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Research Paper

Stress-induced cardiac insufficiency relating to abnormal leptin and FKBP12.6 is ameliorated by CPU0213, an endothelin receptor antagonist, which is not affected by the CYP3A suppressing effect of erythromycin

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Abstract

Objectives Cardiac injury induced by isoprenaline produces stress. This stress can be mediated by the activated endothelin and leptin pathway; thus, the endothelin receptor antagonist CPU0213 may reverse these changes. CPU0213 is metabolized mainly by cytochrome P450 (CYP)3A, thus, erythromycin, an inhibitor of CYP3A, could affect its effects by raising its plasma levels.

Methods Forty rats were divided into five groups. Group 1 rats were normal. Group 2 rats were administered isoprenaline (1 mg/kg, s.c.) for 10 days. Groups 3, 4 and 5 were administered isoprenaline, but group 3 was given erythromycin (100 mg/kg, p.o.) alone on days six to ten, group 4 was given CPU0213 20 mg/kg (s.c.) on days six to ten, whilst group 5 received erythromycin plus CPU0213 on days six to ten. Measurements were conducted to observe changes in the haemodynamics, cardiac weight index, serum lactate dehydrogenase and creatine kinase levels, and expression of endothelin receptor A (ET_A), leptin and its OBRb receptor.

Key findings In isoprenaline-treated rats, cardiac hypertrophy and dysfunction were found. This was associated with upregulated myocardial leptin protein and OBRb receptor mRNA. Immunohistochemical assay of ET_A was upregulated, accompanied with downregulation of FKBP12.6 (calstabin 2) in isoprenaline-treated rats. These effects were significantly reversed by CPU0213. HPLC assay presented an increased plasma level of CPU0213 by erythromycin, but no change in its effects.

Conclusions CPU0213 improved isoprenaline-induced cardiomyopathy by modulating ET_A, leptin and FKBP12.6. However, erythromycin increased plasma levels but did not change its effects.

Keywords CPU0213; CYP3A; endothelin receptor antagonists; ET_A; FKBP12.6

Introduction

Cardiovascular disease is frequently exacerbated by stress, leading to an increase in morbidity and mortality. It would be desirable to be able to alleviate these stress-induced exacerbations in cardiac suffering, which in turn may lead to insights into the molecular events that could be targeted for improving cardiovascular disorder and for novel drug discovery.

Cardiac hypertrophy as the result of stress can be produced by administration of isoprenaline.[1] β-Receptor over-activation results in an excessive production of reactive oxygen species (ROS) and endothelin-1 in the myocardium. These serve as causal factors in developing cardiac failure and arrhythmia, manifesting downregulation/dissociation of FKBP12.6 (the FK506-binding protein, calstabin 2), which contributes a major role to dysfunction of the RyR2 (the ryanodine receptor type 2) macromolecule.[2] Dysfunction of cardiac performance is attributed to FKBP12.6 dissociation/downregulation, leading to calcium leak, which eventually impairs both the systolic and diastolic function. Thus, this downregulated FKBP12.6 is targeted for novel drug discovery in relieving cardiac failure and arrhythmias.[3,4] Upregulation of FKBP12.6 may serve as a surrogate to indicate re-association of FKBP12.6 with RyR2, which ends calcium leak resulting in relief of cardiac arrhythmias and improved performance.[5,6]

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Emerging evidence has shown that inflammatory mediators, including ROS, endothelin and leptin, are actively involved in the mechanisms behind cardiac hypertrophy and insufficiency, and arrhythmias. Inflammatory mediators act paracrinally, adversely affecting the myocardium, which in turn produces more cytokines, thus a vicious cycle is formed exacerbating the pathologies of cardiovascular disease.[7,8] Upregulation of the endothelin pathway is the subsequence of isoprenaline medication, which harms the vasculature by producing an excess of ROS and leptin.[2,9] Leptin, an adipocyte hormone, stimulates energy expenditure by activating the sympathetic nervous system, and it is believed to play a role in the control of appetite by acting on the hypothalamus. However, an excess of leptin and upregulation of its receptors may alter its actions from being beneficial to harmful. This is known as hyperleptinaemia. A rise in leptin may correlate with oxidative stress in the presence of profound stimulation of β-adrenoceptors leading to myocardial injury. However, an over-expression of leptin and its OBRb receptors in the myocardium may be separated from a status of cardiac dysfunction.[5,10] Thus, it is uncertain if leptin plays a role in isoprenaline-induced myocardial injury correlating to changes in the endothelin pathway and cardiac function.

CPU0213 is an endothelin receptor antagonist characterized by dual blockade on the two endothelin receptors and was investigated as a new drug produced by the China Pharmaceutical University.[11] Its profile was similar to bosentan, but was more potent. Its basic pharmacokinetic behaviour was investigated and an interaction with drug enzymes of rat hepatic microsomes was conducted to reveal that cytochrome P450 (CYP)3A was responsible for elimination of CPU0213.[12,13] The effects of CPU0213 were investigated while exposed to the enzyme-inducing drug rifampicin. It was found that it caused a reduction in plasma levels but had no effect on the heart; however; the relationship of the effects of the endothelin receptor antagonist CPU0213 in response to plasma levels has not been fully clarified.[14] It is uncertain whether erythromycin, as the inhibitor of CYP3A, by raising blood concentrations of CPU0213, augments its benefits on stress-induced cardiac injury by improving further abnormal leptin and the calcium modulating protein FKBP12.6.[15]

We hypothesized that isoprenaline developed cardiac hypertrophy and insufficiency by upregulating the endothelin A receptor (ET_A) and leptin to downregulate FKBP12.6, therefore compromising cardiac function, and the endothelin receptor antagonist may counteract these abnormalities in the myocardium. We further hypothesized that the effects produced by the endothelin receptor antagonist may not respond sensitively in parallel to fluctuation of its concentrations in plasma. The purpose of this study was to investigate if the endothelin receptor antagonist CPU0213 could reverse leptin abnormality and abnormality of its receptors, while relieving deteriorated cardiac performance on exposure to sustained isoprenaline injection. For the study a small dose of CPU0213 (20 mg/kg, s.c.) was used to see if the raised plasma levels of CPU0213 resulting from the suppression of CYP3A by erythromycin had an effect on its benefits in relieving cardiac dysfunction and related molecular changes.

Materials and Methods

Insults by isoprenaline

Forty adult male Sprague-Dawley rats (200–220 g) were housed in a controlled environment and allowed free access to tap water and food. Both animal handling and experimental procedures were approved by the University Ethic Committee, in accordance with the regulations of the Jiangsu Provincial Government. These regulations conformed to the Principles of Laboratory Animal Care published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

The rats were randomly divided into five groups of eight. Group 1 was normal. Group 2 was administered isoprenaline (1 mg/kg, s.c.) for 10 days. Group 3 was administered isoprenaline with erythromycin (100 mg/kg, p.o.) given on days six to ten. Group 4 was administered isoprenaline with CPU0213 (20 mg/kg, s.c.) given on days six to ten. Group 5 was administered isoprenaline with erythromycin given 3 h before CPU0213, on days six to ten. CPU0213 and erythromycin were suspended freshly in 0.5% carmellose sodium. Normal and isoprenaline groups received an equal volume of carmellose sodium.

RP-HPLC assay

Measurements were performed in groups 4 and 5, rats administered isoprenaline and treated with CPU0213 without and with erythromycin ($n = 8$), respectively. On the tenth day, 0, 0.5, 1, 2, 4, 6 and 8 h after administration of CPU0213 (20 mg/kg, s.c.), blood samples (0.2 ml) were collected in clean tubes from the post eye venous plexus.

Sample preparation

Dichloromethane (5 ml) was added to 0.3 ml serum containing 5 μl internal standard (CPU0204, 100 μg/ml), the samples were shaken for 5 min and centrifuged at 3500 rev/min for 15 min. The organic phase was separated and evaporated at 55°C to dryness under nitrogen. Residues were dissolved in 50 μl mobile phase and a 20-μl sample was analysed by HPLC with a LC-10AT VP, SPD-10A system (Shimadzu, Japan) and a Hypersil column (C18, 5 μm particle size, 15 cm × 4.6 mm i.d.). The mobile phase consisted of methanol–water (62/38, v/v), at a flow rate of 1 ml/min. The detection wavelength was 220 nm.

Assay validation

Linear regression was carried out based on area ratios of CPU0213 to the internal standard (CPU0204) plotted against CPU0213 concentrations. A calibration curve was prepared by adding appropriate volumes of stock solution of CPU0213 and CPU0204 into centrifuge tubes containing homogenized tissue samples. Final concentrations were in the range 0.02–4 μg/ml.

Haemodynamic changes

On the eleventh day rats were anaesthetized with urethane (1.5 mg/kg, i.p.) and a catheter (PE 50, 0.58 mm i.d., 0.965 mm o.d., Becton Dickinson, San Jose, CA, USA) was inserted into the right carotid artery. This was to enable

measurement of the haemodynamics as described by Na *et al.*,[5] including: the left ventricular systolic blood pressure (LVSP), left ventricular end diastolic blood pressure (LVEDP), maximum uprising rate of left ventricular pressure (LV+dp/dt max), and minimum declining rate of left ventricular pressure (LV−dp/dt min).

The left ventricular weight index

After completion of the haemodynamic measurements, rats were exsanguinated. The hearts were removed and dissected into the left ventricle free wall plus septum (LV) and right ventricle (RV). The left ventricular weight index was assessed as the weight of LV over body weight (LVW/BW).

Serum lactate dehydrogenase and creatine kinase

Serum levels of lactate dehydrogenase (LDH) and creatine kinase were assayed according to the instructions supplied with the assay kits (Nanjing Jiancheng Biotechnological Company, China). LDH is an enzyme for glycolysis and is an important indicator of myocardial injury. Creatine kinase exists in the cytoplasm and mitochondria of the myocardium and a leak of the enzyme into serum indicates an insult to the myocardium quantitatively.

Histological examination

Briefly, a portion of the left ventricle was submerged in 10% formalin in neutral buffered solution for immediate fixation, and 24 h later was embedded in paraffin.[10] Tissue blocks were then sectioned at a thickness of 5 μm, and stained with haematoxylin and eosin (H-E). The sections were examined under light microscopy (× 100).[16]

Immunohistochemistry of ET_A

Hearts were sliced transversely and fixed in 10% neutral formalin for over 24 h. They were then embedded in paraffin and cut into 5-μm thick sections for immunohistochemical staining. The tissue expression of ET_A was assessed immunohistochemically using rabbit polyclonal antibody (diluted 1:100, from Boster Biological Technology Ltd, Wuhan, China). Peroxidase conjugated goat anti-rabbit IgG (from Boster Biological Technology Ltd) was added to visualize the sites of binding in the myocardium (see the black spots in Figure 1a). The sections were examined using light microscopy (× 200, 10 different visual fields).[17]

There were approximately 1000 cells in each section and each group contained four sections. Image-Pro Plus software was used to calculate the percentage of the area of brown/black spots in the slides.

RT-PCR

Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and PCR primers were the same as in Na *et al.*[5] Briefly, a fraction, 5 mg RNA, was used to synthesize the first strand of cDNA using Superscript II RNase H-Reverse Transcriptase (Invitrogen) according to the manufacturer's protocol, and was used as a template in the following PCR reactions.

Western blotting

For quantitative analysis of protein levels of matrix metallopeptidase 9 (MMP-9), NADPH p67phox and phosphorylated protein kinase C (pPKC) in the heart, 100 mg cardiac mass was homogenized in 4 vol extraction buffer and centrifuged at 10 000*g* for 10 min. After determination of protein concentrations, the supernatants were stored at −20°C until use. A sample was heated to 98°C and size fractionated on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The extracted protein was transferred to a nitrocellulose membrane and blocked with nonfat milk (5%, w/v), followed by incubation with the first antibody (for FKBP12.6 from Upstate Biotechnology, New York, NY, USA; for β-actin and leptin from Boster Biological Technology Ltd) for another 1 h. After three washes, the blot was incubated with horseradish peroxidase conjugated secondary antibody IgG (goat anti-rabbit IgG for FKBP12.6, rabbit anti-goat IgG for leptin and β-actin, all the secondary antibodies from Boster Biological Technology Ltd) for an additional 1 h. Antigen was detected with a DAB kit. A linear relationship between the density of blots and the protein load was observed when 20, 40, 60, 80, and 100 μg membrane protein was used per lane.[5]

Statistical analysis

SPSS 11.5 (USA) was used to analyse the results. Data were presented as mean ± SD. One-way analysis of variance was used for statistical evaluation. The Bonferroni's multiple comparison test was used to check the significance of differences. Pharmacokinetic parameters were analysed using the 3P97 program (Chinese Society of Mathematical Pharmacology, Beijing, China). Paired-sample *t*-tests were used for the statistical comparison of means between CPU0213 with and without erythromycin groups. A probability value of $P < 0.05$ was considered statistically significant.

Results

Plasma levels of CPU0213 and the effect of erythromycin

A standard calibration curve of endothelin receptor antagonist CPU0213 (20 mg/kg, s.c.) was tested ($y = 4.99x + 0.121$ ($r = 0.9998$)) and a plasma–time curve was obtained. It was fitted with the 3P97 program for pharmacokinetic analysis and apparent parameters of pharmacokinetics were estimated by a limited number of samplings. This was to ensure that the rats were kept in a good condition to enable evaluation of the haemodynamics 24 h later. Results were in line with a one-compartment model, the maximum plasma concentration (C_{max}) was 1.48 ± 0.24 μg/ml in the control. The addition of erythromycin significantly elevated the value of C_{max}. The details are listed in Table 1 and shown in Figure 2.

Haemodynamic measurements

Compromised haemodynamics in the isoprenaline-treated group 2 were found to be significant for systolic and diastolic function. Compared with group 2, erythromycin provided a mild relief to these changes. CPU0213 (20 mg/kg, s.c.) was more effective at combating the deterioration by

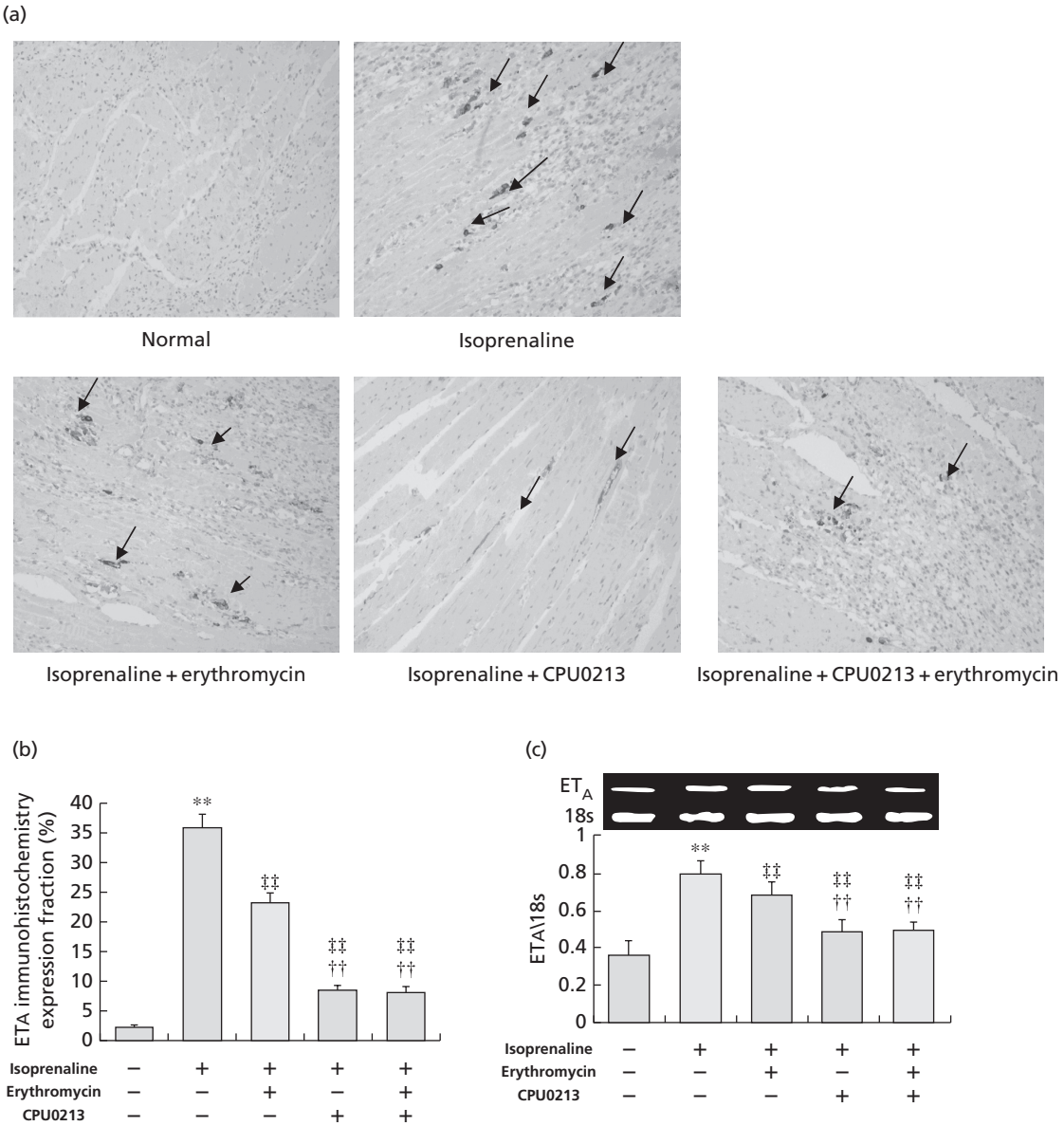

Figure 1 Effects of CPU0213 on immunohistochemistry and mRNA expression of ET_A in the myocardium of rats. Effects were observed in the presence and absence of erythromycin (100 mg/kg, p.o.). (a) Immunohistochemistry expression of ET_A. Sites of binding in the myocardium are indicated by the arrows. (b) Expression fraction of immunohistochemistry of ET_A (× 200). (c) mRNA expression of ET_A. Values are given as mean ± SD, $n = 8$, except for immunohistochemistry when $n = 4$. $^{**}P < 0.01$ compared with normal; $^{\dagger\dagger}P < 0.01$ compared with erythromycin; $^{\ddagger\ddagger}P < 0.01$ compared with isoprenaline.

isoprenaline, and CPU0213 plus erythromycin produced the same protection, despite significantly elevated plasma levels (Table 2).

Myocardial hypertrophy and inflammation

The LVW/BW of the isoprenaline-treated group increased by 45.5% ($P < 0.01$), compared with the normal group. It decreased in groups 4 and 5, the CPU0213 and CPU0213 plus erythromycin groups, but there was no change with erythromycin alone (group 3) (Table 2).

Following an injection of isoprenaline, many inflammatory cells (leucocytes) infiltrated the myocardium, as compared with the normal group. CPU0213 treatment or CPU0213 with co-medication of erythromycin resulted in the disappearance of these changes, whilst administration of erythromycin alone mildly relieved them (Table 2).

Biochemical changes

In the isoprenaline group, serum creatine kinase and LDH levels were higher ($P < 0.01$) compared with the normal group. Treatment with CPU0213 or CPU0213 plus erythromycin decreased creatine kinase and LDH levels compared with the isoprenaline group. However, no significant

Table 1 Pharmacokinetic parameters for CPU0213 following isoprenaline treatment in rats

Pharmacokinetic parameters	CPU0213 (mg/ml)	
	Without erythromycin	With erythromycin
t½ (k_e) (h)	4.31 ± 0.75	6.49 ± 2.23
T (peak) (h)	1 ± 0	2 ± 0
C_{max} (mg/l)	1.48 ± 0.24	3.17 ± 0.32**
AUC ((mg/l) h)	7.47 ± 1.75	28.03 ± 3.538**
CL (mg/kg/h/(mg/l))	2.85 ± 0.60	0.78 ± 0.13**
Vd ((mg/kg)/(mg/l))	16.97 ± 2.39	6.70 ± 1.23**

The dose of CPU0213 was 20 mg/kg (s.c.). Isoprenaline was administered for 10 days, without and with erythromycin 100 mg/kg (p.o.). AUC, area under curve; C_{max}, maximum plasma concentration; CL, clearance; t½, half life; T, time; Vd, volume of distribution. **$P < 0.01$ compared with CPU0213 alone.

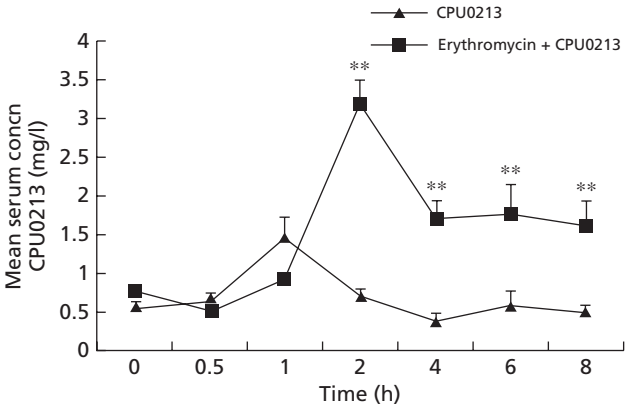

Figure 2 Mean serum concentration of CPU0213 against time in rats. CPU0213 (20 mg/kg, s.c.) was administered without or with erythromycin (100 mg/kg, p.o.). Values are given as mean ± SD (n = 8). **$P < 0.01$ compared with isoprenaline plus CPU0213 group.

difference was found between the two groups and a mild reduction was caused by erythromycin alone (Table 2).

The endothelin system

Abundance of mRNA of ET_A was estimated to show an upregulation of ET_A in the myocardium by isoprenaline, and the increment in ET_A mRNA was markedly decreased by CPU0213 or CPU0213 plus erythromycin (Figure 1). Immunohistochemical spots of ET_A in the myocardium were located mainly on the vascular endothelial cells, vascular smooth muscle, but much less in the myocardial cell membrane. In the normal left ventricle, no visible ET_A expression could be found. The increase in the number of ET_A immunohistochemical spots in the isoprenaline group declined dramatically following medication with CPU0213 or CPU0213 plus erythromycin. A mild effect on ET_A by erythromycin alone was also noted. The semi-quantitative assessment was calculated by using an imaging system and is expressed in Figure 1b. Changes of distribution of imaging ET_A in the cardiac section were in agreement with the findings of mRNA of ET_A (Figure 1).

The leptin system and FKBP12.6

Compared with the normal group, mRNA expression of OBRb and protein expression of leptin were increased in the isoprenaline group ($P < 0.01$), compared with the normal group. Reduction in mRNA expression of OBRb and leptin protein was found slightly with administration of erythromycin alone; however, a dramatic reduction was achieved respectively by either CPU0213 or CPU0213 plus erythromycin ($P < 0.01$), as compared with the isoprenaline group (Figure 3a and b).

A significant downregulation of mRNA of FKBP12.6 was elicited by isoprenaline and it was confirmed with a reduction in protein expression of FKBP12.6 by –51.1% ($P < 0.01$), compared with the normal group. An elevation of mRNA and protein of FKBP12.6 was found predominantly in the CPU0213 and CPU0213 plus erythromycin groups, as

Table 2 Effects of CPU0213 on haemodynamic and other parameters in rat hearts

Parameters	Groups				
	Normal	Isoprenaline	Erythromycin	CPU0213	CPU0213 + erythromycin
LVSP (mmHg)	161.56 ± 12.53	100.59 ± 15.16**	112.54 ± 8.87	139.94 ± 12.84†††‡‡	141.91 ± 19.22†††‡‡
LVEDP (mmHg)	4.10 ± 1.83	12.42 ± 1.52**	9.67 ± 3.01‡	7.24 ± 1.71‡‡	7.05 ± 2.33‡‡
LV+dp/dt max (mmHg/s)	8461.78 ± 2012.28	4209.92 ± 961.34**	5930.48 ± 1581.08‡	8040.00 ± 1917.79†‡‡	8116.93 ± 1160.56†††‡‡
LV–dp/dt min (mmHg/s)	–1810.03 ± 460.24	–832.41 ± 87.42**	–1232.58 ± 369.86‡	–1388.94 ± 193.58‡‡	–1400.31 ± 208.12‡‡
LVW/BW (mg/g)	2.11 ± 0.05	3.08 ± 0.30**	2.90 ± 0.16	2.78 ± 0.18‡	2.80 ± 0.24‡
LDH (U/l)	7300.93 ± 504.12	11293.98 ± 1265.64**	10750.00 ± 958.42	8851.85 ± 1204.21†††‡‡	7111.11 ± 1603.69†††‡‡¶
Creatine kinase (U/ml)	3.72 ± 0.70	6.85 ± 1.43**	6.21 ± 1.33	5.04 ± 1.02‡	4.66 ± 1.30†‡‡
Myocardial leucocyte accumulation	–	+++	++	+	+

Effects of CPU0213 (20 mg/kg, s.c.) were observed in the presence and absence of erythromycin (100 mg/kg, p.o.). LDH, lactate dehydrogenase; LV+dp/dt max, maximum uprising rate of left ventricular pressure; LV–dp/dt min, minimum declining rate of left ventricular pressure; LVEDP, left ventricular end diastolic blood pressure; LVSP, left ventricular systolic blood pressure; LVW/BW, left ventricular weight index. n = 8, except myocardial leucocyte accumulation when n = 4. Values are mean ± SD. **$P < 0.01$ compared with normal. †$P < 0.05$, ††$P < 0.01$ compared with erythromycin. ‡$P < 0.05$, ‡‡$P < 0.01$ compared with isoprenaline. ¶$P < 0.05$ compared with CPU0213.

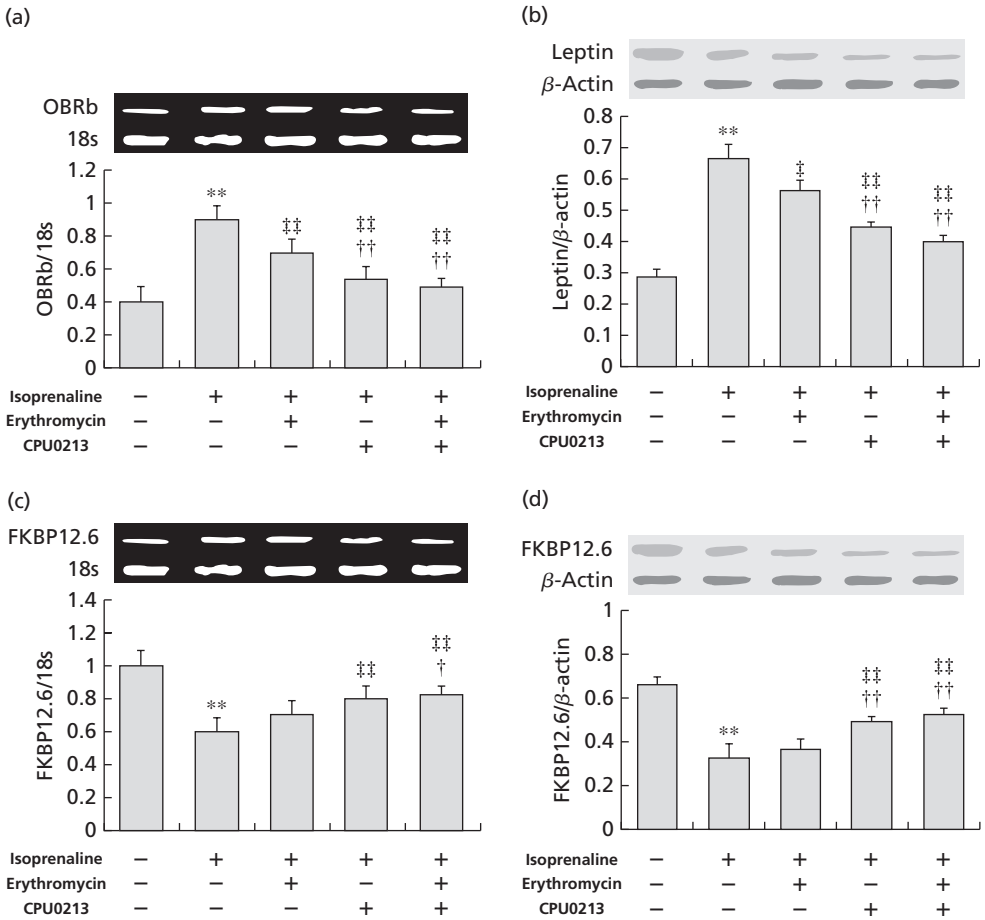

Figure 3 Effects of CPU0213 on mRNA or protein expression of OBRb, leptin and FKBP12.6 in the myocardium of rats. Effects were observed in the in the presence and absence of erythromycin (100 mg/kg, p.o.). (a) OBRb mRNA. (b) Leptin protein. (c) FKBP12.6 mRNA. (d) FKBP12.6 protein. The values shown are mean ± SD, $n = 8$. $^{**}P < 0.01$ compared with normal. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ compared with erythromycin. $^{\ddagger}P < 0.05$, $^{\ddagger\ddagger}P < 0.01$ compared with isoprenaline.

compared with the isoprenaline group, respectively; however, no effect was seen with erythromycin alone (Figure 3c and d).

Discussion

Isoprenaline administration elicited inflammatory reactions and necrosis in the myocardium, most likely by enhancing oxidative stress, which eventually leads to compromised cardiac performance.[1,18] A large amount of ROS produced is mainly from activated NADPH oxidases, which results in mitochondrial energy metabolism dysfunction that mediates cardiac hypertrophy.[8] Over-activation of β-adrenoceptors facilitates production of inflammatory factors, participating in cardiomyocyte apoptosis, necrosis and histological reconstruction/remodelling.[19,20] In this study, evidence of myocardial injury was significant, including ventricular hypertrophy, compromised cardiac performance, leucocyte accumulation and changes in expression of FKBP12.6, leptin and ET_A in the myocardium. Those effects caused by isoprenaline may have correlated with compromised endothelial nitric oxide (NO), resulting in vascular abnormality, and were relieved dramatically by the endothelin receptor antagonism of CPU0213.[9,21]

Leptin was initially believed to be an anti-obesity hormone, owing to its metabolic effects.[22] However, leptin is also an important inflammatory factor implicated in cardiovascular disease.[23,24] An excess of leptin, known as hyperleptinaemia, is associated with the upregulation of OBRb receptors which may not be beneficial, leading to myocardial injury, ventricular hypertrophy and a reduced cardiac contractility.[5,25–27] The findings in this study were in line with those in the literature, relating to NO-related Janus kinase/signal transduction and p38 mitogen-activated protein kinase signalling pathways.[28] In this study, a status of leptin resistance was found due to exposure to isoprenaline by upregulating leptin and its OBRb receptors in the myocardium, which was harmful to the heart.

Endothelin-1 is a potent vasoconstrictor and proliferator, mediated mainly through activation of ET_A and ET_B receptors.[29] Increased endogenous endothelin secretion has been found in the pathology of hypertension, coronary heart disease and heart failure; however, the nonselective endothelin receptor antagonist bosentan reduced myocardial

remodelling in coronary artery ligated-rats, accounting for an improvement of the survival rate.[30] In this study, immunohistochemistry displayed ET_A expression mainly in the vasculature and less in cardiac cells in normal rats. ET_A expression increased in the isoprenaline group, positively correlating with cardiac dysfunction and an upregulation of leptin and its receptors. Suppression of ET_A expression was confirmed by immunohistochemistry and mRNA expression in association with ameliorating detrimental effects of isoprenaline on the heart. A decline in leptin release and reversal of upregulation of OBRb receptors were in parallel with the suppressing effects on endothelin receptors, leading to alleviation of cardiac hypertrophy and compromised cardiac performance. In the process, an activation of NADPH oxidase was actively involved to link the two events, endothelin-1 and leptin. Thus, the endothelin-1 receptor and NADPH oxidase participated importantly in leptin-induced myocardial contractile abnormality.[28]

Reduction in mRNA and protein expression of FKBP12.6 caused instability of RyR2, eliciting calcium leak in diastole, which was considered as the main mechanism underlying catecholaminergic polymorphism ventricular tachycardia (CPVT) and cardiac failure.[31–33] Thus, given that downregulated FKBP12.6 contributed to cardiac failure on exposure to isoprenaline medication, a reversal of cardiac dysfunction and downregulated FKBP12.6 protein, comparable with propranolol, was confirmed by CPU0213 in this study.[34] Activation of the ET–ROS pathway affected the binding capacity of FKBP12.6 with RyR2; either endothelin-1 or ROS was sufficient to dissociate/downregulate FKBP12.6, which was attributed to PKC upregulation.[6] However, hyperphosphorylation of PKCε was actively implicated in the process and this was reversed by the endothelin receptor antagonist CPU0213.[2]

As an antibiotic, erythromycin possesses an anti-inflammatory effect of its own. In this study it presented a partial alleviation of the abnormalities resulting from isoprenaline administration, including compromised haemodynamics, accumulation of leucocytes, upregulation of the ET_A protein and mRNA and leptin system in the myocardium. This was consistent with a previous report were erythromycin relieved transient global cerebral ischaemia.[35] CPU0213 was metabolized mainly through the CYP3A enzyme and CPU0213 plus erythromycin, as expected, caused an elevation of the value of C_{max} (from 1.48 to 3.17 μg/ml, double that without erythromycin). However, the benefits of CPU0213 plus erythromycin to the myocardium were not significantly different from CPU0213 alone.[13] In this study we designed a low dose of CPU0213, 20 mg/kg (s.c.) against 30 mg/kg (s.c.) in the study by Luo *et al.*[14], in which rifampicin caused a decline from 2.795 to 0.939 μg/ml, and a reduction in plasma concentration did not affect the effects on abnormality of FKBP12.6, SERCA2a and ET_A, but a mild modulation of effects on the improved vascular activity. Effects of CPU0213 in the two studies, however, did not respond positively to a fluctuation of plasma levels by the CYP3A inhibitor erythromycin. Thus, we can speculate that the biological effects of the endothelin receptor antagonist CPU0213 may not (or may partly) have been sensitive to fluctuations in plasma levels within 1–3 μg/ml caused by modulating CYP3A activity in the clinical setting. It may improve the treatment of cardiovascular disease in the clinical setting. Patterns of drug effects in relation to plasma levels have been elucidated and discussed by Dai[36] and warrant further investigation.

Conclusions

Leptin resistance mediated cardiac hypertrophy and insufficiency caused by isoprenaline medication, and a nonselective endothelin receptor antagonist CPU0213 effectively reversed the over-active leptin pathway, and ET_A receptors, and the downregulation of FKBP12.6 and compromised cardiac performance. As a CYP3A inhibitor, erythromycin significantly elevated plasma levels of CPU0213 but did not seriously affect its pharmacological effects. However, erythromycin alone, as an antibiotic, provided a mild alleviation to stress-induced cardiac injury and dysfunction.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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