PARP inhibition delays transition of hypertensive cardiopathy to heart failure in spontaneously hypertensive rats

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Aims Oxidative stress followed by abnormal signalling can play a critical role in the development of long-term, high blood pressure-induced cardiac remodelling in heart failure (HF). Since oxidative stress-induced poly(ADP-ribose)polymerase (PARP) activation and cell death have been observed in several experimental models, we investigated the possibility that inhibition of nuclear PARP improves cardiac performance and delays transition from hypertensive cardiopathy to HF in a spontaneously hypertensive rat (SHR) model of HF.

Methods and results SHRs were divided into two groups: one received no treatment (SHR-C) and the other (SHR-L) received 5 mg/kg/day L-2286 (PARP-inhibitor) orally for 46 weeks. A third group was a normotensive age-matched control group (CFY) and a fourth was a normotensive age-matched group receiving L-2286 treatment 5 mg/kg/day (CFY+L). At the beginning of the study, systolic function was similar in both CFY and SHR groups. In the SHR-C group at the end of the study, eccentric hypertrophy with poor left ventricular (LV) systolic function was observed, while PARP inhibitor treatment preserved systolic LV function. Due to these favourable changes, the survival rate of SHRs was significantly improved (P < 0.01) by the administration of the PARP inhibitor (L-2286). The PARP inhibitor used did not affect the elevated blood pressure of SHR rats, but moderated the level of plasma-BNP (P < 0.01) and favourably influenced all the measured gravimetric parameters (P < 0.05) and the extent of myocardial fibrosis (P < 0.05). The inhibition of PARP increased the phosphorylation of Akt-1/GSK-3β (P < 0.01), ERK 1/2 (P < 0.01), and PKC ε (P < 0.01), and decreased the phosphorylation of JNK (P < 0.05), p-38 MAPK (P < 0.01), PKC pan βII and PKC γ/λ (P < 0.01), and PKC α/βII and η (P < 0.05).

Conclusion These data demonstrate that chronic inhibition of PARP induces long-term favourable changes in the most important signalling pathways related to oxidative stress. PARP inhibition also prevents remodelling, preserves systolic function, and delays transition of hypertensive cardiopathy to HF in SHRs.

1. Introduction

The development of left ventricular hypertrophy (LVH) is an adaptive response to pressure overload and is often an initial step in the process leading to congestive heart failure (HF).1 Hypertensive heart disease involves alterations in cardiac structure and function, including hypertrophy of myocytes, ischaemia, and interstitial fibrosis, leading eventually to impaired myocardial performance and coronary haemodynamics and apoptosis. It has been well established that pathogenesis of heart disease associated with hypertension and ageing involves all components of the heart, including myocytes and non-myocytic cells, such as fibroblasts and endothelial cells, extracellular matrix proteins, fibrillar collagen, and coronary vessels.2 It is well established that hypertension induces oxidative stress, but major sources of reactive oxygen species (ROS) are not absolutely certain, raising the possibility that NADPH oxidase, nitric oxide synthase, lipoxygenases, cyclo-oxygenases, xanthine oxidase,
and cytochrome P450 enzymes, and the mitochondrial respiratory chain may be important ROS producers.\(^3,4\) Oxidative stress-induced unfavourable processes, such as DNA damage\(^5\) and changes in signalling pathways,\(^6\) can contribute considerably to the process of cardiac remodelling\(^7,8\) and to the development of end-stage HF.\(^9,10\) DNA damage induces the activation of nuclear enzyme poly(ADP-ribose)polymerase-1 (PARP), which can deplete NAD\(^+\) and ATP pools and induce unfavourable changes in kinase cascades, promoting apoptotic or necrotic cell death.\(^5\) Activation of PARP was demonstrated in ischaemic heart disease models\(^1\) and in biopsies from human subjects with HF,\(^12\) and it was shown in several studies that PARP inhibitors protect the heart from ischaemia–reperfusion-induced damage. However, no data are available concerning the potential protective effects of PARP inhibitors in hypertensive rats in any phase of the development of HF.

We suggested previously that PARP inhibitors beneficially affected stress-responsive signalling pathways including ERK 1/2, p38 MAP kinase, JNK, and PI-3-kinase-Akt-GSK-3\(\beta\) pathways\(^13–15\) which play a critical role in cardiac hypertrophic growth and/or ventricular dilatation.\(^4,16,17\) The spontaneously hypertensive rat (SHR) is an experimental model for studying the relationship between long-term high blood pressure and consequent hypertensive cardiopathy and HF.\(^1\) Here we tested the hypothesis that the PARP activation adversely affects cardiac remodelling, a well-established cardiomyopathic/HF model of SHR, and demonstrated that chronic inhibition of PARP by L-2286 (Figure 1) reversed maladaptive cardiac remodelling through effects on cardiac structure and function by inducing favourable modulation of stress-responsive kinase cascades.

2. Methods

2.1 Animal model and invasive blood pressure measurement

Thirty-week-old male SHR rats obtained from Charles River Laboratories (Budapest, Hungary), a compensatory hypertrophic stage, were randomly divided into two groups. One group received no treatment (\(n=47\), SHR-C), whereas the other group received L-2286 5 mg/b.w. in kg/day for 46 weeks\(^14,15,18,19\) (2-(2-Piperidine-1-ylethyl)thio)quinazolin-4(3H)-one), a water-soluble PARP inhibitor (SHR-L, \(n=47\)). The third group was an age-matched normotensive control group (CFY, \(n=22\), Charles River Laboratories, Budapest, Hungary). The fourth was a normotensive age-matched group receiving L-2286 treatment 5 mg/b.w. in kg/day (CFY-L, \(n=22\)). The dose of L-2286 was based on our previous results with this PARP inhibitor.\(^14,15\) According to these data, L-2286 can exert protective effects against oxidative cell damage in concentration of 10 \(\mu\)M. The serum concentration of L-2286 in the applied dose (5 mg/kg/day) with an estimated average bioavailability is approximately 10 \(\mu\)M in rats. L-2286 was dissolved in drinking water on the basis of preliminary data about the volume of daily consumption, but the water was provided ad libitum throughout the experiment. SHR rats over 40 weeks of age were observed daily, to achieve a description of normal activity, responsiveness to manipulation, weight, respiration, and general aspect. Several rats exhibited the following symptoms: lethargy, subcutaneous oedema, and increased respiratory rate.\(^20\) Deaths were recorded daily. Before the administration of L-2286 and at the end of the 46-week treatment period, echocardiographic measurements were performed. Invasive blood pressure measurement was carried out at the beginning (at the age of 30 weeks) and at the age of 53 weeks and at the end of the study. Five rats from each group were anaesthetized with ketamine hydrochloride intraperitoneally (Richter Gedeon Ltd., Budapest, Hungary) and a polyethylene catheter (Portex, London, UK) was inserted into their left arteria femoralis. Blood pressure was measured by CardioMed System CH-2005 (Medi-Stim AS, Oslo, Norway). Animals were euthanized with an overdose of ketamine hydrochloride intraperitoneally and heparinized with sodium heparin (100 IU/rat i.p., Biochemie GmbH, Kundl, Austria). They were sacrificed, and their blood was collected to determine the concentration of plasma BNP. Their hearts were removed, the atria and great vessels were trimmed from the ventricles, and weight of the ventricles was measured, which was then normalized to the body mass and to the length of the right tibia (indices of cardiac hypertrophy). The lung wet weight-to-dry weight ratio (an index of pulmonary congestion) was also measured in the animals.\(^14\) Hearts were freeze-clamped and were stored at \(-70^\circ\)C or fixed in 10% formalin. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and was approved by the Animal Research Review Committee of the University of Pecs Medical School.

2.2 Determination of plasma B-type natriuretic peptide

Blood samples were collected into the Lavender Vacutainer tubes containing EDTA and aprotinin (0.6 TIU/mL of blood) centrifuged at 1600 g for 15 min at 4°C to separate plasma. Supernatants were collected and kept at \(-70^\circ\)C. Plasma B-type natriuretic peptide-45 levels (BNP-45) were determined by enzyme immunoassay method as the manufacturer proposed (BNP-45, Rat EIA Kit, Phoenix Pharmaceuticals Inc., CA, USA).

2.3 Histology

Ventricles fixed in formalin were sliced and embedded in paraffin. Five micrometre thick sections were cut from base to apex. Slices were stained with Masson’s trichrome staining to detect the interstitial fibrosis as described earlier.\(^15\) Sections were quantified with the...
2.4 Western blot analysis
Fifty milligrams of heart samples were homogenized in ice-cold 50 mM Tris-buffer, pH 8.0 (containing protease inhibitor cocktail 1:1000, and 50 mM sodium vanadate, Sigma-Aldrich Co., Budapest) and harvested in 2× concentrated SDS-polyacrylamide gel electrophoresis sample buffer. Proteins were separated on 10 or 12% SDS-polyacrylamide gel electrophoresis sample buffer. After blocking (2 h with 3% non-fat milk in Tris-buffered saline), membranes were probed overnight at 4°C with antibodies recognizing the following antigens: phospho-specific Akt-1/protein kinase B-α Ser473 (1:1000), anti-actin (1:10000), phospho-specific glycogen synthase kinase (GSK)-3β Ser9 (1:1000), phospho-specific extracellular signal-regulated kinase (ERK 1/2) Thr183,Tyr185 (1:1000), phosphorylated p38 mitogen-activated protein kinaseThr180,Gly182 (p38 MAPK) (1:1000), phospho-specific c-Jun N-terminal kinase (JNK) (1:1000), phospho-specific protein kinase C (PKC) (pan) (1:1000), phospho-specific protein kinase C α/βII (PKC α/βII) Thr364/365 (1:1000), phospho-specific protein kinase C δ (PKC δ)Thr498 (1:1000), phospho-specific protein kinase C ζ (PKC ζ)Thr110/113 (1:1000), phospho-specific protein kinase C ε (PKC ε) Ser273 (1:1000), anti poly(ADP-ribose) (anti-PAR, 1:5000). Antibodies were purchased from Cell Signaling Technology, Beverly, MA, USA except anti-actin, which was bought from Sigma-Aldrich Co., Budapest, Hungary. Phospho-specific PKC ε was purchased from Upstate, UK and anti-PAR from Alexix Biotechnology, London, UK. Membranes were washed six times for 5 min in Tris-buffered saline (pH 7.5) containing 0.2% Tween (TBST) before addition of goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:3000 dilution, 0.2% Tween (TBST) before addition of goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (1:3000 dilution, Bio-Rad, Budapest, Hungary). The antibody-antigen complexes were visualized by means of enhanced chemiluminescence. After scanning, results were quantified by NIH ImageJ program.

2.5 Non-invasive evaluation of cardiac function
At baseline, all animals were examined by echocardiography to exclude rats with any heart abnormalities. Transthoracic two-dimensional echocardiography was performed under inhalation anaesthesia at the beginning of the experiment and on the day of sacrifice. Rats were lightly anaesthetized with a mixture of 1.5% isoflurane and 98.5% oxygen. The chest of the animals was shaved, acoustic coupling gel was applied, and warming pad was used to maintain normothermia. Rats were imaged in the left lateral decubitus position. Cardiac dimensions and functions were measured from short- and long-axis views at the mid-papillary level by a VEVO 770 high-resolution ultrasound imaging system (VisualSonics, Toronto, Canada)—equipped with a 25 MHz transducer. LV fractional shortening (FS), ejection fraction (EF), left ventricular (LV) end-diastolic volume (VEDV), LV end-systolic volume (LVESV), and the thickness of septum and posterior wall were determined. FS (%) was calculated by: 100 × ((LVIDd − LVIDs)/LVIDd) (LVID is LV inside dimension); EF (%) was calculated by: 100 × ((LVVol – LVol0)/LVol0); relative wall thickness (RWT) was calculated by: (PW thickness + interventricular septal thickness)/LV interior dimension.

2.6 Statistical analysis
All data are expressed as mean ± SEM. Comparisons among groups were made using Student’s t-test or one-way ANOVA (SPSS for Windows 11.0). To post hoc comparison, Bonferroni test was chosen. Survival was depicted graphically using Kaplan–Meier survival curves and compared using log-rank test. Values of P < 0.05 were considered statistically significant.

3. Results

3.1 Effect of PARP inhibition on gravimetric parameters and survival rate
At the beginning of the study, the body weight of CFY rats was significantly higher than the SHR rats (CYF: 387.5 ± 4.78 g, SHR: 345.25 ± 8.71 g, SHR-L: 334.44 ± 8.67 g, P < 0.05, 30-week-old rats). Similar results were obtained at the end of the study (CYF: 408.75 ± 6.14 g, SHR: 356.8 ± 5.61 g, SHR-L: 367.67 ± 9.7 g, P < 0.05 CFY vs. SHR groups, 76-week-old rats). Treatment with PARP inhibitor somewhat increased the body weight (SHR-L vs. SHR-C groups), but the changes were not significant. At the end of the study (76-week-old rats), heart weights (HW) and weights of ventricles (LV) were significantly increased in the SHR group compared with the CFY group (HW; CFY: 0.57 ± 0.04, SHR: 1.61 ± 0.05, SHR-L: 1.41 ± 0.03, P < 0.01 CFY vs. SHR, P < 0.05 SHR-L vs. CFY, SHR-C, WV: (g): CYF: 0.041 ± 0.03, SHR-L: 0.129 ± 0.03, P < 0.01 CFY vs. SHR, P < 0.05 SHR-L vs. CYF, SHR-C, these parameters were significantly decreased by L-2286 treatment. The ratios of weight of ventricles to body weight (WV/BW) and weight of ventricles to length of right tibia (WV/TL) were also significantly increased (WV/BW (g)/CFY vs. SHR-C) moderated by L-2286 (HW (g): CFY: 2.71 ± 0.09, SHR-C: 3.99 ± 0.06; WV/TL (mg/mm): CFY: 23.53 ± 0.77, SHR-C: 31.16 ± 0.68, P < 0.01 CFY vs. SHR-C compared with the CFY group, and were diminished significantly by this PARP inhibitor (WV/BW (g)/SHR-L: 3.52 ± 0.09, WV/TL (mg/mm): SHR-L: 28.26 ± 0.65, P < 0.01 vs. CFY and P < 0.05 vs. SHR-C). The ratio of the lung wet weight-to-dry weight was enhanced in the SHR groups significantly (CFY: 4.65 ± 0.1 g/g, SHR-C: 5.25 ± 0.07 g/g, P < 0.05 CFY vs. SHR-C), and was favourably influenced by L-2286 (SHR-L: 5.03 ± 0.04 g/g, P < 0.01 vs. CFY, and P < 0.05 vs. SHR-C) (Table 1). These parameters of the SHR groups indicated not only the presence of cardiac hypertrophy, but also the presence of pulmonary congestion.20,21 During the sacrifice, HF diagnosis was confirmed by the presence of ascites, internal congestion, hepatomegaly, and pleural effusion,20 which occurred very frequently in the SHR-C group.

The survival rate was significantly (P < 0.01) improved by the administration of L-2286. Only the curves of the two SHR groups were compared, because there was no mortality in the two CFY groups (Figure 2).

3.2 Effect of long-term L-2286 administration on plasma-BNP level and interstitial fibrosis
The level of plasma-BNP was significantly elevated in the SHR group (CFY: 2.03 ± 0.06 ng/mL, SHR-C: 2.71 ± 0.07 ng/mL, SHR-L: 2.21 ± 0.06 ng/mL, P < 0.01 CFY vs. SHR groups) compared with the CFY group and this elevation was significantly decreased by L-2286 treatment (P < 0.01 SHR-C vs. SHR-L) (Table 1). In case of myocardial fibrosis, the highest value was obtained from SHR-C rats (CFY: 17.153 ± 0.8, SHR-C: 23.048 ± 0.74, SHR-L: 19.4 ± 1.01, P < 0.01 vs. CFY), and the deposition of collagen was significantly (P < 0.05 vs. SHR-C) moderated by L-2286 (Table 1 and Figure 2).

3.3 Effect of long-term administration of L-2286 on echocardiographic parameters and blood pressure
There was no significant difference in LV systolic functions (EF and FS) between the CFY and SHR groups at the age of
30 weeks (EF (%): CFY: 68.10 ± 0.90 and SHR30w: 68.33 ± 1.75; FS (%): CFY: 38.45 ± 4.38 and SHR30w: 39.10 ± 3.33). Heart rate did not differ significantly during anaesthesia (CFY: 269 ± 18, SHR 272 ± 15 heart rate was decreased because of the isoflurane) between the groups. However, LVESV and LVEDV, the thickness of the PW and septum, RWT, and LV mass (indicating ventricular hypertrophy) also increased significantly (P < 0.05 CFY vs. SHR) (Table 2).

At the end of our study (76-week-old rats), we performed echocardiographic examination again. Heart rate did not differ significantly among the groups (CFY: 266 ± 19, SHR-C: 269 ± 22, SHR-L: 261 ± 25). The long-term administration of L-2286 could not influence significantly the elevated blood pressure of SHR rats. The worst LV systolic functions (EF (%): CFY: 71.07 ± 1.89, SHR-C76w: 52.41 ± 1.85, SHR-L76w: 69.11 ± 1.95; FS (%): CFY: 41.9 ± 1.71, SHR-C76w: 28.08 ± 1.23, SHR-L76w: 40.25 ± 1.64) were found in the SHR-C group (P < 0.01 vs. CFY and SHR-L). Although the systolic LV function was lower in the SHR-L group compared with the CFY group, this difference was not significant. Left ventricular end-diastolic diameter, left ventricular end-systolic diameter, (LVEDV and LVESV) were significantly enlarged in the SHR-C group compared with other groups (P < 0.01).

Table 1 Effect of L-2286 treatment on gravimetric parameters and on plasma-BNP in CFY and SHR

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CFY</th>
<th>SHR-C</th>
<th>SHR-L</th>
<th>CFY-L</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW30w (g)</td>
<td>387.5 ± 4.78</td>
<td>345.25 ± 8.71*</td>
<td>334.44 ± 8.67*</td>
<td>381.9 ± 5.96</td>
</tr>
<tr>
<td>BW76w (g)</td>
<td>408.75 ± 6.14</td>
<td>356.8 ± 5.61*</td>
<td>367.67 ± 9.7*</td>
<td>405.11 ± 7.41</td>
</tr>
<tr>
<td>HW76w (g)</td>
<td>1.22 ± 0.04</td>
<td>1.61 ± 0.05**</td>
<td>1.41 ± 0.03*</td>
<td>1.21 ± 0.03</td>
</tr>
<tr>
<td>WV76w (g)</td>
<td>1.11 ± 0.03</td>
<td>1.43 ± 0.04**</td>
<td>1.29 ± 0.03*</td>
<td>1.09 ± 0.03</td>
</tr>
<tr>
<td>WV/BW76w (g/g)</td>
<td>2.71 ± 0.09</td>
<td>3.99 ± 0.06**</td>
<td>3.52 ± 0.09*</td>
<td>2.69 ± 1.01</td>
</tr>
<tr>
<td>WV/TL76w (mg/mm)</td>
<td>23.53 ± 0.77</td>
<td>31.16 ± 0.68**</td>
<td>28.26 ± 0.65**</td>
<td>22.9 ± 0.82</td>
</tr>
<tr>
<td>Lung weight/dry weight76w (g/g)</td>
<td>4.65 ± 0.1</td>
<td>5.25 ± 0.07**</td>
<td>5.03 ± 0.04**</td>
<td>4.63 ± 0.08</td>
</tr>
<tr>
<td>p-BNP76w (ng/mL)</td>
<td>2.03 ± 0.06</td>
<td>2.71 ± 0.07**</td>
<td>2.21 ± 0.06**</td>
<td>2.02 ± 0.07</td>
</tr>
</tbody>
</table>

CFY: normotensive age-matched control rats, n = 7; SHR-C: spontaneously hypertensive rats (SHR) 76-week-old SHR control rats, n = 7; SHR-L: 76-week-old SHR treated with L-2286, for 46 weeks, n = 26; CFY-L: normotensive age-matched control rats treated with L-2286, for 46 weeks, n = 7. BW30w, 76w: body weight of 30-week-old and 76-week-old rats; HW76w: heart weight of 76-week-old rats; WV76w: weights of ventricles of 76-week-old rats; TL76w: length of right tibia of 76-week-old rats; p-BNP76w: plasma brain natriuretic peptide of 76-week-old rats. Values are means ± SEM.

* P < 0.05 (vs. CFY group), ** P < 0.01 (vs. CFY group), *** P < 0.05 (vs. SHR-C), **** P < 0.01 (vs. SHR-C).

Figure 2 L-2286 treatment influence the deposition of interstitial collagen. Representative histologic sections stained with Masson’s trichrome (n = 4). Scale bar is 48 μm. Magnifications 20× fold. CFY (A): normotensive age-matched control rats; SHR-C (B): 76-week-old spontaneously hypertensive rats; SHR-L (C): 76-week-old spontaneously hypertensive rats treated with L-2286 for 46 weeks. (D) Arrows show blue stained, thickened collagen fibres. Densitometric evaluation of sections is shown. * P < 0.01 vs. CFY, ** P < 0.05 vs. CFY, *** P < 0.05 vs. SHR-C.
The thickness of septum and PW was significantly elevated in the SHR groups when compared with the CFY group (PW: 
P < 0.05, septum: 
P < 0.05 vs. SHR-C group, and 
P < 0.01 vs. SHR-L group). The LV wall thickness (septum and PW) did not differ significantly between the two SHR groups. LV mass (uncorrected) was also increased in the SHR groups ( 
P < 0.01) compared with the CFY group, and L-2286 treatment decreased significantly ( 
P < 0.01) compared with the SHR-C group, similar to the LV mass index (LV mass/body weight). Finally, the significantly elevated RWT ( 
P < 0.05 SHR groups vs. CFY group) was not moderated by L-2286 administration (Table 3).

Time course change of blood pressure was measured by the invasive method. The significantly elevated blood pressure of SHRs ( 
P < 0.01 CFY vs. SHR-C and SHR-L groups) was not influenced by L-2286 treatment. In healthy rats, blood pressure was also not affected by L-2286 administration (Figure 3).

### 3.4 Effect of long-term L-2286 administration heart poly-ADP-ribosylations

To determine whether our PARP-inhibitor administration was effective, we detected the poly-ADP-ribosylation of the samples. Western blot analysis of heart proteins revealed that the ADP-ribosylation of SHR-C heart samples were the highest. This elevation was significantly decreased ( 
P < 0.05 SHR-C vs. SHR-L group) by L-2286 treatment (Figure 4).

### 3.5 Effect of long-term L-2286 treatment on Akt-1/ GSK-3β phosphorylation and MAPK, PKC pathways

Akt-1Ser473 was activated in elderly CFY rats ( 
P < 0.01 vs. SHR groups), while its activity was downregulated in SHR-C rats compared with CFY rats, but the phosphorylation of it was increased in SHR-L ( 
P < 0.01) animals. Similar results were obtained from the analysis of GSK-3βSer9 phosphorylation, which is one of the downstream target of Akt-1Ser473 (Fig 4).

In case of MAPK pathways, the modest phosphorylation of the examined—p38-MAPKThr180-Gly-Tyr182, JNK, and ERK 1/2Thr183-Tyr185—MAPKs pathways occurred in CFY rodents ( 
P < 0.01 vs. SHR groups). The phosphorylation of p38-MAPKThr180-Gly-Tyr182 (Figure 5) and JNK (data not shown) in SHR-C rats was the highest, and these alterations were attenuated by L-2286 treatment significantly ( 
P < 0.01).

The phosphorylation of ERK 1/2Thr183-Tyr185 was raised ( 
P < 0.01) in SHR-C group compared with the CFY group, and this was further enhanced ( 
P < 0.01) by L-2286 administration compared with SHR-C (Figure 4).

The phosphorylation of pan PKCβIIβIII, PKCα/βI, βIIThr505, SER729, IISer660, PKCα/βI was the lowest in CFY rodents ( 
P < 0.01 vs. SHR groups). The activity of pan PKCβ, α/β, δ, and ε was augmented in SHR-C rodents, but these elevations were

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### Table 2 Echocardiographic parameters of 30-week-old male normotensive CFY Sprague-Dawley (CFY, 

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CFY30w</th>
<th>SHR30w</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF (%)</td>
<td>68.1 ± 0.9</td>
<td>68.33 ± 1.75</td>
</tr>
<tr>
<td>FS (%)</td>
<td>38.45 ± 4.38</td>
<td>39.1 ± 3.38</td>
</tr>
<tr>
<td>LVEDV (mL)</td>
<td>278.55 ± 15.78</td>
<td>339.84 ± 14.65*</td>
</tr>
<tr>
<td>LVESV (mL)</td>
<td>86.45 ± 9.02</td>
<td>98.23 ± 10.4*</td>
</tr>
<tr>
<td>PW (mm)</td>
<td>1.6 ± 0.07</td>
<td>2.11 ± 0.09*</td>
</tr>
<tr>
<td>Septum (mm)</td>
<td>1.48 ± 0.06</td>
<td>1.83 ± 0.08*</td>
</tr>
<tr>
<td>RWT</td>
<td>0.4 ± 0.05</td>
<td>0.51 ± 0.02*</td>
</tr>
<tr>
<td>LV mass (uncorr, mg)</td>
<td>999.57 ± 61.3</td>
<td>1250.58 ± 78.78*</td>
</tr>
<tr>
<td>LV mass/BW (mg/g)</td>
<td>2.59 ± 0.9</td>
<td>3.69 ± 0.06*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. EF, ejection fraction; FS, fractional shortening; LV, left ventricular; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; Septum, thickness of septum; PW, thickness of posterior wall; RWT, relative wall thickness; LV mass, weights of LV; BW, body weight. *P < 0.05 (vs. CFY group).

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### Table 3 L-2286 treatment influenced echocardiographic parameters in spontaneously hypertensive rats (SHR)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CFY-L76w</th>
<th>SHR-C76w</th>
<th>SHR-L76w</th>
<th>CFY-L76w</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF (%)</td>
<td>71.07 ± 1.89</td>
<td>52.41 ± 1.85*</td>
<td>69.11 ± 1.95</td>
<td>69.68 ± 1.66</td>
</tr>
<tr>
<td>FS (%)</td>
<td>41.9 ± 1.71</td>
<td>28.08 ± 1.23*</td>
<td>40.25 ± 1.64</td>
<td>41.94 ± 1.83</td>
</tr>
<tr>
<td>EDD (mm)</td>
<td>7.1 ± 0.13</td>
<td>8.23 ± 0.2*</td>
<td>7.27 ± 0.06**</td>
<td>7.08 ± 0.2</td>
</tr>
<tr>
<td>ESD (mm)</td>
<td>4.08 ± 0.06</td>
<td>5.92 ± 0.18*</td>
<td>4.35 ± 0.12**</td>
<td>4.11 ± 0.09</td>
</tr>
<tr>
<td>LVEDV (mL)</td>
<td>278.5 ± 5.94</td>
<td>368.49 ± 7.13*</td>
<td>279.04 ± 5.07**</td>
<td>280.5 ± 6.47</td>
</tr>
<tr>
<td>LVESV (mL)</td>
<td>86.03 ± 11.37</td>
<td>175.41 ± 12.29*</td>
<td>186.18 ± 6.58**</td>
<td>85.02 ± 9.86</td>
</tr>
<tr>
<td>PW (mm)</td>
<td>1.72 ± 0.04</td>
<td>2.06 ± 0.09***</td>
<td>2.15 ± 0.05*</td>
<td>1.76 ± 0.07</td>
</tr>
<tr>
<td>Septum (mm)</td>
<td>1.79 ± 0.04</td>
<td>2.12 ± 0.08***</td>
<td>2.21 ± 0.05*</td>
<td>1.81 ± 0.06</td>
</tr>
<tr>
<td>RWT</td>
<td>0.42 ± 0.01</td>
<td>0.49 ± 0.02***</td>
<td>0.6 ± 0.01*</td>
<td>0.5 ± 0.01</td>
</tr>
<tr>
<td>LV mass (uncorr., mg)</td>
<td>1002.33 ± 53.47</td>
<td>1438.12 ± 97.36*</td>
<td>1273.63 ± 41.76*</td>
<td>1024.06 ± 68.78</td>
</tr>
<tr>
<td>LV mass/BW (mg/g)</td>
<td>2.51 ± 0.06</td>
<td>3.94 ± 0.21*</td>
<td>3.48 ± 0.13*</td>
<td>2.47 ± 0.15</td>
</tr>
<tr>
<td>SAP270w (mmHg)</td>
<td>142 ± 10</td>
<td>228 ± 7*</td>
<td>224 ± 9*</td>
<td>140 ± 8</td>
</tr>
<tr>
<td>DAP270w (mmHg)</td>
<td>97 ± 5</td>
<td>173 ± 9*</td>
<td>168 ± 8*</td>
<td>92 ± 6</td>
</tr>
<tr>
<td>MAP270w (mmHg)</td>
<td>112 ± 7</td>
<td>192 ± 6*</td>
<td>187 ± 7*</td>
<td>108 ± 8</td>
</tr>
</tbody>
</table>

CFY: normotensive age-matched control rats, n = 7; SHR-C76w: 76-week-old SHR control rats, n = 7; SHR-L76w: 76-week-old SHR treated with L-2286 for 46 weeks, n = 25; CFY-L76w: normotensive age-matched control rats treated with L-2286, for 46 weeks, n = 7. SAP, DAP, MAP: systolic, diastolic, and mean arterial blood pressure at 76-week-old age (n = 5 from each group). Values are mean ± SEM. 

EF, Ejection fraction; FS, Fractional shortening; EDD, End-diastolic diameter; ESD, End-systolic diameter; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; Septum, thickness of septum; PW, thickness of posterior wall; RWT, relative wall thickness; LV mass, weights of LV; BW, body weight. *P < 0.01 (vs. CFY group), **P < 0.05 (vs. SHR-C), ***P < 0.05 (vs. CFY group), ****P < 0.01 (vs. SHR-C).
significantly moderated by L-2286 treatment ($P < 0.01$ pan PKC $\beta$II$^{S660}$ and $\alpha/\beta$II$^{T410/403}$; $P < 0.05$ PKC $\alpha/\beta$II$^{T638/641}$, $\delta$II$^{T505}$) (Figures 5 and 6).

On the other hand, the enhanced phosphorylation of PKC $\varepsilon$Ser$^{729}$ was further increased significantly ($P < 0.01$) by L-2286 administration (Figure 6).

4. Discussion

The major findings of this study are that chronic inhibition of nuclear PARP enzyme reduces ADP-ribosylation of nuclear proteins and thus prevents the development of HF from cardiac hypertrophy with inducing reverse remodelling with restoration of cardiac structure and function while changing the altered patterns of signal transducing processes. We used the SHR which provides an animal model of high blood pressure that is similar to essential hypertension in humans. They are characterized by the fact that they suffer from pre-hypertension (systolic blood pressure 100–120 mmHg) during the first 6–8 weeks of their life, and develop sustained hypertension over the next 12–14 weeks. Effect of L-2286 on healthy rats was also examined, but it was inconclusive on the intact hearts (data not shown) as it was expected. Our study began in the compensated phase of hypertensive cardiopathy in SHR with signs of LVH (at 30 weeks old) and after 46 weeks, the obvious signs of HF could be detected in SHRs. The development of HF from long-term hypertension can be explained by different mechanism in the literature, but oxidative stress and abnormal signalling are generally respected as the molecular basis of the disease.

In the case of ischaemia–reperfusion induced cardiac damages and PARP activation is respected as one of the main mechanisms of the damage of cardiomyocytes, it is well documented that PARP inhibitors have beneficial effects. However, in case of long-term hypertension induced cardiac hypertrophy followed by HF, there is no data available about the possible role of PARP. Furthermore, our previous works demonstrated that inhibition of PARP has a major effect on signalling in oxidative stress in cardiomyocytes, by activating the PI-3-kinase-Akt pathway and inhibiting JNK and p38 MAP kinases. One of the PARP inhibitors used in our previous studies was L-2286 which had a protective effect in ischaemia–reperfusion injury and in isoproterenol induced cardiac remodelling without having any detectable side effects in animal models. In this study, we tested the effect of PARP inhibition in ageing SHRs having cardiac hypertrophy and fibrosis related to higher mechanical and oxidative stress and had typical signs of HF (gravimetric parameters and observation daily) and impaired systolic LV function. These conditions have important role in the pathogenesis of diastolic and systolic dysfunctions in hypertensive heart disease.

Both in animal models and in humans, increased blood pressure has been associated with oxidative stress in the vasculature, i.e. with an excessive endothelial production of ROS, which may be both a cause and a consequence of hypertension. ROS-induced DNA damages can lead to the activation of PARP enzyme. The effect of PARP inhibitors on oxidative stress has been investigated in several studies. In our experiment, PARP-inhibitor administration did not influence the elevated blood pressure in SHRs, just like in human studies, where the use of antioxidants had only limited effects on hypertension or cardiovascular endpoints. Therefore, although SHR is an increased afterload model of hypertension, in this study, the observed beneficial alterations cannot be explained by a blood pressure lowering effect. Chronic untreated hypertension often leads to excessive collagen deposition as part of the process of cardiovascular remodelling that includes LVH and endothelial dysfunction. This remodelling leads to a more rigid myocardium and ultimately to HF with impaired diastolic and systolic LV function. In our experiment, the level of plasma-BNP was elevated in both SHR groups and

![Figure 3](image-url) Effect of L-2286 treatment on blood pressure. (A) CFY, normotensive control group, (B) SHR-C: spontaneously hypertensive rats receiving only placebo, (C) SHR-L: spontaneously hypertensive rats treated with L-2286 for 46 weeks, (D) CFY-L: normotensive rats receiving L-2286 for 46 weeks. Values are means ± SEM, n = 5 from each group: ●: systolic blood pressure, ■: diastolic blood pressure, ▲: mean blood pressure.
this finding was similar to de Bold’s,\textsuperscript{30} who examined the mRNA level of plasma BNP in SHR rats at different ages.

Exalted BNP production and release by cardiocytes occur in hypertension and have been considered to be a compensatory mechanism against ventricular overload.\textsuperscript{30} The Framingham study demonstrated that an increase in BNP predicted the risk of death and cardiovascular events.\textsuperscript{31} This alteration could be mitigated by PARP inhibition and in accordance with this, the survival rate of treated rats was also significantly better.

If the heart experiences extended periods of elevated workload, it undergoes a hypertrophic enlargement in response to increased demand. A number of signalling modulators in the vasculature milieu are known to regulate heart muscle mass, including those that influence gene expression, apoptosis, cytokine release, and growth factor signalling.\textsuperscript{16} One of them is the Akt-1/GSK-3β pathways, which were favourably influenced by PARP inhibitor. In our experiment, the downregulated phosphorylation of Akt-1/ GSK-3β in SHR-C samples was increased by PARP inhibitor. Similar effects of PARP inhibition were seen in differential experimental systems indicating that Akt activation is an important step in the cytoprotective effects of PARP inhibition.\textsuperscript{13,17} Akt-1 is well known to play a central role in the development of physiologic hypertrophy, but also has an important role in cardiac angiogenesis through the activation of mammalian target of rapamycin (mTOR). It is likely that ineffective angiogenesis might contribute to the transition from LVH to HF.\textsuperscript{32} The protecting effect of PARP inhibitors against the development of HF from LVH can be mediated at least partly through the Akt-1/mTOR signalling.

MAPKs are ubiquitously expressed, and their specific functions in the heart have been a focus of intensive study.\textsuperscript{33} Growing evidence suggests that modulation of the complex network of MAPKs cascades could be a rewarding approach to treatment of cardiomyocyte hypertrophy and HF.\textsuperscript{32} In our experiment, the elevated activations of p38, JNK in the SHR-C groups were decreased, whereas the activation of ERK was increased by L-2286. While the ERKs are particularly implicated in growth-associated responses, the p38 MAPK and JNKs are generally activated by cytotoxic stress factors.\textsuperscript{32} JNK directly phosphorlates the pro-apoptotic factor Bad, enhancing cell death through the intrinsic pathway. Qin
et al.\cite{28} found that the increase in p38 activity was associated with the increased oxidative stress in the remote non-infarcted myocardium after myocardial infarction. In vivo a number of reports have shown protection from ischaemia-induced apoptosis and cardiac dysfunction through the acute use of p38 inhibitors.\cite{34} Activation of ERK causes cardiac hypertrophy and increases survival, whereas inactivation of ERK contributes to myocyte apoptosis.\cite{28} Cardiac-specific expression of constitutively activated MEK1 promotes cardiac hypertrophy without compromised function or long-term animal survival, suggesting that the activation of ERK activity promotes a compensated form of hypertrophy.\cite{33}

There is now considerable evidence that a variety of PKC isoforms also act as major modulators of the myocyte death machinery, having both pro and anti-apoptotic effects.

In our study, the phosphorylation of PKC pan \(\text{Ser}^{660}\), \(\alpha/\beta\text{Thr}^{638/641}\), and \(\zeta/\lambda\text{Thr}^{410/413}\) were attenuated in SHR-L compared with SHR-C by PARP inhibitor. Several reports suggest that PKC \(\alpha\) and \(\beta\) are involved in the development of cardiac hypertrophy and HF.\cite{35} PKC \(\alpha\) and \(\beta\) increased in the failing human heart due to dilated and ischaemic cardiomyopathy.\cite{36} Activation of PKC \(\delta\) appears to contribute to ischaemic injury in cardiac myocytes.\cite{34} Koide et al.\cite{35} revealed that PKC \(\alpha\), \(\beta\), and \(\delta\) were up-regulated from the stage of cardiac hypertrophy extending to congestive HF. The hypothesis that PKC \(\delta\) is pro-apoptotic while PKC \(\epsilon\) is anti-apoptotic is also supported by a number of in vivo reports.\cite{34,35,37,38} The activation of PKC \(\epsilon\) was upregulated by L-2286 treatment in SHR-L group.

In this experiment, there were no differences in LV systolic functions (EF and FS) at baseline (the age of 30 weeks). These parameters were preserved in the CFY and SHR-L groups, but moderated in the SHR-C group at the end of the study. L-2286 increased EF by reducing end-systolic dimensions.\cite{3}

During the development of hypertension, alterations in LV geometry may occur as an adaptation to increasing pressure and volume load. In hypertensive patients, LV geometry can be classified into four patterns on the basis of LV mass index and RWT.\cite{1} In conformity with this classification, eccentric hypertrophy was found in SHR-C group (increased LV mass/BW and normal RWT), while L-2286 administration could preserve concentric hypertrophy (increased LV mass/BW and increased RWT) state, which could be detected at the beginning of the study in both SHR groups. Therefore, the inefficaciousness of L-2286 on thickness of septum and PW can be considered as a favourable effect because it can add to maintaining of concentric hypertrophy.

In conclusion, in this study the long-term administration of PARP inhibitor could beneficially influence gravimetric parameters, cardiac fibrosis, and several echocardiographic parameters and delay the onset of hypertension-induced HF without lowering blood pressure. In the background of these results can be the beneficial effect of PARP inhibition on myocardial fibrosis and the activation of prosurvival
intracellular signalling pathways PI-3-kinase-Akt-1Ser473 and PKCεSer729 pathways.

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