Angiotensin (1-7) and apelin co-therapy: New strategy for heart failure treatment in rats

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Abstract

Objective: Isoproterenol (ISO)-induced heart failure is a standardized model for the study of beneficial effects of various drugs. Both apelin and angiotensin 1–7 have a cardiac protective effect. We assumed that co-therapy with apelin and angiotensin 1–7 [Ang (1–7)] may have synergistic cardioprotective effects against isoproterenol-induced heart failure.

Methods: The rats were randomly assigned to one of eight groups, 7 animals in each, as follows: (1) Control I (saline; IP injection), (2) Control II (saline; via mini-osmotic pump), (3) ISO (5 mg/kg; IP), (4) Apelin (20 µg/kg; IP), (5) Ang (1–7) (30 µg/kg/day; via mini-osmotic pump), (6) Apelin+ISO, (7) Ang (1–7)+ISO, and (8) Apelin+Ang (1–7)+ISO. Rat myocardial injury was induced by intraperitoneal injection of 5 mg/kg of ISO for 10 days. Apelin and Ang (1–7) were administered 30 minutes before the ISO injection.

Results: A decrease in the systolic blood pressure [SBP (p<0.001)], diastolic blood pressure [DBP (p=0.024)], left ventricular systolic pressure [LVSP (p<0.001)], left ventricular contractility [dP/dt max. (p<0.001)], relaxation [dP/dt min. (p<0.001)], and an increase in left ventricular end-diastolic pressure [LVEDP, (p<0.001)] were observed in ISO-treated rats. Plasma LDH and myocardial and plasma MDA were higher in the ISO heart than in controls (p<0.001). Histopathological examination of the cardiac tissue showed myocardial fibrosis and leukocyte infiltration in ISO-treated rats as compared to control. Co-therapy with apelin and Ang (1–7) was more effective than either agent used alone in restoring these parameters to that of control rats.

Conclusion: The results of this study showed that the combination of apelin and Ang (1–7) had a more cardioprotective effect than either used alone against ISO-induced heart failure, and co-therapy may be a useful treatment option for myocardial injuries and heart failure. *(Anatol J Cardiol 2020; 23: 209-17)*

Keywords: apelin, Ang (1–7), heart failure, isoproterenol, rat

Introduction

Heart failure (HF) poses a significant public health problem. The prevalence of HF is over 5.8 million in the United States and over 23 million worldwide (1, 2). Various cardiovascular diseases will eventually lead to HF. Currently, HF treatment research focuses on limiting and reversing cardiac remodeling, and one of the leading research directions in this area is the treatment of myocardial fibrosis. Isoproterenol (ISO) is a synthetic catecholamine and β-adrenoceptor agonist. It can produce cardiac dysfunction, including cardiac hypertrophy, fibroblast proliferation, connective tissue accumulation with decreased myocardial compliance, and inhibition of diastolic and systolic functions (3). These symptoms are similar to the pathological changes in human heart failure. Ang (1–7) plays a cardiovascular protective role in the renin–angiotensin system (RAS), opposite to cardiovascular toxic effects of Ang II (4). Ang (1–7), by acting via the Mas receptor and releasing nitric oxide (NO) and prostaglandin, causes vasodilation, inhibition of cell growth, and cell proliferation (5). In the healthy rat heart, an acute perfusion of Ang (1–7) increases the cardiac output, stroke volume, and improves the endothelial function of the aorta. The coronary perfusion of Ang (1–7) can also improve the cardiac function in rats with coronary artery ligation-induced HF (4). In addition to the reduction in the myocyte size, infusion of Ang (1–7) can attenuate ventricular dysfunction and remodeling after myocardial infarction (6). Chronic administration of Ang (1–7) improves cardiac hypertrophy and fibrosis in rats (7).

Apelin also has a cardioprotective effect. It has been known as an endogenous ligand for the APJ receptor (8). Both apelin

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and its receptor are widely present in different parts of the body, for example, in the heart (cardiac myocytes and vascular smooth muscle cells) (9). The apelin receptor is often co-expressed with angiotensin II type-1 receptor and acts as an endogenous counter-regulator for this receptor (10). In acute myocardial injury and HF, apelin can act as an endogenous cardioprotective agent (11). The endogenous apelin level is found to be insufficient during severe HF (12). Ang (1–7) signaling is suppressed in apelin-deficient hearts (13). Apelin induces the expression of angiotensin-converting enzyme 2 (ACE2). ACE2 is the main enzyme that produces Ang (1–7). However, the cardioprotective effects of ACE2 may be limited because ACE2 hydrolyzes apelin (14).

Considering the above, 1) there is a partial improvement of cardiac function by Ang (1–7) and apelin, 2) there is a reduction in the apelin levels during HF, and 3) apelin induces angiotensin-converting enzyme 2 (ACE2) to improve the heart function in Ang (1–7) manner (15), and thus we assumed that combined administration of apelin and Ang (1–7) would have more beneficial effects than the administration of either of these agents alone.

Methods

Animals

Male Sprague-Dawley rats weighing 180 to 250 g were kept under strict 12-hour dark/light cycles in single cages at a constant room temperature (24°C) and moisture (70%). Rats had free access to deionized water and a standard diet. Rats were assigned to the different experimental groups after 7 days to adapt to the environment. Experiments have been conducted and approved by the National Ethics Committee in accordance with all applicable international, national, and institutional guidelines for animal studies (IR.FUMS.RES 1395.9).

For induction of HF, ISO (5 mg/kg) diluted in normal saline was administered for 10 days, once daily by intraperitoneal (IP) injection. This dose of ISO causes severe histological changes in the cardiac tissue and subsequent cardiac dysfunction after 10 days of injection (16). Control animals received normal saline.

Experimental procedure

The rats were randomly divided into eight groups (n=7 per group):

Group I: Control I (0.9% normal saline, IP) for 10 days,

Group II: Control II (0.9% normal saline) via a mini-osmotic pump (Alzet 2001, Cupertino, CA) implanted subcutaneously between the scapula for 10 days,

Group III: ISO (5 mg/kg, IP),

Group IV: Apelin (20 µg/kg, IP) (11),

Group V: Ang (I–7) (30 µg/kg/day via a mini-osmotic pump) (17),

Group VI: ISO (5 mg/kg, IP)+Apelin (20 µg/kg, IP),

Group VII: ISO (5 mg/kg, IP)+Ang (1–7) (30 µg/kg/day via a mini-osmotic pump),

Group VIII: ISO (5 mg/kg, IP)+Apelin (20 µg/kg, IP)+Ang (1–7) (30 µg/kg/day via a mini-osmotic pump),

Ang (1–7) was obtained from Tocris Bioscience, apelin from Phoenix Pharmaceuticals, and isoproterenol hydrochloride from Sigma Aldrich, Germany. All injections continued for 10 days. Apelin was administered 30 min before the ISO injection.

Measurement of hemodynamic parameters

The rats were anesthetized with sodium pentobarbital (50 mg/kg, IP). Two catheters filled with heparin saline (500 U/mL) were inserted into the right femoral and right carotid arteries to measure the arterial blood pressure and left ventricular (LV) pressure, and the catheter in the right carotid artery was further inserted into the left ventricle. The blood pressure, heart rate, dP/dt max, dP/dt min, LVSP, and LVED were recorded via Powerlab (4S, Australia), as described previously (18-20).

Assay of the malondialdehyde level and lactic dehydrogenase activity in plasma

After hemodynamic parameters were measured, blood was collected from the LV in heparinized syringes and transferred to the tubes. The blood was immediately centrifuged, and plasma samples were assayed for malondialdehyde (MDA) and lactic dehydrogenase (LDH). The LDH level was measured using the Sigma lactate dehydrogenase assay kit (MAK066), and the MDA level in heart and plasma was measured using the enzyme-linked immunosorbent assay kit (Sigma-Aldrich, UK) in accordance with the manufacturer's instructions.

Pathological examination of the myocardium

The rats were executed, and their hearts were quickly removed. A histological examination of myocardial sections in the cardiac apex of the myocardium was performed using the hematoxylin–eosin (H&E) staining. The heart, immersed in 10% formaldehyde for fixation, in separately labeled bottles, was then transversely sectioned under the mitral value level. The thickness of each tissue slice was approximately 4 mm, inserted in a similarly labeled tissue capsule. All the capsules were collected in 10% solution of formaldehyde and sent for tissue processing (tissue processor did sabz; Ds 2080/H). Gradual dehydration started with the alcohol grade 70%, 1h in a shaking state and continued in the same way with the alcohol grade 80%, 90%, 95%, and 100%. For cleaning steps, xylene was used in two subsequent bottles. In the last step, two melted paraffin waxes (63°C) are used for infiltration (impregnation). Each of the processed tissue slices were inserted at the bottom of proper sized metallic molds, which were then filled with melted paraffin wax. The tissue paraffin blocks were immediately supported by plastic cassettes (tissue teks) placed on the top of the molds and, if necessary, filled with melted paraffin wax.

The molds were transported in the cold plate for rapid hardening. The block was stored in the freezer before sectioning, which was done by rotary microtome (Microm HM 325, Thermo Scientific). The sections then floated on the surface of the water bath to prevent folding, after sectioning on the slides,

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transport in the oven 70°C–75°C for 50 minutes, for drying and dewaxing.

The H&E staining was done via the routine method, which started by the immersion in three bottles of xylene for complete dewaxing, then rehydration with a gradually increased grade of alcohol (90-80-70%), Harris hematoxylin (30 minutes), acid alcohol for destaining, eosin 1% and dehydration again by use of gradual decreased grade of slides. Except for the last steps, we use tap water to wash between the steps. Finally, the cleaning step was completed using xylene. The slides were mounted with a 24x60 glass cover by an entellan adhesion solution. The slides of each group were microscopically evaluated (Olympus BX-53 microscope) for all endocardial, myocardial, and epicardial criteria.

Assessment of myocardial hypertrophy

The thorax was opened, the heart was exposed, and the pericardium and large vessels removed. The heart was washed with normal saline and dried on filler paper. Body weight (BW) and left ventricular weight (LVW) were determined using an electronic balancing system. The interventricular septum remained a part of the left ventricle (LV). The LVW/BW ratio (g/kg) was then calculated for the assessment of macroscopic hypertrophy. Microscopic hypertrophy assessment was based on the assessment of transverse myocyte diameter, haphazard disarray of myocyte bundles, myofiber disarray, myocyte nuclear volume, inflammatory cell infiltration, and interstitial fibrosis.

Statistical analysis

Statistical analysis was performed using the Prism software. The results obtained are expressed as the mean±standard deviation. All datasets were first tested for normality using the D'Agostino and Pearson omnibus normality tests. Data were then analyzed using one-way analysis of variance with Tukey's test for post-hoc comparisons. P values <0.05 were considered statistically significant.

Results

Since apelin and Ang (I–7) alone did not have any effect on any of the measured parameters, and the results of the normal saline implanted osmotic pump did not differ from the IP-injected control group, only the results of the remaining groups were compared with the IP injected control group and the ISO groups.

ISO-induced heart failure and myocardial injury in rats

During this study, the total mortality after 10 days of ISO administration was 45%. The BW was slightly reduced by ISO treatment. In the ISO-treated rats as compared with control group, a decrease in SBP [96.7±10.7 vs. 117.6±6 mm Hg, (p=0.002)], DBP [62.7±7.4 vs. 74.3±5.6 mm Hg, (p=0.024)] (Fig. 1 and Table 1), dp/dtmax [1695±302.7 vs. 4578±274.9 mm Hg/s, (p<0.001)], dp/dtmin [−1808±352.4 vs. −3653±225.2 mm Hg/s, (p<0.001)] (Fig. 2 and Table
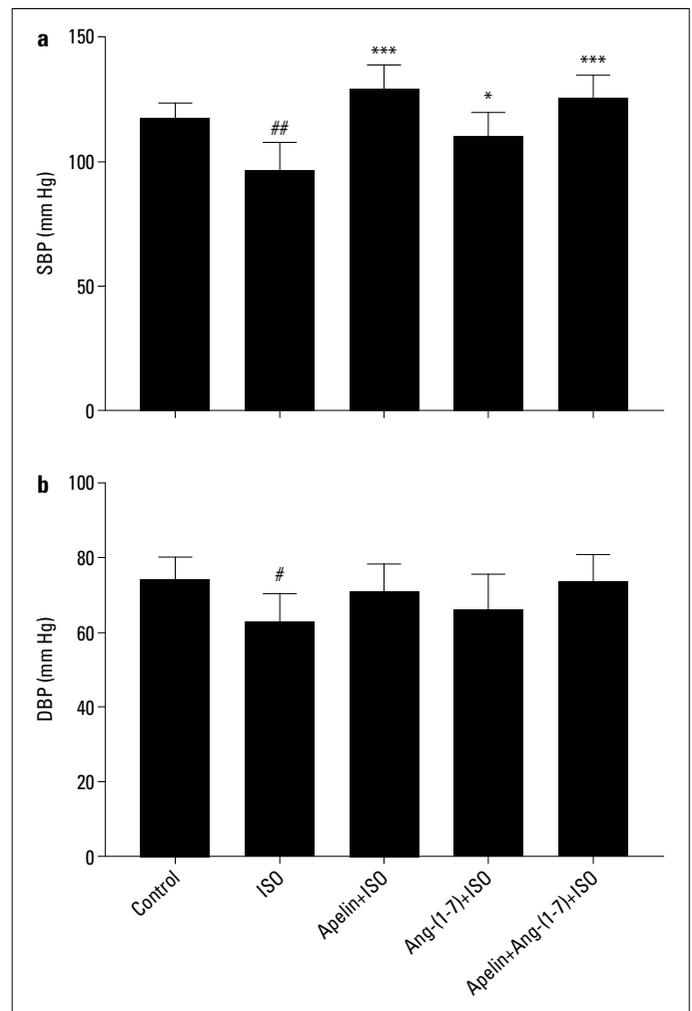

Figure 1. Systolic blood pressure (SBP) (a) and diastolic blood pressure (DBP) (b) in isoproterenol (5 mg/kg/day IP)-induced heart failure (ISO group) treated with apelin (20 µg/kg/day), angiotensin (I–7) (30 µg/kg/day), and apelin+angiotensin (1–7) compared to the control group. Results represent mean values±standard deviation of 7 animals; #P<0.05, ##P<0.01 control group; *P<0.05, ***P<0.001 vs ISO group (one-way analysis of variance followed by Tukey's test)

1), LVSP [95.4±9.8 vs. 119.3±7.7 mm Hg (p<0.001)], and an increase in LVEDP [19.3±1.6 mm Hg vs. 2.6±0.8 (p<0.001)] (Fig. 3 and Table 1) was observed, respectively. The heart rate was not significantly altered. Plasma LDH activity significantly increased (p<0.001) (Table 1). In addition, the content of the lipid peroxide product MDA increased (p<0.001) in the myocardium and plasma, and the left ventricle was significantly hypertrophied (Tables 1 and 2).

Vascular congestion as well as chronic inflammation and fibrotic foci were increased in ISO-treated hearts compared to other groups. Histopathological findings of a hypertrophic muscle are also more common than in other groups (Fig. 4 and Table 2).

Effect of apelin on cardiac function and myocardium injury induced by isoproterenol

After a 10-day treatment with apelin as compared to ISO-treated rats, an increase in SBP [129.3±9.3 vs. 96.7±10.7 mm Hg

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Table 1. Parameters of isoproterenol-induced heart failure in rats treated with apelin and angiotensin (1–7), or their combination as compared to the control group

	Control	ISO	Apelin+ISO	Ang (1–7)+ISO	Apelin+Ang (1–7)+ISO
Heart rate (bpm)	387±25	398±28	390±24	386±32	384±19
Systolic blood pressure (mm Hg)	117.6±6	96.7±10.7##	129.3±9.3***	110.4±9*	125.9±8.5***
Diastolic blood pressure (mm Hg)	74.3±5.6	62.7±7.4#	70.9±7.1	66±9.3	73.7±2.6
dp/dtmax (mm Hg/s)	4578±274.9	1695±302.7###	3310±403.5***	2699±350.8***	4322±355.3***
dp/dtmin (mm Hg/s)	-3653±225.2	-1808±352.4###	-2930±367***	-2733±317.8***	-3878±386***
LVSP (mm Hg)	119.3±7.7	95.4±9.8###	130.8±9.3***	111.8±9.3*	124.7±9.8***
LVEDP (mm Hg)	2.6±0.8	19.3±1.6###	8.2±1.1***	11.2±2.1***	3.9±1.3***
MDA content in heart (nmol/g protein)	2.93±0.29	5.42±0.97###	3.61±0.70***	3.28±0.84***	2.86±0.76***
MDA content in plasma (nmol/mL)	2.63±0.40	4.48±0.90###	3.83±0.76#	3.63±0.85	3.12±0.70*
LDH activity in plasma (IU/L)	383±49	1120±199###	842±193### *	706±199##***	508±152###***
LV-to-BW (g/kg)	2.4±0.27	3.6±0.44###	3±0.41	2.9±0.54	2.5±0.62**

Data are expressed as mean±standard deviation; n=7 for each treatment group. #P<0.05, ##P<0.01, ###P<0.01 vs. controls, respectively; *P<0.05, **P<0.01, ***P<0.01 vs. ISO, respectively; dP/dt max - left ventricular contractility; dP/dt min - left ventricular relaxation; LVSP - left ventricular systolic pressure; LVEDP - left ventricular end-diastolic pressure; MDA - malondialdehyde; LD - lactate dehydrogenase; LV-to-BW - left-ventricular-weight-to-body-weight ratio

Table 2. Histopathologic assessment of the heart in different groups

	Disruption of fiber/vesicular large nuclei with mild indented borders	Striation loss	Vascular congestion	Cell swelling change	Fatty degeneration	Chronic Inflammatory cells infiltration (mononuclear leukocyte)	Subendocardial fibrosis
Control	-	-	-	-	-	-	-
ISO	++	-	++	-	-	+++	+++
Ang (1–7)+ISO	+	-	-	-	-	+++	++
Apelin+ISO	+	-	-	-	-	++	++
Apelin+Ang (1–7)+ISO	-	-	-	-	-	±	±

The grades are intact (-), minimal (±), slight (+), moderate (++), and moderate to severe (+++)

(p<0.001)] (Fig. 1 and Table 1), dp/dt max [3310±403.5 vs. 1695±302.7 mm Hg/s (p<0.001)], dp/dt min [−2930±367 vs. −1808±352.4 mm Hg/s (p<0.001)] (Fig. 2 and Table 1), and LVSP [130.8±9.3 vs. 95.4±9.8 mm Hg (p<0.001)] and a significant decrease in LVEDP [8.2±1.1 vs. 19.3±1.6 mm Hg (p<0.001)] (Fig. 3 and Table 1) were observed, respectively. Apelin induced a non-significant increase in DBP as compared to ISO-treated rats [70.9±7.1 vs. 62.7±7.4 mm Hg (p=0.139)] (Fig. 1 and Table 1). Treatment with apelin as compared to ISO-treated rats significantly decreased the myocardial MDA content (p<0.001), as a lipid peroxidation marker. Myocardial injury, such as the myocardial LDH leakage, improved (p=0.015) (Table 1); apelin had no significant effect on the LV-to-BW ratio (Table 1). A histological analysis revealed that the treatment with apelin considerably reduced the ISO-induced myocardial infiltration of inflammatory cells and fibrosis (Fig. 4 and Table 2).

Effect of Ang (1–7) on cardiac function and myocardium injury induced by isoproterenol

Ang (1–7) treatment in ISO-administered rats led to an increase in SBP [110.4±9 vs. 96.7±10.7 mm Hg (p=0.049)] (Fig. 1 and Table 1), dp/dt (max) [2699±350.8 vs. 1695±302.7 mm Hg/s (p<0.001)], dp/dt (min) [−2733±317.8 vs. −1808±352.4 mm Hg/s, (p<0.001)] (Fig. 2 and Table 1), LVSP [111.8±9.3 vs. 95.4±9.8 mm Hg (p=0.018)], and a decrease in LVEDP [11.2±2.1 vs. 19.3±1.6 mm Hg (p<0.001)] (Fig. 3 and Table 1), respectively. Ang (1–7) induced a non-significant increase in DBP as compared to the ISO group [66±9.3 vs. 62.7±7.4 mm Hg (p=0.816)] (Fig. 1 and Table 1). Ang (1–7) also decreased MDA content in the heart tissue and plasma level of LDH in ISO-induced heart failure rats (p<0.001) (Table 1). Histological sections showed that Ang (1–7) partially decreased leukocytosis infiltration and fibrosis and cardiac hypertrophy (Fig. 4 and Table 2).
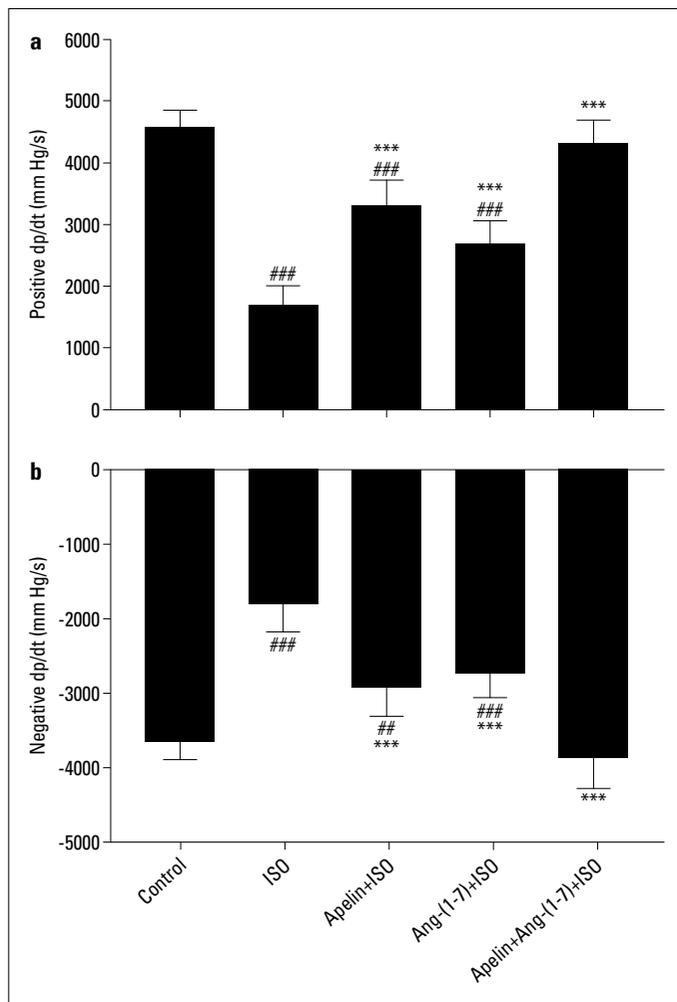
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Figure 2. +dP/dt(max) (a) and −dP/dt(max) (b) in isoproterenol (5 mg/kg/day IP)-induced heart failure (ISO group) treated with apelin (20 µg/kg/day), angiotensin (I−7) (30 µg/kg/day) and apelin+angiotensin (1−7) compared to the control group. Results represent mean values±standard deviation of 7 animals; ##P<0.01, ###P<0.01 vs. control group; ***P<0.001 vs. ISO group (one-way analysis of variance followed by Tukey's test)

Figure 3. Left ventricular systolic pressure (LVSP) (a) and left ventricular end-diastolic pressure (LVEDP) (b) in isoproterenol (5 mg/kg/day IP)-induced heart failure (ISO group) treated with apelin (20 µg/kg/day), angiotensin (I−7) (30 µg/kg/day), and apelin+angiotensin (1−7) compared to the control group. Results represent mean values±standard deviation of 7 animals; ###P<0.01 vs. control group; *P<0.05, ***P<0.001 vs. ISO group (one-way analysis of variance followed by Tukey's test)

The protective effects of co-administration of angiotensin-(1−7) and apelin on cardiac performance and myocardial injury in isoproterenol-induced heart failure in rats

In the present study, we also observed that combined treatment with apelin and Ang (1−7) in ISO-treated rats resulted in an increase in SBP [125.9±8.5 vs. 96.7±10.7 mm Hg (p<0.001)] (Fig. 1 and Table 1), dp/dt max [4322±355.3 vs. 1695±302.7 mm Hg/s (p<0.001)], dp/dt min [−3878±386 vs. −1808±352.4 mm Hg/s (p<0.001)] (Fig. 2 and Table 1), LVSP [124.7±9.8 vs. 95.4±9.8 mm Hg (p<0.001)], a decrease in LVEDP [3.9±1.3 vs. 19.3±1.6 mm Hg (p<0.001)] compared to rats treated with ISO alone (Fig. 3 and Table 1), and restored DBP to values observed in the control group [73.7±6.9 vs. 74.3±5.5 mm Hg (p=0.999)] (Fig. 1 and Table 1). This combined treatment was more effective than the individual treatment of the ISO group. Co-administration of apelin and Ang (1−7) also decreased cardiac
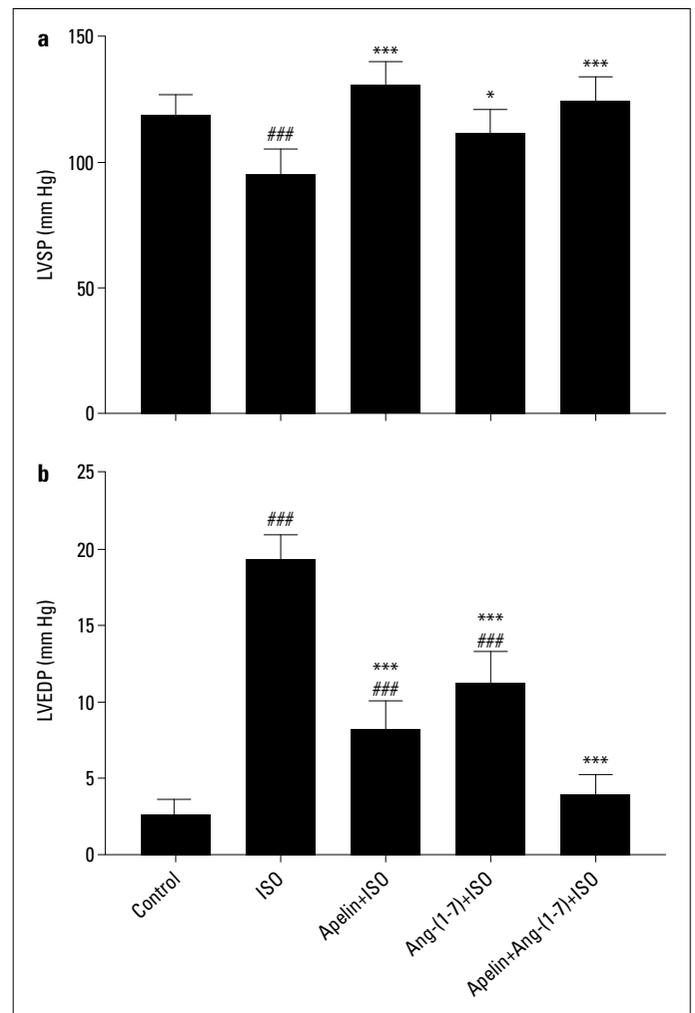
and plasma MDA and plasma LDH activity, which was higher than either apelin or Ang (1−7) therapy alone (Table 1). Apelin and Ang (1−7) alone do not have a significant effect on the ISO-induced LV-to-BW ratio, but the combined administration of apelin and Ang (1−7) resulted in a significant reduction of this ratio (Table 1). This reduction is considerably higher than that of Ang (1-7) alone. Histological sections have shown a significant decrease in chronic inflammatory and fibrotic foci in the myocardial and some subendocardial regions (Fig. 4 and Table 2).

Discussion

In this study, we observed that the co-administration of apelin and Ang (1−7) has more beneficial effects than either of these peptides in ISO-induced HF.
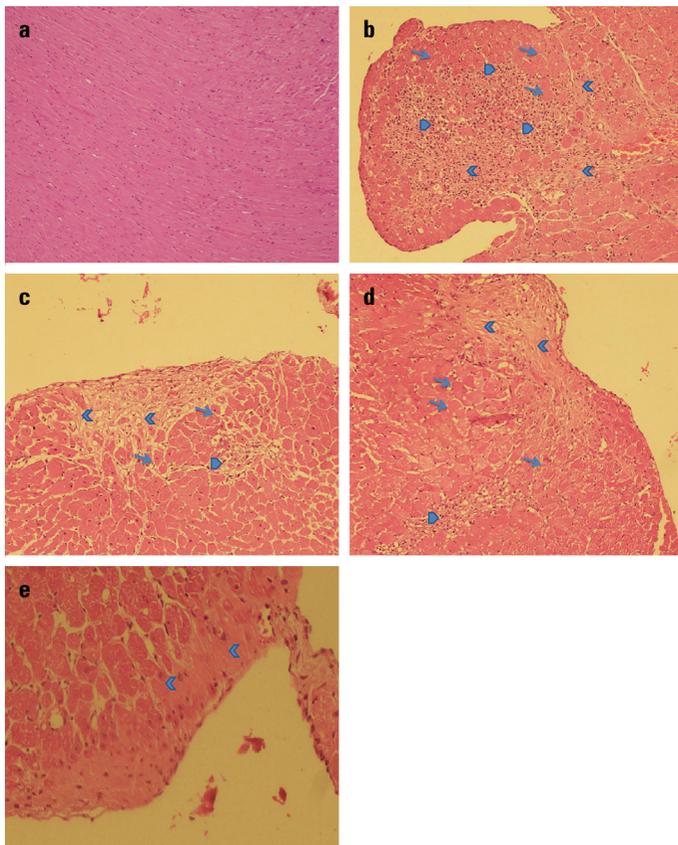
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Figure 4. Pathological data for damaged myocardium in rat cardiac tissue induced by ISO treatment (hematoxylin&eosin stain, ×200); a: cardiac tissue of the control group; b: cardiac tissue of ISO-treated rats; c: cardiac tissue of rats receiving ISO and apelin; d: cardiac tissue of rats receiving ISO and angiotensin (1–7); e: cardiac tissue in rats receiving ISO and angiotensin (1–7) and apelin. Fibrosis (◄), leukocytes infiltration (▶), hypertrophy (↘).

Supramaximal ISO doses produce severe myocardial necrosis and interstitial fibrosis (21). In our experiment, ISO-treated rats had severe HF, decreased dp/dtmax, dp/dtmin, SBP and LVESP, and increased LVEDP. Also, ISO induced myocardial injury, increased plasma LDH activity, and MDA content (Table 1). Histological assessments revealed extensive subendocardial necrosis, capillary dilation, and leukocytic infiltration (Fig. 4 and Table 2).

Co-therapy with apelin and Ang (1–7) was more effective than when they were given alone against ISO-induced HF. Co-treatment improved the ISO-induced reduction in myocardial contractile function, increased the dp/dtmax, dp/dtmin, SBP, and LVESP, and reduced the LVEDP value. Injury with myocardial ischemia, such as myocardial LDH leakage and lipid peroxidation (MDA), has been significantly reduced. Pathological reactions during the development of HF include myocyte hypertrophy and cardiac fibrosis, which is strongly influenced by Ang II signaling (15). Apelin has been shown to decrease Ang II-induced cardiac dysfunction and pathological remodeling and to antagonize endogenous Ang II-mediated heart contractility impairment in mice (15). Apelin suppresses *in vitro* cardiomyocyte cell hypertrophy

and pro-fibrotic gene expression, providing direct evidence that endogenous apelin is critical to antagonizing the Ang II–AT1R axis of cardiac muscle cells (15). It has been suggested that the gene expression of apelin and its APJ receptor have been reduced in the injured myocardium. The hemodynamic mechanism of apelin has been reported to include the activation of signaling transduction pathways, such as phosphorylation of Akt/eNOS and Erk1/2 (22).

Apelin has been shown to exert its inotropic effect by increasing myofilament sensitivity to Ca^{2+} rather than increasing intracellular Ca^{2+} transients (23). Apelin lowers blood pressure (24), but in our study, by increasing contractility, systolic pressure increased. In our research, Ang (1–7) like apelin did not have a direct effect on LV functional performance in healthy rats. On the other hand, the administration of Ang (1–7) slightly increased SBP, LVSP, dp/dt max, and dp/dt min and decreased LVEDP relative to the ISO-treated group. It has been shown that Ang (1–7) significantly increased the L-type Ca^{2+} flow in myocytes (25). Ang (1–7) may increase the left ventricular contraction and relaxation via the Ang (1–7)/Mas receptor axis, coupled with the nitric oxide/bradykinin-mediated mechanism (26). In this study, we found that the protective effect of co-administration of apelin and Ang (1–7) in a HF is significantly higher than that of apelin or Ang (1–7) alone. Increased sensitivity to calcium by apelin and an increase in intracellular calcium by Ang (1–7) can contribute to increased contractility in the combination treatment group. ISO-induced myocardial injury was mainly caused by the accumulation of free radical oxygen resulting in lipid peroxidation (high production of MDA) (11). Elevated MDA represents an increase in membrane permeability that could result in cardiomyocyte leakage of myocardial enzymes (CKMB and LDH) (27). Apelin increases the activity of superoxide dismutase and suppresses the production and release of reactive oxygen species (28). Ang (1–7) suppresses the production and release of reactive species of oxygen (29). Our findings showed that co-therapy with apelin and Ang (1–7) reduced plasma LDH production and reduced plasma and myocardium MDA production. These findings indicate that co-therapy could protect from myocardial injury by inhibiting ISO-induced lipid peroxidation.

Histological sections in the ISO-treated group revealed myocardial fibrosis and leukocytic infiltration. Either Ang (1–7) or apelin alone partially reduced these signs. Antifibrotic properties of these two peptides have already been shown (17, 30). It has also been shown that, in contrast to Ang (1–7) (31), apelin treatment reduced neutrophil infiltration (32). Reducing the LVEDP by apelin and Ang (1–7) helps to increase the blood flow to the subendocardial area and as a result, decreases the necrosis and consequently fibrosis.

Microscopic measurement of individual hypertrophic myocytes in many studies is usually based on the 1) transverse myocyte diameter; 2) haphazard disarray of myocyte bundles and myofiber disarray; and 3) increased nuclear volume simi-
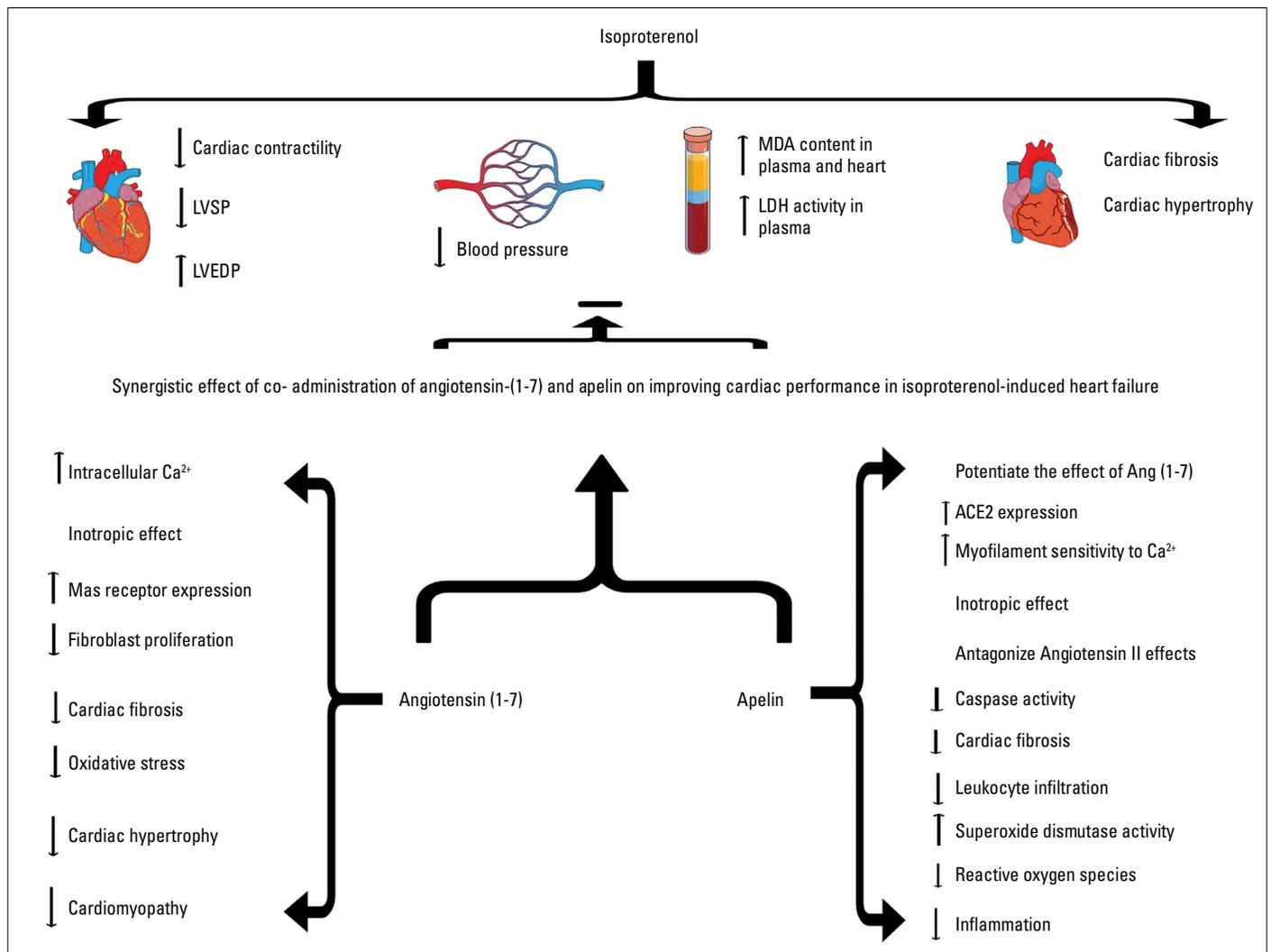
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Figure 5. Graphic abstract summarizing the impact of co-administration of angiotensin (1–7) and apelin on cardiac performance in isoproterenol-induced heart failure

lar to cell size. The nucleus becomes large vesicular with mild indentation of nuclear borders (33). In our study, the last one, i.e., large vesicular and mild indented nuclei were seen in ISO-treated rats.

Microscopic findings "between muscle fiber hypertrophy" are usually based on 1) chronic inflammatory cell infiltration, mainly mononuclear leukocytes (monocytes more than lymphocytes) and 2) interstitial fibrosis (34). In our study, both of these findings were seen in ISO-treated rats (Table 2).

The ratio of left ventricular weight to body weight increased in ISO-treated rats as a macroscopic finding of hypertrophy. Since systolic pressure decreased in ISO-treated rats, cardiac hypertrophy is not caused by increased afterload.

Administration of Ang (1–7) partially reduced the ISO effects on cardiac hypertrophy. This result is consistent with previous studies demonstrating that Ang (1–7) reduces cardiac hypertrophy (35). Interestingly, co-administration of apelin and Ang (1–7) had a greater effect on the reduction of cardiac hypertrophy than Ang (1–7) alone.

In the HF induced by ISO, the plasma level of apelin was decreased (11). There is a growing interest in the protective role of ACE2, Ang (1–7), and apelin in HF. ACE2 is a major enzyme that determines the magnitude and duration of action of apelin in the cardiovascular system (22). ACE2 can form Ang (1–7) from Ang II; on the other hand, ACE2 hydrolyzes apelin to Ang II. Therefore, the protective function of ACE2 as a negative physiological regulator of the renin–angiotensin system is limited.

Thus, targeting Ang (1–7)/apelin signaling rather than ACE2 may lead to the development of a novel therapeutic approach for patients with HF and other vascular disorders associated with cardiovascular remodeling. An overview of the effects of Ang (1–7) and apelin on cardiac performance is shown in Figure 5.

Study limitations
The limitations of the current study are that the plasma levels of Ang (1–7), apelin, and ACE2 were not measured, and a single dose of Ang (1–7) and apelin was used; further studies of different doses and long-term drug use are needed.

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Conclusion

Combined administration of apelin and Ang (1–7) improved the heart function and myocardial injury in ISO-damaged hearts. Developing a therapeutic strategy to stimulate apelin/Ang (1–7) signaling, which has positive inotropic and protective effects in the heart, would help to create a new class of cardiovascular medicine for elderly people.

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