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Effect of carvedilol against myocardial injury due to ischemia–reperfusion of the brain in rats

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ABSTRACT

We have previously reported the mechanism behind the myocardial injury and the activation of autonomic nervous system during the ischemia–reperfusion (IR) of the rat brain. This study was planned to investigate the effect of carvedilol, a β-blocker, in improving the myocardial injury caused by IR of the rat brain. We have used a whole cerebral IR model in rats by clamping both the right and left common carotid arteries. Rats were divided into five groups; Sham surgery group (Group-Sham), carvedilol treatment before ischemia group (Group-Is + C), vehicle control group (Group-Is + V), carvedilol treatment before reperfusion group (Group-Re + C) and the vehicle control group (Group-Re + V). We have measured the blood pressure and heart rate via a catheter, myocardial tissue β1-adrenaline receptor (β1-AR) levels, phosphor-p38 mitogen-activated protein kinase (p-p38 MAPK) signaling factor, malondialdehyde (MDA), and apoptosis (TUNEL assay and expression of caspase-7 protein). The results indicated that the increased expressions of β1-AR, p-p38 MAPK, caspase-7, apoptotic cells and MDA level in the myocardial tissue due to brain ischemia–reperfusion were significantly reduced by carvedilol treatment. From these observations we can suggest that, with the advantage of its antioxidant and β blocking action, carvedilol had played the improvement of myocardial injury in ischemia–reperfusion of the brain.

1. Introduction

The relevance between the brain and the heart is known and discussed for a long time. Clinically, brain injury leads to changes in autonomic function and increased catecholamines in the blood causing cardiac tissue injury and further progressing to arrhythmia and cardiac malfunction (Davis and Natelson, 1993; White et al., 1995). Several animal experiments using severe brain injury animal models also reported the activation of sympathetic nervous system causing pathological changes including abnormal hemodynamics of the heart (Min et al., 2009; Ozisik et al., 2004; Shanlin et al., 1988; Shivalkar et al., 1993). In in vitro experiments, rat cardiomyocyte apoptosis was induced by treatment with a β-adrenergic receptor agonist isoproterenol, and this apoptosis was amplified by a β2 selective blocker ICI 118.551, and suppressed by β1 selective blocker CGP 20712A (Communal et al., 1999), suggesting that this apoptosis was mediated via β1 adrenergic receptors. The functional relationship between the brain and heart is vital in cases where the new born infants were exposed to cerebral hypoxia/ischemia due to problems with delivery or post-delivery respiratory management (Armstead et al., 2010).

β-adrenergic receptor blockers are used in the treatment of hypertension, tachyarrythmia and ischemic heart diseases and have been proved to reduce morbidity and improve survival in patients with cardiac disease (Du et al., 2014). Carvedilol, a nonselective β-blocker, is one of the widely used therapeutic agents for the treatment of cardiovascular ailments. In recent years, it has attracted attention because of the reports showing inhibitory effects on oxidative stress and apoptosis in laboratory animals (Arozal et al., 2011; Sahu et al., 2014; Xu et al., 2014).

Based on these above discussed evidences, we hypothesized that carvedilol treatment would protect the rats from brain ischemia–reperfusion (I–R) induced cardiac injury.

2. Materials and methods

2.1. Animal

Male Sprague–Dawley (SD) rats weighing 500–600 g were divided into the following groups; Sham surgery group (Group-Sham, n = 4); pre-ischemic carvedilol treatment group (Group-Is + C, n = 4) and its vehicle control group (Group-Is + V, n = 4), before reperfusion

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carvedilol treatment group (Group-Re + C, n = 4) and its vehicle control group (Group-Re + V, n = 4). Anesthesia state was maintained by halothane inhalation to measure the mean blood pressure (mBP) and heart rate (HR) from the femoral artery. Drug administration and blood sampling were done by jugular vein catheterization. I–R model was created using the method by Ichikawa and Konishi (2002). Briefly, both the right and left common carotid arteries were clipped for 85 min to induce cerebral ischemia (Is), followed by their removal to resume the perfusion (Re). After 45 min of clip removal, the rats were sacrificed to collect the total heart. Carvedilol (2 mg/kg i.v.) administration was done either 5 min before ischemia (Group-Is + C) or 5 min before reperfusion (Group-Re + C). The same amount of vehicle was injected to their control groups at similar time duration.

2.2. Measurement of the average blood pressure and heart rate

To measure the BP and HR, we have inserted the catheter (AD Instruments Castle Hill, NSW, Australia) into the femoral artery. Using a Power System, mBP and HR were measured at various time points such as 5 min before Is (−5 min), just after clipping the carotid arteries (0 min), just before reperfusion (84 min) and during the end of the study (45 min).

2.3. TTC staining of brain sections

TTC (2,3,5-triphenyltetrazolium chloride; Wako, Osaka, Japan) staining of brain slices was performed as previously described (Ichikawa and Konishi, 2002). The brains removed were frozen for 10 min at −20 °C, and then six coronal brain sections, 3 mm thick, were incubated in phosphate buffered saline containing 1% TTC at 37 °C for 10 min, then, kept in 10% buffered formalin. The infarct area of each section was traced and percentage of infarction was measured using an ImageJ analysis system.

2.4. Measurement of malondialdehyde (MDA) by TBARS method

To 50 mg of the heart tissue, 1 ml of normal saline was added and homogenized using a Polytron homogenizer (KINEMATICA, Switzerland). Then the homogenates were centrifuged at 3000 rpm and 4 °C for 10 min. The final supernatant heart tissue extracts were analyzed using the TBARS Assay Kit (ZeptoMetrix Corporation, New York, USA) to measure the concentration of MDA as per the manufacturer's protocol.

2.5. Protein expression analysis by Western blotting

The excised heart tissues were pulverized by a Polytron homogenizer in lysis buffer (50 mM Tris hydrochloride, 200 nM sodium chloride, 20 mM sodium fluoride, 1.0 mM sodium vanadate, 1.0 mM dithiothreitol) and then centrifuged at 3000 rpm, 4 °C for 10 min. The supernatants were analyzed for their total protein content using bicinchoninic acid method. Then the loading samples were prepared (Arumugam et al., 2012a) and used for further experiment. Briefly, the proteins were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to a nitrocellulose membrane. The membranes were blocked with 5% skim milk in tris-buffered saline with tween (TBS-T, 20 nM Tris, 137 mM NaCl, 0.5% Tween20) at room temperature, and reacted overnight with the primary antibody in a cold room. Then the membranes were washed with TBS-T and further incubated with the secondary antibody to detect the binding sites of the primary antibodies using ECL plus (Amersham). Following primary antibodies were used in the present study; caspase-7, phosphor-p38 mitogen-activated protein kinase (MAPK) and p38 MAPK (Cell Signaling Technology, Tokyo, Japan) and β1-adrenergic receptor (β1-(AR), Santa Cruz Biotechnology, Inc., TX, USA) antibodies.

Table 1
Effect of carvedilol on heart rate (bpm) in cerebral ischemia–reperfusion induced rats.

	Sham	Is + V	Is + C	Re + V	Re + C
−5 min	300 ± 20.49	329 ± 14.96	309 ± 12.96	293 ± 12.9	314 ± 19.04
0 min	298 ± 19.62	346 ± 25.24	270 ± 24.76	285 ± 12.98	320 ± 23.56
84 min	294 ± 29.15	324 ± 24.11	269 ± 25.13	368 ± 20.4	287 ± 6.29
45 min	289 ± 28.0	384 ± 10.01	284 ± 23.72	360 ± 19.74	248 ± 15.67

Data are expressed as mean ± SE (n = 4). Is + V and Is + C, rats underwent ischemia–reperfusion and treated with vehicle or carvedilol respectively before ischemia, Re + V and Re + C, rats underwent ischemia–reperfusion and treated with vehicle or carvedilol respectively before reperfusion.

Table 2
Effect of carvedilol on mean blood pressure (mm Hg) in cerebral ischemia–reperfusion induced rats.

	Sham	Is + V	Is + C	Re + V	Re + C
−5 min	77 ± 7.53	95 ± 6.9	96 ± 4.26	81 ± 9.29	97 ± 7.63
0 min	81 ± 6.92	116 ± 12.53	87 ± 8.02	93 ± 12.96	104 ± 13.91
84 min	82 ± 8.08	98 ± 7.13	71 ± 5.11	99 ± 8.3	69 ± 9.43
45 min	98 ± 3.75	112 ± 9.03	84 ± 5.79	108 ± 3.0	79 ± 2.72

Data are expressed as mean ± SE (n = 4). Is + V and Is + C, rats underwent ischemia–reperfusion and treated with vehicle or carvedilol respectively before ischemia, Re + V and Re + C, rats underwent ischemia–reperfusion and treated with vehicle or carvedilol respectively before reperfusion.

2.6. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay

Paraffin-embedded sections of the ventricle were deparaffinized and incubated with 20 mg/ml proteinase K. Then the slides were washed with PBS and reacted for 5 min with 3% H_2O_2 to block the endogenous peroxidase. Further staining was carried out using TUNEL staining kit (Takara Bio, Shiga, Japan) as per the manufacturer's protocol. The sealed sections were observed under a light microscope to identify the apoptotic cells (Arumugam et al., 2012b).

2.7. Statistical analysis

The statistical analysis was carried out using GraphPad Prism software and the results are expressed as mean ± standard error (SE), using Student *t*-test. P values less than 0.05 are considered statistically significant.

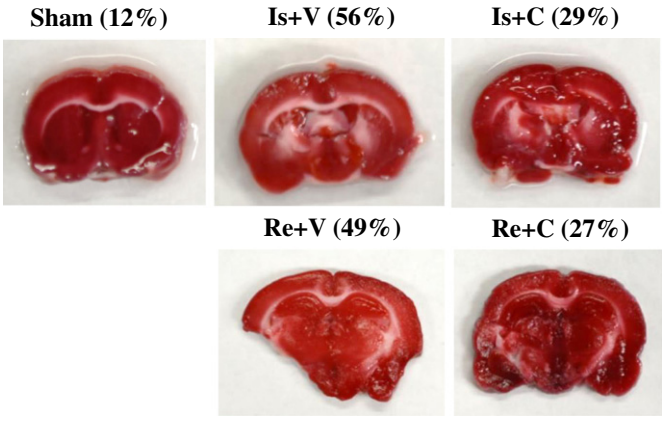

Fig. 1. Ischemia–reperfusion injury of brain using TTC staining. TTC staining showed a white infarction in the brain of various groups of rats. The percentage infarction area is given in the brackets. Is + V and Is + C; Re + V and Re + C, rats underwent ischemia–reperfusion and treated with vehicle or carvedilol (2 mg/kg i.v.) respectively before ischemia or reperfusion.