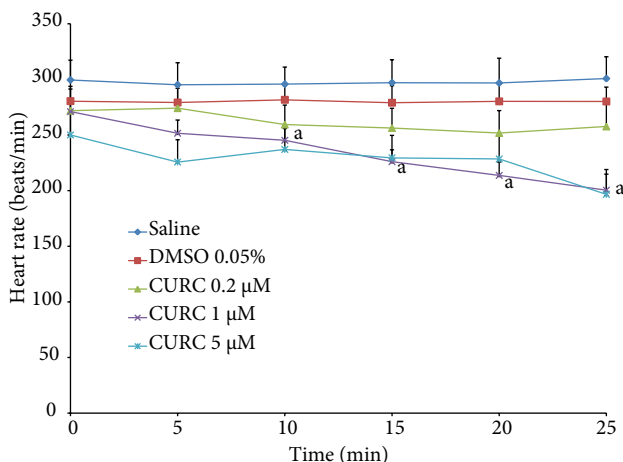


Figure 1. Time-dependent effects of saline, DMSO, and different doses of curcumin infusion on heart rate. a: $P < 0.05$, denoting significant differences compared to control values. $N = 7$ for each group.

heart. Curcumin at a dose of 1 μM significantly increased LVEDP (from 7.0 ± 0.4 to 54.6 ± 7.9 mmHg, $P < 0.001$) (Figure 3). Curcumin at a dose of 5 μM caused a decrease in $+dP/dt_{\max}$ (from 3255 ± 351 to 2436 ± 253 mmHg s^{-1} , $P < 0.05$) (Figure 4) and $-dP/dt_{\min}$ (from 1965 ± 232 to 1209 ± 128 mmHg s^{-1} , $P < 0.05$); on the other hand, it caused a significant increase in the LVEDP (from 6.8 ± 0.6 to 48.9 ± 7.7 mmHg, $P < 0.01$) (Figure 3). Moreover, it stopped the heart 40 min after infusion at a dose of 5 μM . The maximum effect of curcumin occurred 25 min after the initial exposure (Table 1).

While atropine at the dose of 1 μM ($n = 7$) significantly increased both LVDP (from 74.0 ± 3.8 to 88.3 ± 3.9 mmHg, $P < 0.05$) and $+dP/dt_{\max}$ (from 2177 ± 77.6 to 2608 ± 174 mmHg s^{-1} , $P < 0.05$) (Figure 5) in the heart, there was no significant increase in HR. It did not alter the other hemodynamic variables of the heart. When atropine (1 μM) and curcumin (1 μM) were administered together to the heart, no significant effect was observed on HR (Figure 6) or the other hemodynamic variables of the heart ($n = 7$). L-NAME alone, at a dose of 100 μM ($n = 7$), increased both LVEDP (from 7.1 ± 0.7 to 15.1 ± 1.7 mmHg, $P < 0.05$) (Figure 7) and $+dP/dt_{\max}$ (from 3205 ± 237 to 3828 ± 236 mmHg s^{-1} , $P < 0.05$) (Figure 5). On the other hand, the combination of L-NAME (100 μM) with curcumin (1 μM) ($n = 7$) increased LVEDP (from 7.6 ± 0.5 to 13.8 ± 1.9 mmHg, $P < 0.05$) (Figure 7). However, the combination reduced $+dP/dt_{\max}$ (from 3692 ± 257 to 2964 ± 185 , $P < 0.05$) (Figure 5). The HR, RPP, and coronary flow were not significantly altered by atropine, atropine + curcumin, L-NAME, or L-NAME + curcumin (Table 2). The effects of the drugs on HR, LVDP, LVEDP, coronary flow, $+dP/dt_{\max}$, $-dP/dt_{\min}$, and RPP are shown in Table 2.

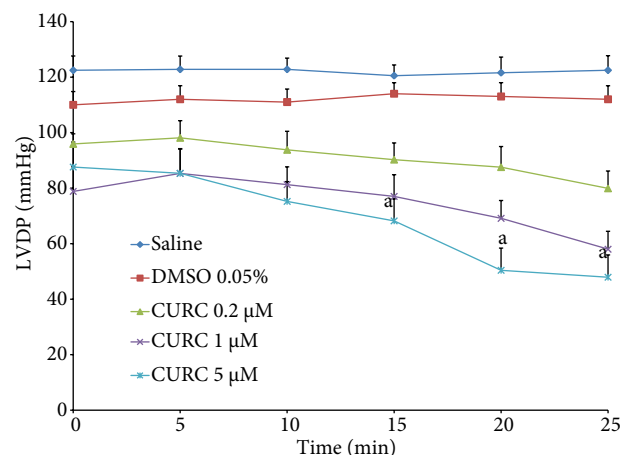


Figure 2. Time-dependent effects of saline, DMSO, and different doses of curcumin infusion on left ventricular developed pressure (LVDP). a: $P < 0.05$, denoting significant differences compared to control values. $N = 7$ for each group.

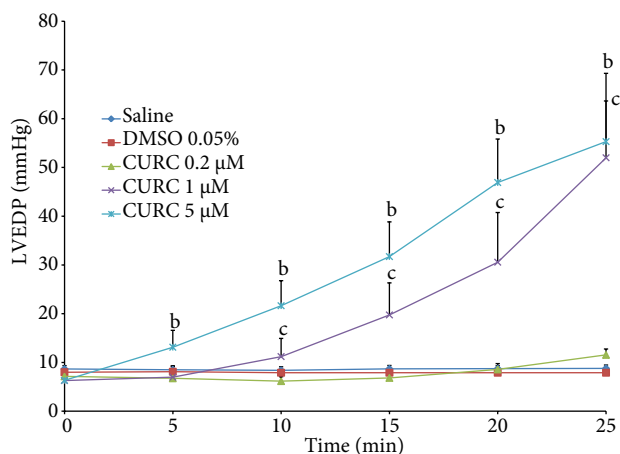


Figure 3. Time-dependent effects of saline, DMSO, and different doses of curcumin infusion on left ventricular end-diastolic pressure (LVEDP). b: $P < 0.01$, c: $P < 0.001$, denoting significant differences compared to control values. $N = 7$ for each group.

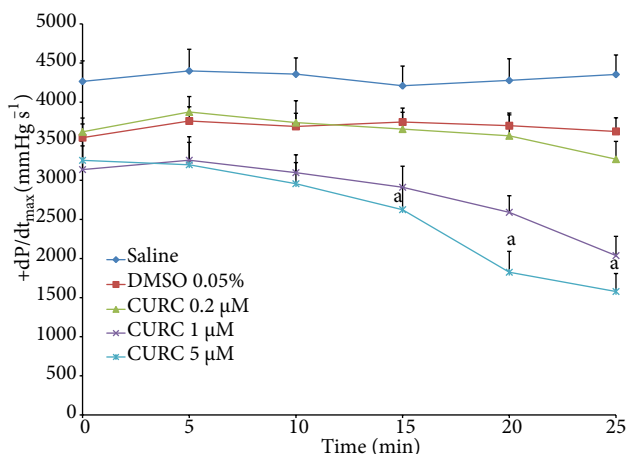


Figure 4. Time-dependent effects of saline, DMSO, and different doses of curcumin infusion on $+dP/dt_{max}$ (maximum rate of increase in the left ventricular pressure). a: $P < 0.05$, denoting significant differences compared to control values. $N = 7$ for all doses.

Table 1. Maximal changes in heart rate, LVDP, $+dP/dt_{max}$, LVEDP, and RPP induced by different doses of the drugs and their combinations.

Drugs	Doses (μM)	Heart rate Δ % max	LVDP Δ % max	$+dP/dt_{max}$ Δ % max	LVEDP Δ % max	RPP Δ % max
Curcumin	0.2 μM	-4.4 ± 0.1	-6.2 ± 0.2	$+0.04 \pm 0.1$	$+11.6 \pm 0.4$	-8.2 ± 0.4
	1 μM	-16 ± 0.6^a	-5.9 ± 0.2	-11.4 ± 0.3	$+283.1 \pm 5.3^c$	-4.6 ± 0.2
	5 μM	-10.6 ± 0.3	-25.3 ± 0.7^a	-25.1 ± 0.7^a	$+432.8 \pm 6.7^b$	-5.4 ± 0.3
Atropine	1 μM	-14.4 ± 0.5	$+19.3 \pm 0.6^a$	$+19.8 \pm 0.6^a$	$+12.0 \pm 0.4$	$+1.5 \pm 0.4$
Atropine + curcumin	1 μM + 1 μM	$+0.3 \pm 0.2$	-12 ± 0.3	-7.4 ± 0.2	$+57.2 \pm 3.1$	-10.3 ± 0.3
L-NAME	100 μM	-2.4 ± 0.1	-1.4 ± 0.09	$+19.4 \pm 0.5^a$	$+71.8 \pm 4.1^a$	-3.7 ± 0.1
L-NAME + curcumin	100 μM + 1 μM	-7.4 ± 0.2	-16.4 ± 0.6	-19.7 ± 0.6^a	$+82.2 \pm 4.8^a$	-22.9 ± 0.7

RPP Δ % max, heart rate Δ % max, LVDP Δ % max, $+dP/dt_{max}$ Δ % max, and LVEDP Δ % max are maximal changes in RPP, heart rate, LVDP, $+dP/dt_{max}$, and LVEDP as percentage of baseline values, respectively. RPP: rate pressure product, LVDP: left ventricular developed pressure, $+dP/dt_{max}$: maximum rate of increase in the left ventricular pressure, LVEDP: left ventricular end-diastolic pressure, and L-NAME: N^G -nitro-L-arginine methyl ester. a: $P < 0.05$, b: $P < 0.01$, and c: $P < 0.001$, denoting significant differences compared to control values.

4. Discussion

There are several studies about therapeutic effects of curcumin (6,18); however there are only a few studies about the dose–response relationship of the effects of curcumin (13). The adverse effects and dose–response relationship of the effects of curcumin when it is used as a drug need to be studied further. Therefore, for the first time, in the present study, we observed the effects and dose–response relationship of curcumin on the hemodynamic variables of the heart in isolated perfused rat hearts.

In the present study, we found that the efficacious dose of curcumin was 1 μM . This information can contribute to future studies on the effects of curcumin on the heart. It has been extensively studied, particularly the therapeutic effects of curcumin in the treatment of cancer and inflammatory disorders (4,13,18). In addition, it was also widely reported that curcumin protected a wide variety of cell types against oxidative damage (19).

However, very little is known regarding the effect of curcumin on cardiovascular disease (2). There is a

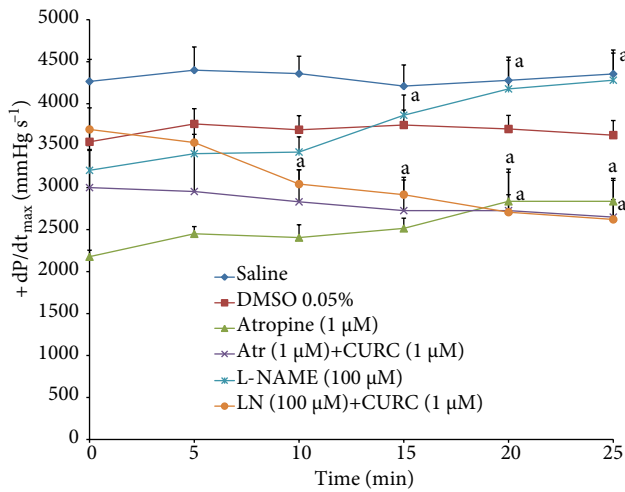


Figure 5. Time-dependent effects of saline, DMSO, and drug combinations infusion on $+dP/dt_{max}$ (maximum rate of increase in the left ventricular pressure). Atr: atropine, CURC: curcumin, LN: L-NAME (N^G -nitro-L-arginine methyl ester). a: $P < 0.05$, denoting significant differences compared to control values. $N = 7$ for all groups.

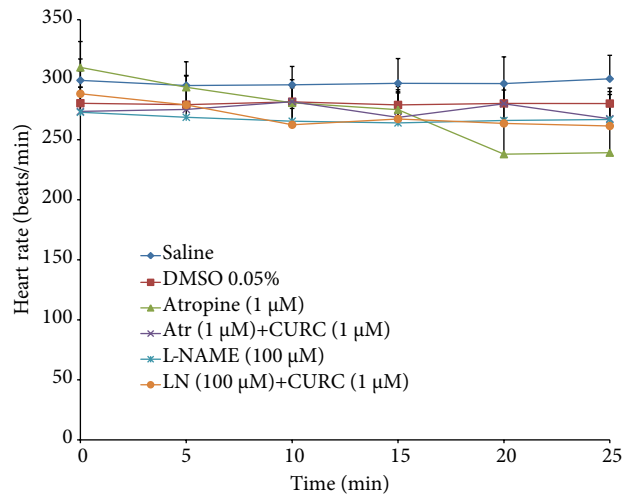


Figure 6. Time-dependent effects of saline, DMSO, and drug combinations infusions on heart rate. Atr: atropine, CURC: curcumin, LN: L-NAME (N^G -nitro-L-arginine methyl ester). $N = 7$ for all groups.

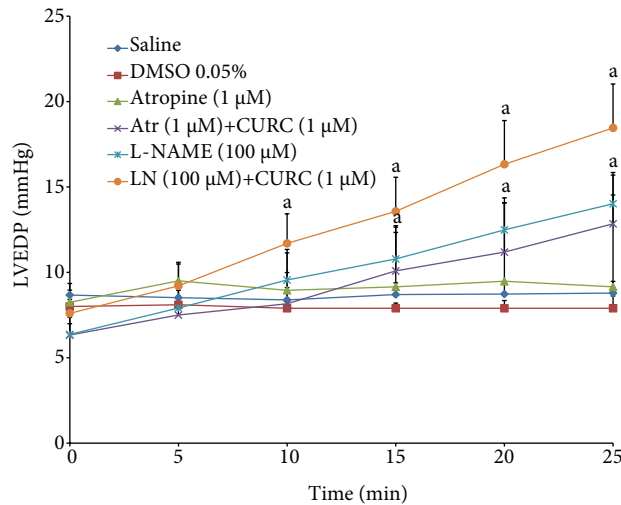


Figure 7. Time-dependent effects of saline, DMSO, and drug combinations infusions on left ventricular end-diastolic pressure (LVEDP). Atr: atropine, CURC: curcumin, LN: L-NAME (N^G -nitro-L-arginine methyl ester). a: $P < 0.05$, denoting significant differences compared to control values. $N = 7$ for all groups.

shortage of information about the effects of curcumin on the hemodynamic parameters of the heart in the literature. Therefore, the results obtained from the present study may be important in terms of the effects of curcumin on hemodynamic parameters of the heart. Swamy et al. (4) reported on the cardioprotective effect of curcumin against doxorubicin-induced cardiotoxicity in rats. Furthermore, Ansari et al. (18) also showed that curcumin had a protective role in myocardial oxidative damage induced

by isoproterenol in rats. Curcumin represses hypertrophic responses in cardiomyocytes (20).

We found the optimum dose of curcumin on the hemodynamic variables of the heart before exploring the effect mechanisms of curcumin on the variables. The concentration of $1 \mu M$ was the optimum dose of curcumin. At a dose of $0.2 \mu M$, curcumin had no effect on most of the hemodynamic variables of the heart, and curcumin at a dose of $5 \mu M$ adversely affected almost all of the variables.

Table 2. Effects of the drugs on heart rate, LVDP, LVEDP, coronary flow, $+dP/dt_{max}$, $-dP/dt_{min}$, and RPP.

	Curcumin			Atropine	Atr + Curc	L-NAME	L-N + Curc
	0.2 μ M	1 μ M	5 μ M	1 μ M	1 μ M + 1 μ M	100 μ M	100 μ M + 1 μ M
Heart rate (beats/min)		↓ ^a					
LVDP (mmHg)			↓ ^a	↑ ^a			
RPP (bpm mmHg)							
LVEDP (mmHg)		↑ ^c	↑ ^b			↑ ^a	↑ ^a
Coronary flow (mL/min)							
$+dP/dt_{max}$ (mmHg/s)			↓ ^a	↑ ^a		↑ ^a	↓ ^a
$-dP/dt_{min}$ (mmHg/s)	↓ ^a		↓ ^a				

Atr: atropine, LN: L-NAME (N^G -nitro-L-arginine methyl ester), Curc: curcumin, \uparrow : increase, \downarrow : decrease, LVDP: left ventricular developed pressure, RPP: rate pressure product, LVEDP: left ventricular end-diastolic pressure, $+dP/dt_{max}$: maximum rate of increase in the left ventricular pressure, and $-dP/dt_{min}$: maximum rate of pressure decrease in left ventricle. a: $P < 0.05$, b: $P < 0.01$, and c: $P < 0.001$, denoting significant differences compared to control values. $N = 7$ for all doses.

Additionally, the dose of 5 μ M stopped the heart 40 min after infusion.

We found that curcumin produced dose-dependent decreases in HR. However, none of the doses of curcumin caused significant alterations in the RPP. RPP is an index of myocardial work load, and increased RPP is reported as a risk for the cardiovascular system (21). Thus, we can say that there is neither a therapeutic nor an adverse effect of curcumin on the mechanical workings of the heart.

Curcumin at 5 μ M did not affect coronary flow; on the other hand, while it decreased the heart rate, LVDP, $+dP/dt_{max}$, and $-dP/dt_{min}$, it increased LVEDP. Thus, we can conclude that curcumin at high doses has adverse effects on the hemodynamic variables of the heart.

Atropine is a competitive antagonist for the muscarinic acetylcholine receptor types M_1 , M_2 , M_3 , M_4 , and M_5 . Thus, atropine is an anticholinergic drug (parasympatholytic) and blocks binding sites of acetylcholine on the muscarinic acetylcholine receptors. It is known that atropine reverses the effects of acetylcholine on the heart. Atropine causes increases in the firing of the sinoatrial node and in conduction through the atrioventricular node of the heart. Atropine also has a positive inotropic effect on the myocardium (15,22). We used atropine at the concentration of 1 μ M because atropine produces its optimum effects at this dose (15,16). On the other hand,

the intent behind the use of atropine and L-NAME (nitric oxide-synthase inhibitor N^G -nitro-L-arginine methyl ester) in the present study was to detect the mechanisms of curcumin's effect on isolated perfused rat hearts.

The expected effects of atropine when administered alone were increases in the HR, LVDP, and $+dP/dt_{max}$. However, while there was an increase both in LVDP and $+dP/dt_{max}$, a significant increase in HR did not occur. When atropine and curcumin were administered in the heart together, effects of the curcumin (1 μ M) were not observed. Thus, we concluded that curcumin might act like acetylcholine on the muscarinic acetylcholine receptors, directly activate K^+ channels, or cause leakage of K^+ ions out from the cells via intracellular second messengers in cardiac myocytes and the autorhythmic cells of the heart.

Cheng et al. (23) reported that curcumin could activate muscarinic M_1 cholinceptors (M_1 -mAChR) through the PLC-PKC pathway in urinary bladder isolated from rats. Based on the results of these studies, we suggest that curcumin may directly activate K^+ channels or cause leaking of K^+ ions from the cells by triggering intracellular second messengers in cardiac myocytes and the cells of the electrical conduction system of the heart.

The bradycardic effect of curcumin at the dose of 1 μ M showed that curcumin may be effective for treatment of pathologic tachycardia. On the other hand, it must be

taken into consideration that curcumin (1 μM) caused a significant increase in left ventricular end-diastolic pressure ($P < 0.001$), which is a risk factor in cardiac diseases since an increase in the LVEDP may lead to pulmonary congestion and edema. However, it may be significant that curcumin decreased the heart rate without adverse changes in vitality of the heart. Thus, curcumin may be used as a specific bradycardic agent with its other efficacious effects in the treatment of pathologic tachycardia. Impairment of cardiac contractility alters the diastolic pressure–volume relationship and leads to an increase in LVEDP. Furthermore, the increase in LVEDP is associated with decreased coronary flow (24). Our study indicated that curcumin did not significantly alter the coronary flow ($P > 0.05$) under constant coronary perfusion pressure (60 mmHg) and constant flow condition (16 mL/min). In our study, the increase in LVEDP observed may have been due to the decrease in LVDP.

Results obtained from the present study have shown that curcumin produces a negative inotropic and chronotropic effect with a decrease in HR and LVDP and an increase in LVEDP. In accordance with our results, prior studies (25,26) investigated the effect of turmeric extract on the arterial pressure and HR in nonanesthetized rats. In conclusion, they revealed the hypotensive and bradycardic effects of the extract, as well as its dose-dependency.

In an I/R and ischemic preconditioning study by Sato et al., isolated rat hearts were perfused with 100 μM curcumin (27). They reported that curcumin blocked the I/R- and preconditioning-mediated increase in c-Jun NH₂-terminal kinase 1 and c-Jun protein levels and reduced the I/R-mediated increased myocardial infarction. However, when we applied a 5 μM curcumin to the hearts, the curcumin stopped the hearts 40 min after the infusion. Such a difference between the results from the applied doses of curcumin is interesting. We also suggest that a high dose of curcumin, such as 5 μM , may be toxic for the heart, based on the results of our study.

Nitric oxide (NO) is a ubiquitous intercellular messenger found in a wide variety of cell types, tissues, and organ systems (28). NO is involved in a substantial reduction in cardiac myocyte contraction via a cGMP-dependent mechanism (29). L-NAME blocks formation of NO in the cells. In the present study, L-NAME administered alone induced an increase in LVEDP and

+dP/dt_{max}. It was expected that L-NAME would induce an increase in LVEDP, but it was very surprising that it also increased +dP/dt_{max}. When L-NAME and curcumin were administered in the heart together, there was an increase in LVEDP. However, there was also a decrease in +dP/dt_{max}. Thus, L-NAME did not block the effects of curcumin. These observations suggest that curcumin may probably affect the heart differently than NO.

Brouet and Ohshima (30) demonstrated that curcumin could interfere with the formation of NO by inhibiting induction of nitric oxide synthase in activated macrophages. Some authors suggested that curcumin might prevent the intracellular Ca²⁺ release channels (31). However, Bilmen et al. (32) reported that curcumin inhibited the ATPase activity of sarcoendoplasmic reticulum Ca-ATPase (SERCA) in the skeletal and cardiac myocytes. Several authors also reported that, while curcumin at a concentration between 1 and 10 mM (33) or 1 and 3 μM (34) increased Ca²⁺ transport, the ATPase activity was inhibited by higher curcumin concentrations in the sarcoplasmic reticulum of skeletal and cardiac muscle. Lee et al. (35) reported the first case of a complete atrioventricular (AV) block associated with overdose of curcumin intake. In the conditions of the complete AV block, the impulse generated in the sinoatrial node in the atrium could not propagate to the ventricles. M₂-mAChR receptors, located in the heart, reduce the HR and conduction velocity of the AV node by slowing the speed of depolarization after a cholinergic agonist such as acetylcholine binds to it. The receptors also attenuate contractile forces of the atrial and ventricular cardiac muscles. Additionally, our results with L-NAME indicate that curcumin acts through a different mechanism than NO on the heart cells. Taken together, curcumin may not only cause K⁺ ions to leak out of the autorhythmic cells and myocytes in the heart but also may increase Ca²⁺ transport by triggering the ATPase activity of SERCA. Both of these also cause a decrease in contractility and HR in the heart. Therefore, we think that further studies are needed to clarify the molecular mechanisms of curcumin.

Acknowledgments

We thank Dr Bilgin Kaygısız for his help in laboratory assistance and Dr Fatma Töre for her help in statistical analysis.

References

1. Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: a short review. *Life Sci* 2006; 78: 2081–2087.
2. Srivastava G, Mehta JL. Currying the heart: curcumin and cardioprotection. *J Cardiovasc Pharmacol Ther* 2009; 1: 22–27.
3. Goel A, Kunnumakkara AB, Aggarwal BB. Curcumin as “curecumin”: from kitchen to clinic. *Biochem Pharmacol* 2008; 75: 787–809.

4. Swamy AV, Gulliaya S, Thippeswamy A, Koti BC, Manjula DV. Cardioprotective effect of curcumin against doxorubicin-induced myocardial toxicity in albino rats. *Indian J Pharmacol* 2012; 1: 73–77.
5. Manikandan P, Sumitra M, Aishwarya S, Manohar BM, Lokanadam B, Puvanakrishnan R. Curcumin modulates free radical quenching in myocardial ischaemia in rats. *Int J Biochem Cell Biol* 2004; 36: 1967–1980.
6. Srivastava R, Dikshit M, Srimal RC, Dhawan BN. Anti-thrombotic effect of curcumin. *Thromb Res* 1985; 40: 413–417.
7. Li HL, Liu C, De Couto G, Ouzounian M, Sun M, Wang AB, Huang Y, He CW, Shi Y, Chen X et al. Curcumin prevents and reverses murine cardiac hypertrophy. *J Clin Invest* 2008; 118: 879–893.
8. Nirmala C, Puvanakrishnan R. Effect of curcumin on certain lysosomal hydrolases in isoproterenol-induced myocardial infarction in rats. *Biochem Pharmacol* 1996; 51: 47–51.
9. Venkatesan N. Curcumin attenuation of acute adriamycin myocardial toxicity in rats. *Br J Pharmacol* 1998; 124: 425–427.
10. Schoonderwoerd BA, Smit MD, Pen L, Van Gelder IC. New risk factors for atrial fibrillation: causes of not-so-lone atrial fibrillation. *Europace* 2008; 10: 668–673.
11. Quiles JL, Mesa MD, Ramírez-Tortosa CL, Aguilera CM, Battino M, Gil A. *Curcuma longa* extract supplementation reduces oxidative stress and attenuates aortic fatty streak development in rabbits. *Arterioscler Thromb Vasc Biol* 2002; 22: 1225–1231.
12. Bronte E, Coppola G, Di Miceli R, Sucato V, Russo A, Novo S. Role of curcumin in idiopathic pulmonary arterial hypertension treatment: a new therapeutic possibility. *Med Hypotheses* 2013; 81: 923–926.
13. Sharma RA, Euden SA, Platton SL, Cooke DN, Shafayat A, Hewitt HR, Marczylo TH, Morgan B, Hemingway D, Plummer SM et al. Phase I clinical trial of oral curcumin: biomarkers of systemic activity and compliance. *Clin Cancer Res* 2004; 10: 6847–6854.
14. Kaygısız Z, Kaygısız B, Kılınç E. The effect of Des-Arg9-bradykinin and bradykinin-potentiating peptide C on isolated rat hearts. *Acta Physiol Hung* 2013; 100: 280–288.
15. Deng XF, Mulay S, Chemtob S, Varma DR. Mechanisms of the atrium-specific positive inotropic activities of quinidine- and atropine-like agents in rats. *J Pharmacol Exp Ther* 1997; 281: 322–329.
16. Legssyer A, Ziyat A, Mekhfi H, Bnouham M, Tahri A, Serhrouchni M, Hoerter J, Fischmeister R. Cardiovascular effects of *Urtica dioica* L. in isolated rat heart and aorta. *Phytother Res* 2002; 16: 503–507.
17. Pabla R, Curtis MJ. Effect of endogenous nitric oxide on cardiac systolic and diastolic function during ischemia and reperfusion in the rat isolated perfused heart. *J Mol Cell Cardiol* 1996; 28: 2111–2121.
18. Ansari MN, Bhandari U, Pillai KK. Protective role of curcumin in myocardial oxidative damage induced by isoproterenol in rats. *Hum Exp Toxicol* 2007; 26: 933–938.
19. Srinivasan K. Antioxidant potential of spices and their active constituents. *Crit Rev Food Sci Nutr* 2014; 54: 352–372.
20. Chowdhury R, Nimmanapalli R, Graham T, Reddy G. Curcumin attenuation of lipopolysaccharide induced cardiac hypertrophy in rodents. *ISRN Inflamm* 2013; 1–8.
21. White WB. Heart rate and the rate-pressure product as determinants of cardiovascular risk in patients with hypertension. *Am J Hypertens* 1999; 12: 50–55.
22. Buccino RA, Sonnenblick EH, Cooper T, Braunwald E. Direct positive inotropic effect of acetylcholine on myocardium. Evidence for multiple cholinergic receptors in the heart. *Circ Res* 1966; 19: 1097–1088.
23. Cheng TC, Lu CC, Chung HH, Hsu CC, Kakizawa N, Yamada S, Cheng JT. Activation of muscarinic M-1 cholinceptors by curcumin to increase contractility in urinary bladder isolated from Wistar rats. *Neurosci Lett* 2010; 473: 107–109.
24. Doi Y, Masuyama T, Yamamoto K, Mano T, Naito J, Nagano R, Kondo H, Hori M. Coronary back flow pressure is elevated in association with increased left ventricular end-diastolic pressure in humans. *Angiology* 1996; 47: 1047–1051.
25. Zhang C, Zha DG, Du RS, Hu F, Li SH, Wu XY, Liu YL. Evaluation of left ventricular diastolic function in canine acute myocardial ischemia using velocity vector imaging and quantitative tissue velocity imaging. *Nan Fang Yi Ke Da Xue Xue Bao* 2009; 29: 1333–1336.
26. Adaramoye OA, Anjos RM, Almeida MM, Veras RC, Silvia DF, Oliveira FA, Cavalcante KV, Araújo IG, Oliveira AP, Medeiros IA. Hypotensive and endothelium independent vasorelaxant effects of methanolic extract from *Curcuma longa* L. in rats. *J Ethnopharmacol* 2009; 124: 457–462.
27. Sato M, Cordis GA, Maulik N, Das DK. SAPKs regulation of ischemic preconditioning. *Am J Physiol Heart Circ Physiol* 2000; 279: 901–907.
28. Conti V, Russomanno G, Corbi G, Izzo V, Vecchione C, Filippelli A. Adrenoreceptors and nitric oxide in the cardiovascular system. *Front Physiol* 2013; 4: 1–11.
29. Loscalzo J, Welch G. Nitric oxide and its role in the cardiovascular system. *Prog Cardiovasc Dis* 1995; 38: 87–104.
30. Brouet I, Ohshima H. Curcumin, an anti-tumour promoter and anti-inflammatory agent, inhibits induction of nitric oxide synthase in activated macrophages. *Biochem Biophys Res Commun* 1995; 206: 533–540.
31. Brini M, Carafoli E. Calcium pumps in health and disease. *Physiol Rev* 2009; 89: 1341–1378.
32. Bilmen JG, Khan SZ, Javed MH, Michelangeli F. Inhibition of the SERCA Ca²⁺ pumps by curcumin. Curcumin putatively stabilizes the interaction between the nucleotide-binding and phosphorylation domains in the absence of ATP. *Eur J Biochem* 2001; 268: 6318–6327.
33. Logan-Smith MJ, Lockyer PJ, East JM, Lee AG. Curcumin, a molecule that inhibits the Ca²⁺-ATPase of sarcoplasmic reticulum but increases the rate of accumulation of Ca²⁺. *J Biol Chem* 2001; 276: 46905–46911.
34. Sumbilla C, Lewis D, Hammerschmidt T, Inesi G. The slippage of the Ca²⁺ pump and its control by anions and curcumin in skeletal and cardiac sarcoplasmic reticulum. *J Biol Chem* 2002; 277: 1390–1396.
35. Lee SW, Nah SS, Byon JS, Ko HJ, Park SH, Lee SJ, Shin WY, Jin DK. Transient complete atrioventricular block associated with curcumin intake. *Int J Cardiol* 2011; 150: 50–52.