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Research Paper

CPU228, a derivative of dofetilide, relieves cardiac dysfunction by normalizing FKBP12.6, NADPH oxidase and protein kinase C ε in the myocardium

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Abstract

Objectives The aim of this study was to determine if CPU228, a derivative of dofetilide, is more effective than dofetilide in attenuating isoproterenol-induced heart failure by recovering downregulated FK506 binding protein (FKBP12.6), and suppressing oxidative stress, upregulated NADPH oxidase and protein kinase C ε (PKCε) hyperphosphorylation in the myocardium.

Methods Heart failure was induced by isoproterenol (1 mg/kg s.c. for 5 days) in male Sprague-Dawley rats. Intervention with either CPU228 or dofetilide (2 mg/kg on Days 3–5) was then conducted *in vivo* and *in vitro*.

Key findings Isoproterenol produced compromised left ventricular systolic pressure, left ventricular pressure rise (dp/dt_{max}) and fall (dp/dt_{min}), and left ventricular end-diastolic pressure, associated with oxidative stress, abnormal FKBP12.6, NADPH oxidase p67phox and PKCε in the myocardium. CPU228 was more effective in attenuating these changes than dofetilide *in vivo*. Dofetilide produced a prolonged QTc to replace a shortened one. In primary neonatal cardiomyocytes, cultured with isoproterenol and treated with either CPU228 or dofetilide at 10^{-8}, 10^{-7} and 10^{-6} mol/l, isoproterenol produced a hyperadrenergic state characterized by downregulated FKBP12.6, upregulated NADPH oxidase p67phox and PKCε *in vitro*. CPU228 was more effective than dofetilide in recovering these changes in a dose-dependent manner without a prolonged QTc.

Conclusions CPU228 was more effective than dofetilide in attenuating heart failure by normalizing isoproterenol-induced changes, including downregulation of FKBP12.6, upregulation of NADPH oxidase and PKCε hyperphosphorylation *in vivo* and *in vitro*.

Keywords CPU228; dofetilide; FKBP12.6; isoproterenol; NADPH oxidase; protein kinase C ε

Introduction

Malignant ventricular arrhythmias frequently occur in failing hearts and share the same molecular changes as those underlying heart failure. Sudden cardiac death has become one of the main causes of mortality worldwide.[1] The possible causes underlying lethal arrhythmias are complex, relating to ion channelopathy in the sarcolemma and abnormal calcium modulating proteins, such as defective ryanodine receptor type 2 (RyR2)–FK506 binding protein (FKBP12.6; calstabin 2) complex in the sarco/endoplasmic reticulum. Inherited channelopathy is caused by genetic mutations of ion channels (K^+, Na^+, Ca^{2+} channels), manifesting in long QT syndrome,[2] short QT syndrome[3] and catecholaminergic polymorphic ventricular tachyarrhythmia (CPVT), attributed to mutations of calcium-releasing protein RyR2 and calcium-holding protein calsequestrin 2[4] in the sarco/endoplasmic reticulum.[4,5] There is substantial clinical evidence that the selective class III antiarrhythmic drug (I_{Kr} blocking agent) D-sotalol failed in clinical trials because of an increase in mortality compared with the placebo.[6] Therefore, there is a real need to find less torsadogenic multi-ion channel blockers as potential candidates for the treatment of arrhythmias.[7,8] Dofetilide (Figure 1), a first generation pure class III antiarrhythmic drug, selectively suppresses I_{Kr} channels, leading to profound prolongation of action potential duration in single cardiac myocytes and QT interval in electrocardiography (ECG)

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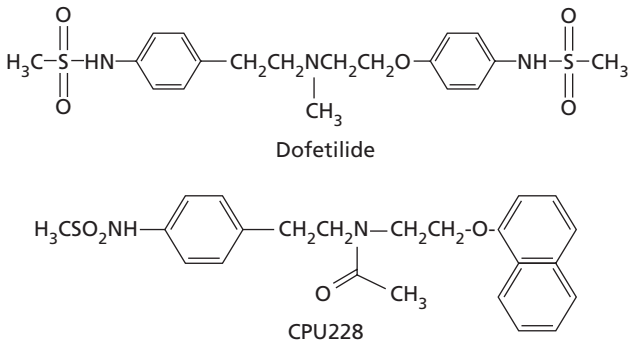

Figure 1 Chemical structure of dofetilide and its derivative CPU228

recordings, and thus an increased susceptibility to torsades de pointes (TDP) ventricular arrhythmias,[9] eventually deteriorating to ventricular fibrillation. The increased incidence of life-threatening TDP greatly limits the clinical application of dofetilide and it was ultimately withdrawn from the British market in 2004 because of its torsadogenic potential.[10] Given its unsuccessful clinical application, chemical modification of its moiety by introducing a group with L-type calcium channel blocking activity was conducted and the derivative CPU228 (Figure 1) was produced. CPU228 possesses a multi-ion channel blocking profile, with enhanced antiarrhythmic activity and a greatly reduced occurrence of TDP.[11]

Downregulated FKBP12.6 is a major factor implicated in both cardiac arrhythmias and heart failure. Emerging evidence demonstrates that downregulated/dissociated FKBP12.6 (calstabin 2) causes instability of the intracellular Ca^{2+} releasing channel (RyR2) in the sarco/endoplasmic reticulum, resulting in substantial diastole calcium leak, leading to susceptibility to arrhythmogenesis and compromised cardiac function.[12] Calcium leak, caused by downregulation of FKBP12.6 involved in the occurrence of CPVT,[4] can be reproduced by profound stimulation of β-adrenoceptors (hyperadrenergic state) following overdoses of L-thyroxine in rats, associated with an increase in bursts of ventricular fibrillation.[13] Therefore, downregulated FKBP12.6 can be taken as a substitute for diastole calcium leak, providing a molecular basis for the genesis of a failing heart and malignant arrhythmias.

Oxidative stress has emerged as a causal factor in triggering cardiac failure[14,15] and arrhythmogenesis,[16] and activation of NADPH oxidase, a major source of reactive oxygen species (ROS), is responsible for downregulation of FKBP12.6 in the presence of isoproterenol, resulting in hyperphosphorylation of protein kinase C ε (PKCε).[17] As a consequence of β-adrenoceptor activation, an increase in calcium influx appears to predominate, playing an aetiological role in worsening cardiac function and the risk for arrhythmias through downregulating FKBP12.6 and therefore diastole calcium leak.[18] Calcium antagonism, by counteracting the calcium influx, brings about a recovery of cardiac failure,[19] and improved survival of patients suffering CPVT which occurs with physical exercise is probably via attenuating the downregulation of FKBP12.6.[4]

In this study, isoproterenol treatment was used to induce a hyperadrenergic state,[12] causing vulnerability of the myocardium to impaired cardiac function based on downregulation of FKBP12.6 and escalating expression of NADPH oxidase and PKCε (hyperphosphorylated PKCε),[17,20,21] which may contribute to arrhythmogenesis.[22] We aimed to determine if CPU228 is more effective than dofetilide in relieving cardiac insufficiency by suppressing isoproterenol-induced molecular changes, including downregulated FKBP12.6 and upregulated NADPH oxidase and PKCε *in vivo* and *in vitro*.

Materials and Methods

Animals and chemicals

All experiments were approved by the ethics committee of China Pharmaceutical University and were in accordance with the Guidelines for the Care and Use of Laboratory Animals by the Science-Technology Bureau, Jiangsu Province, China.

Male Sprague-Dawley rats, 220–240 g, were provided by the Animal House of the University. CPU228 and dofetilide were obtained from the Center of New Drugs Research, China Pharmaceutical University. Isoproterenol injections were purchased from Shanghai Harvest Pharmaceutical Company (Shanghai, China).

Isoproterenol-induced cardiac dysfunction

Rats (n = 32) were randomly divided into four groups: group 1 was the normal control; group 2 was treated with isoproterenol (1 mg/kg s.c.) for 5 days; groups 3 and 4 were treated with isoproterenol (1 mg/kg s.c.) and co-treated with either CPU228 or dofetilide (2 mg/kg s.c.) for the last 3 days, respectively.

Rats were anesthetized with urethane and an intracardiac catheter was inserted via the left common carotid into the left ventricle to measure instant changes in haemodynamic parameters (left ventricular systolic pressure, left ventricular pressure rise (dp/dt_{max}) and fall (dp/dt_{min}) and left ventricular end-diastolic pressure) with a computer-aided instrument, as previously reported,[13] and the lead II ECG was recorded to measure the QTc ($QTc = QT/(R\text{-}R)^{1/2}$). Rats were then killed via carotid artery bleeding, the heart was harvested and weighed to determine the cardiac weight index (heart weight/bodyweight). A portion of cardiac tissue was processed for RT-PCR and Western blot analysis of FKBP12.6 and NADPH oxidase p67phox.

Serum biochemical measurements

Serum was collected by centrifuging blood samples to determine biochemical parameters of oxidative stress, malondialdehyde and glutathione peroxidase, after isoproterenol injection using a commercial kit according to the manufacturer's instructions (Jian Chen Biochemical Co, Nanjing, China).[13]

Cell culture with isoproterenol

Cardiac myocytes were isolated from neonatal Sprague-Dawley rats as previously described,[17] and the primary cardiomyocytes were cultured for 72 h in the following

groups: group 1 was the normal control; in group 2, isoproterenol was added (10^{-6} mol/l) to stimulate ventricular myocytes *in vitro*; in groups 3, 4 and 5, the isoproterenol (10^{-6} mol/l) treated cells were co-treated with dofetilide at 10^{-7} mol/l, 10^{-6} mol/l and 10^{-5} mol/l, respectively; in groups 6, 7 and 8, isoproterenol treated cells were co-treated with CPU228 at 10^{-7} mol/l, 10^{-6} mol/l and 10^{-5} mol/l, respectively. Cells were incubated for 24 h after adding drugs and then harvested and extracted for RT-PCR or Western blot analysis.

RT-PCR

RT-PCR was performed to determine the mRNA abundance of FKBP12.6, NADPH oxidase p67phox and PKCε as previously described.[13,17] Briefly, after extraction of total RNA from frozen cardiac tissue with Trizol reagent (Promega, Corporation, Madison, USA), RNA (5 μg) was used to synthesize the first strand of cDNA for the following PCR reactions (Eppendorf Mastercycler; Eppendorf International, Hamburg, Germany). Samples were processed in parallel with β-actin and the density of the bands was analysed using Labworks imaging acquisition and analysis software (GDS8000; Syngene, Cambridge, England).

Western blotting

For the quantitative analysis of proteins of FKBP12.6, NADPH oxidase p67phox and PKCε, a sample of cardiac tissue was homogenized and centrifuged at 10 000g for 10 min, as previously described.[13,17] Briefly, after determining the protein concentration, an aliquot of the supernatants was heated to 95°C and size fractionation was conducted via sodium dodecyl sulfate polyacrylamide gel electrophoresis. After transfer onto nitrocellulose and blocking with non-fat milk (5% wt/vol), proteins were incubated with the appropriate first antibody: polyclonal goat anti-FKBP12.6-IgG (Santa Cruz Biotechnology Inc., Santa Cruz, USA), polyclonal rabbit anti-phosphorylated-PKCε(Ser729)-IgG (Millipore Corporation, Billerica, USA), polyclonal rabbit anti-actin-IgG (Wuhan Boster Biological Technology Ltd., Wuhan, China) and NADPH oxidase p67phox (Affinity Bioreagents, Rockford, USA). Horseradish peroxidase-conjugated IgG (Wuhan Boster) was added as the second antibody. Antigen was detected with a 3,3′-diaminobenzidine (DAB) kit (Wuhan Boster). A linear relationship of the blot density with protein loaded was observed while 20, 40, 60, 80 and 100 μg of membrane protein were used per lane.

Statistical analysis

SigmaPlot 9.0 (SPSS Inc, Chicago, USA) was applied for data processing and the results were presented as means ± SD. Student's t-test was used to determine the statistical significance for differences between the two means, and one-way analysis of variance was followed to compare means between all experimental groups. Differences were considered statistically significant at a value of $P < 0.05$.

Results

Cardiac dysfunction

After isoproterenol treatment for 5 days, cardiac hypertrophy developed relative to the normal control ($P < 0.01$). Compromised cardiac function, assessed by altered parameters in systolic (left ventricular systolic pressure, left ventricular pressure rise dp/dt_{max}) and diastolic (left ventricular end-diastolic pressure, left ventricular pressure fall dp/dt_{min}) activity, was predominant ($P < 0.01$) relative to the normal control (Table 1). Both dofetilide and CPU228 significantly recovered the changes in cardiac function, but the effect of CPU228 was greater than that of dofetilide.

By monitoring the lead II ECG, a shortened QTc in the presence of isoproterenol was found ($P < 0.05$) relative to the normal control, indicating a shortened repolarization in the myocardium. This favours the initiation of cardiac arrhythmias based on the re-entrant mechanism, one of the mechanisms underlying arrhythmogenesis elicited by isoproterenol such as in CPVT.[4] A normal QTc interval is necessary to suppress the arrhythmogenetic property in the myocardium relating to the hyperadrenergic state. In this respect, dofetilide produced a prolonged QTc to replace the shortened one. In contrast, after the application of CPU228, the shortened QTc was completely eliminated and no prolongation of QTc was found, approaching the situation in the normal control (Table 1).

Malondialdehyde and glutathione peroxidase

In serum, malondialdehyde was elevated in isoproterenol treated rats, in association with a reduction in the antioxidant molecule glutathione peroxidase ($P < 0.01$) relative to the normal control. CPU228 was more effective in reversing these changes ($P < 0.01$), compared with isoproterenol untreated and dofetilide treated rats (Table 1).

FKBP12.6 and NADPH oxidase *in vivo*

Compromised cardiac function was evidenced by changes in both systolic and diastolic function due to isoproterenol treatment, confirmed at a molecular level by measuring the expression of mRNA and protein of FKBP12.6 in the myocardium. We found that downregulation of mRNA (Table 1) and protein abundance (Figure 2) was significant, indicating cardiac dysfunction. NADPH oxidase subtype p67phox was also focused on, and upregulation of both mRNA (Table 1) and protein abundance (Figure 2) was significant, ($P < 0.01$), relative to the normal control. The changes were dramatically reversed by dofetilide and CPU228. As compared with dofetilide, CPU228 was again more effective in relieving isoproterenol-induced abnormalities (Table 1; Figure 2).

FKBP12.6, NADPH oxidase and PKC *in vitro*

The effects of dofetilide and CPU228 at three different concentrations on isoproterenol-induced downregulation of FKBP12.6 in isolated cardiac myocytes were determined *in vitro*. The reversal of downregulated FKBP12.6 mRNA by dofetilide and CPU228 was significant and the magnitude of the drug effect was related to the dose used (Table 1). The

Table 1 Isoproterenol-induced changes in cardiac function and mRNA expression of the myocardium

Cardiac function	Normal control	Isoproterenol	Dofetilide	CPU228
Heart weight/bodyweight (mg/g)	4.05 ± 0.55	5.59 ± 0.799**	4.64 ± 0.50††	3.93 ± 0.29††,‡
Left ventricular systolic pressure (kPa)	18.6 ± 3.4	15.1 ± 2.0**	18.2 ± 1.6†	19.4 ± 1.4††
Left ventricular end-diastolic pressure (kPa)	0.67 ± 0.12	1.66 ± 0.42**	1.14 ± 0.28††	0.77 ± 0.16††,‡
Left ventricular dp/dt_{max} (kPa/s)	1062 ± 144	727 ± 63**	918 ± 71††	1050 ± 84††,‡
Left ventricular dp/dt_{min} (kPa/s)	821 ± 70	575 ± 60**	690 ± 47††	801 ± 85††,‡‡
QTc (ms)	52.6 ± 8.3	39.2 ± 6.5**	78.2 ± 10.1††	53.3 ± 12.3††,‡‡
Malondialdehyde (nmol/ml)	0.84 ± 0.13	2.03 ± 0.55**	1.35 ± 0.32††	0.90 ± 0.21††,‡
Glutathione peroxidase (U)	1043 ± 137	691 ± 80**	821 ± 89†	950 ± 69††,‡
In-vivo study				
$p67_{phox}/\beta$-actin mRNA	0.30 ± 0.06	0.67 ± 0.05**	0.54 ± 0.07††	0.40 ± 0.07††,‡‡
FKBP12.6/β-actin mRNA	0.52 ± 0.06	0.25 ± 0.03**	0.33 ± 0.05†	0.45 ± 0.10††,‡‡
In-vitro study			Dofetilide (mol/l)	CPU228 (mol/l)
$p67_{phox}/\beta$-actin mRNA	0.27 ± 0.05	0.68 ± 0.08**	10^{-7}, 0.67 ± 0.06	10^{-7}, 0.56 ± 0.09
			10^{-6}, 0.50 ± 0.04††	10^{-6}, 0.32 ± 0.03††,‡‡
			10^{-5}, 0.41 ± 0.07††	10^{-5}, 0.25 ± 0.07††,‡‡
FKBP12.6/β-actin mRNA	0.68 ± 0.07	0.23 ± 0.04**	10^{-7}, 0.26 ± 0.03	10^{-7}, 0.32 ± 0.04
			10^{-6}, 0.34 ± 0.07††	10^{-6}, 0.50 ± 0.07††,‡‡
			10^{-5}, 0.51 ± 0.04††	10^{-5}, 0.63 ± 0.04††,‡‡
Protein kinase C ε/β-actin mRNA	0.30 ± 0.07	0.73 ± 0.03**	10^{-7}, 0.75 ± 0.08	10^{-7}, 0.70 ± 0.06
			10^{-6}, 0.55 ± 0.04††	10^{-6}, 0.43 ± 0.05††,‡‡
			10^{-5}, 0.42 ± 0.07††	10^{-5}, 0.31 ± 0.04††,‡‡

Cardiac function in rats *in vivo* and *in vitro* was improved by dofetilide and CPU228. CPU228 had a greater effect than dofetilide in normalizing the changes induced by isoproterenol. Data are mean ± SD, $n = 8$. FKBP12.6, FK506 binding protein. $^{**}P < 0.01$, significantly different compared with the normal control; $^{†}P < 0.05$, $^{††}P < 0.01$, significantly different compared with the isoproterenol; $^{‡}P < 0.05$, $^{‡‡}P < 0.01$, significantly different compared with dofetilide.

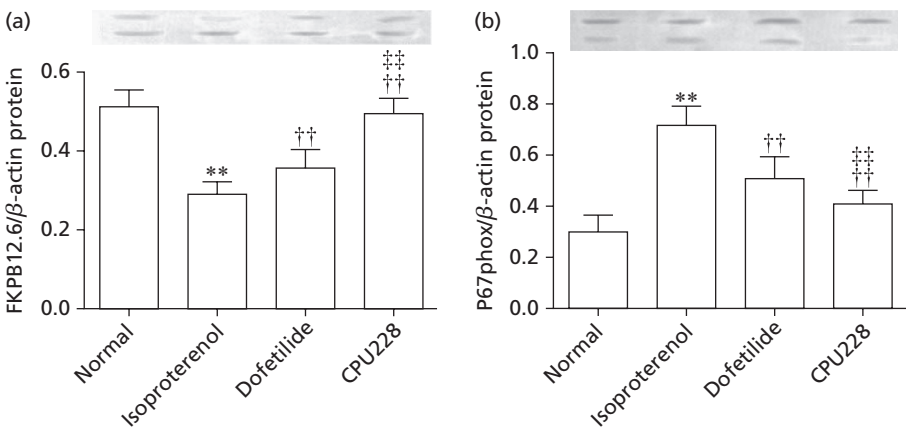

Figure 2 Effects of dofetilide and CPU228 on isoproterenol-induced changes in cardiac function *in vivo*. In the presence of isoproterenol, FK506 binding protein (FKBP12.6) was significantly decreased (a). In contrast, protein expression of NADPH oxidase p67phox was significantly upregulated (b). CPU228 was more effective than dofetilide in normalizing these changes. Values are mean ± SD, $n = 8$. $^{**}P < 0.01$ significantly different compared with the normal control; $^{††}P < 0.01$ significantly different compared with isoproterenol; $^{‡‡}P < 0.01$ significantly different compared with dofetilide.

response of the depressed protein abundance of FKBP12.6 to the two compounds was dose dependent (Figure 3), almost the same as the changes in mRNA levels. The ability of CPU228 to elevate the depressed FKBP12.6 protein *in vitro* was greater than that of dofetilide (Table 1; Figure 3). In the myocyte culture with isoproterenol, mRNA expression of NADPH oxidase p67phox and PKCε was significantly upregulated, and was suppressed dose-dependently by dofetilide and CPU228 (Table 1). The upregulated protein of NADPH oxidase p67phox and hyperphosphorylated PKCε responded to three doses of the two compounds, and the responses to CPU228 were greater than those of dofetilide (Table 1; Figure 3).

Discussion

In response to isoproterenol, both the intact rat hearts and isolated cardiomyocytes presented downregulation of FKBP12.6, a key step in impairing cardiac function by disturbing the calcium releasing ability of RyR2 and leading to calcium leak.[1,20,21] Isoproterenol raises the amount of cAMP and protein kinase A (PKA), which in turn adversely

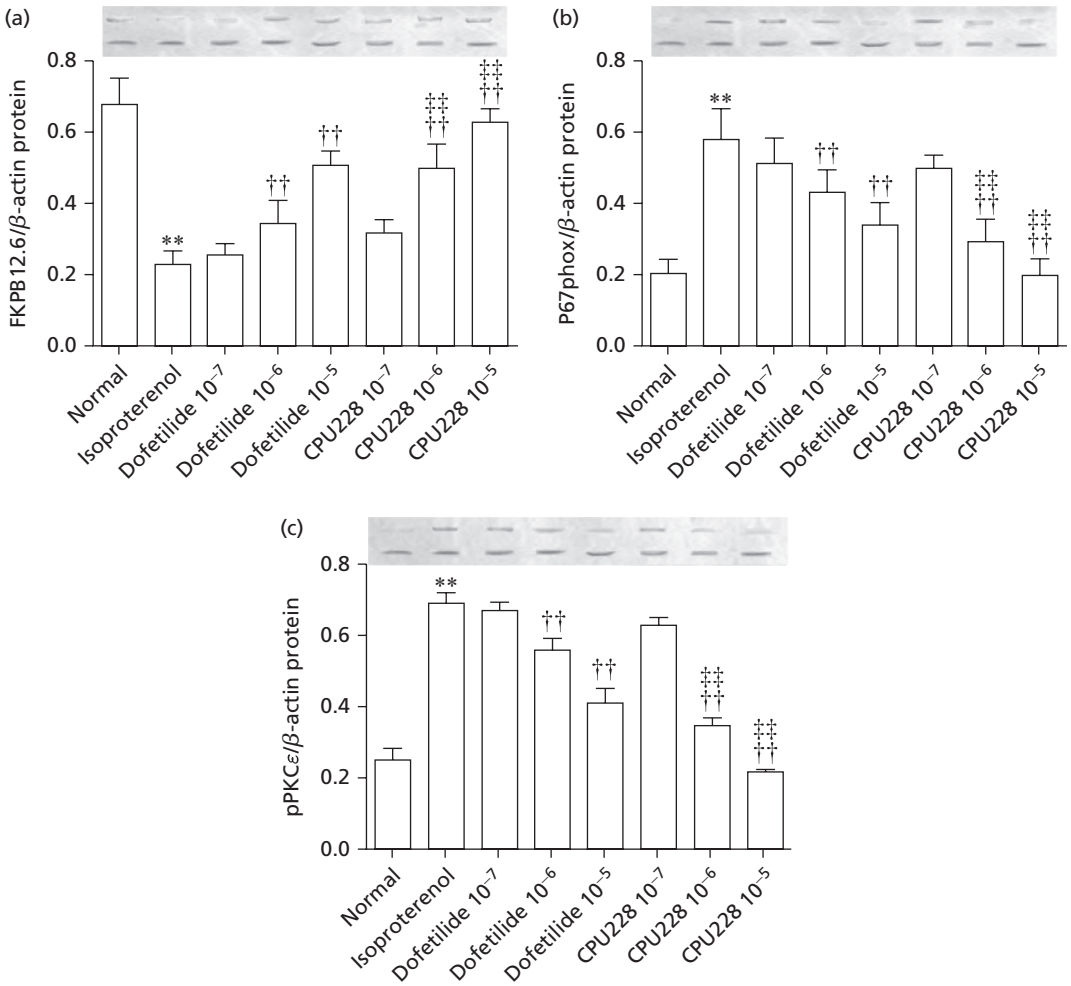

Figure 3 Effects of dofetilide and CPU228 on isoproterenol-induced changes in cardiac function *in vitro*. Primary myocytes were incubated with isoproterenol and there was significant downregulation of FK506 binding protein (FKBP12.6) protein expression (a), upregulation of NADPH oxidase p67phox protein (b) and hyperphosphorylated protein kinase C ε (pPKCε) (c). CPU228 was more effective than dofetilide in normalizing these changes. Values are mean ± SD, $n = 6$. $^{**}P < 0.01$ significantly different compared with normal; $^{\dagger\dagger}P < 0.01$ significantly different compared with isoproterenol; $^{\ddagger\ddagger}P < 0.01$ significantly different compared with dofetilide.

affects RyR2 phosphorylation, consequently inducing cardiac failure[21] and an increase in the risk of sudden cardiac death.[22] Based on accumulated data, RyR2 phosphorylation is caused not only by PKA resulting from β-adrenergic stimulation,[23] but also calmodulin-dependent protein kinase II,[24] possibly relevant to the activation of PKC. In this study, isoproterenol reproduced cardiac insufficiency attributed to downregulated FKBP12.6 via profound activation of β-adrenoceptors. Treatment with dofetilide and CPU228 restored normal expression of FKBP12.6 at the sarco/endoplasmic reticulum, alleviating cardiac dysfunction in isoproterenol treated rats.

Abnormal FKBP12.6 expression on exposure to isoproterenol is probably linked with elevated expression of NADPH oxidase, which manifests in overt oxidative stress in the myocardium.[17] Oxidative stress has been substantially implicated as an important causal factor inducing cardiac dysfunction by activating NADPH oxidase,[15,16] and serves in downstream events involved in β-adrenoceptor stimulation by isoproterenol.[25] Heart failure is a multifactorial syndrome caused by inflammation, ROS, endothelial dysfunction, and a reduced energy supply in the myocardium.[26] Upregulated NADPH oxidase plays a critical role in cardiac dysfunction, and was more markedly suppressed by CPU228 than dofetilide. An involvement of excess oxidants in heart failure appears to be in line with a recovery in mitochondrial oxidative capacity leading to a return in cardiac performance.[27]

A prolonged QT indicates an increased susceptibility of the heart to the development of TDP, a dangerous ventricular tachyarrhythmia frequently found in patients on selective I_{Kr} blockers.[28,29] Evidence suggests that arrhythmias may be related to a rise in Ca^{2+} waves released from the sarcoplasmic reticulum, propagating along cardiac cells. Patients with a mutation in RyR2 suffering from CPVT may present a decreased Ca^{2+} threshold at the sarco/endoplasmic reticulum

to deliver Ca^{2+} release waves, and these can also be seen in failing hearts.[30] With downregulation of FKBP12.6, calcium leak occurs, leaving the calcium store of the sarco/endoplasmic reticulum depleted. This phenomenon happens with isoproterenol treatment,[18] with less calcium released at the systole and a decrease in Ca^{2+}-excitation coupling of the cardiomyocytes. A decreased threshold of calcium wave production is attributed to the presence of β-adrenoceptor stimulation. In this respect, PKCε initiates phosphorylation of the RyR2 macromolecule at sites other than those phosphorylated by PKA,[31] and PKC has been considered as a target for heart failure[32] and broadly acting PKC inhibitors such as ruboxistaurin are likely to become a novel therapeutic approach in treating heart failure.[33] In agreement with these findings, we demonstrated in the present study that CPU228 suppressed PKCε hyperphosphorylation, to be beneficial for ameliorating cardiac dysfunction in isoproterenol treated rats.

Hyperphosphorylation of RyR2 on isoproterenol exposure produces abnormalities resembling those in patients with CPVT by mutation,[34] in association with upregulated NADPH oxidase and consequently a decrease in glutathione peroxidase and an increase in malondialdehyde. This may indicate that a common mechanism in its pathology provides an intimate link between cardiac failure and arrhythmogenesis.

Torsadogeneity, a potential for a dangerous tachyventricular arrhythmia, is linked with prolonged QT in patients with failing hearts.[9] Although, in this study, some relief to the isoproterenol-induced failing heart was achieved with dofetilide treatment, a prolonged QTc was produced by its selective inhibition on I_{Kr}, with the potential risk of TDP. In contrast, CPU228 significantly corrected the shortened QTc, but produced no prolonged QTc, suggesting that CPU228 was superior to dofetilide in this regard, in line with our previous findings.[11]

An accumulation of calcium in myocardial cells correlates with isoproterenol-induced mitochondrial dysfunction, manifesting the activation of NADPH oxidase and the consequent production of ROS mainly through abnormal electron transport chain in the affected mitochondria, resulting in damage to the myocardium.[35,36] It was interesting to find that dofetilide possessed an antioxidant role in combating cardiac abnormalities caused by isoproterenol treatment. The antioxidant activity of CPU228 was more effective, partly stemming from a calcium influx blocking effect in the moiety following chemical modification. CPU228 significantly reduced the generation of ROS, cutting off the cascade of ischaemic reactions in the myocardium. CPU228, possessing potent antioxidant activity, contributes a critical role in elevating the depressed FKBP12.6,[17] and abolishing diastole calcium leak.[11] Thus, we suggest that a reduction in myocardial Ca^{2+} levels by CPU228, accounting for its improvement of isoproterenol-induced heart failure, is relevant to a reduction of Ca^{2+} in the mitochondria,[36] which in turn further diminishes oxidative stress in the myocardium. Limitation of calcium influx has been proven to be effective in prolonging the life span of patients with a type of malignant ventricular tachyarrhythmia, CPVT.[34,37] The findings of the present study offer evidence of calcium antagonism in relieving isoproterenol-induced changes, including downregulated FKBP12.6, upregulated NADPH oxidase and PKCε hyperphosphorylation, which contribute to CPVT. Indeed, verapamil, a compound with calcium antagonistic effects, has found a place in dealing with CPVT in clinical settings.[38]

An improvement of cardiac dysfunction caused by profound stimulation of the β-adrenoceptors is markedly revealed by dofetilide. There are several reasons to explain this interesting therapeutic outcome. Dofetilide may be able to reverse the apoptotic death and necrosis caused by isoproterenol. Heart failure is a pleiotropic disorder that involves both apoptosis and necrotic loss of myocytes in association with dysregulated Ca^{2+} handling and β-adrenergic receptor stimulation.[39] The antioxidant effect of dofetilide reverses the apoptotic death and necrosis caused by isoproterenol treatment. Oxidants cause more calcium influx, being an important causal factor in heart failure.[35] That dofetilide enhances cardiac contractility through its stimulating activity of Na^{+}–Ca^{2+}exchangers[40] may be another reason for its ability to improve a failing heart. Convincingly, an additional Ca^{2+} influx blockade by CPU228 manifested a more promising effect on the molecular basis of isoproterenol-induced cardiac insufficiency and isoproterenol-related arrhythmogenesis such as in CPVT.

Conclusions

In this study, CPU228, as a complex class III antiarrhythmic drug capable of inhibiting both I_{Kr} and $I_{Ca.L}$ simultaneously,[11] was more effective than dofetilide at correcting the isoproterenol-induced abnormal expression of the calcium handling protein FKBP12.6, NADPH oxidase and PKCε signalling pathway. Dofetilide had a mild effect in relieving heart failure relating to its antioxidant activity, but with an unacceptable QTc prolonging effect. Its derivative CPU228 was more effective in attenuating heart failure but without QTc prolongation. Thus, CPU228 may find a role in clinical practice in dealing with heart failure and arrhythmogenesis relating to β-adrenoceptor activation.

Declarations

Conflict of interest

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

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