INVESTIGATION OF POTENTIAL PHARMACOLOGICAL DRUG TARGETS IN THE DEVELOPMENT AND PROGRESSION OF HYPERTENSIVE ORGAN DAMAGES

PhD thesis

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ABBREVIATIONS

AKT	protein kinase B (PKB)
BNP	B-type natriuretic peptide
BW	body weight
DAP	diastolic arterial blood pressure
EF	ejection fraction
ERK ½	extracellular signal-regulated kinase
FS	fractional shortening
GSK-3β	glycogen synthase kinase-3β
HF	heart failure
IMT	intima-media thickness
IR	ischemia-reperfusion
IVS (d)	thickness of interventricular septum in diastole
IVS (s)	thickness of interventricular septum in systole
JNK	c-jun N-terminal kinase
LVEDV	left ventricular end-diastolic volume
LVESV	left ventricular end-systolic volume
LVID (d)	left ventricular end-diastolic diameter
LVID (s)	left ventricular end-systolic diameter
MAP	mean arterial blood pressure
MAPK	mitogen activated protein kinase
NAD+	nicotinamide adenine dinucleotide
NIH	National Institute of Health
NSAID	non-steroidal anti-inflammatory drug
PARP	poly(ADP-ribose) polymerase
PI3K	phosphatidylinositol-3-kinase
PKC	protein kinase C
PW (d)	thickness of left ventricular posterior wall in diastole
PW (s)	thickness of left ventricular posterior wall in systole
ROS	reactive oxygen species
RWT	relative wall thickness
SAP	systolic arterial pressure
SHR-C	spontaneously hypertensive rat trated with placebo
SHR-L	spontaneously hypertensive rat trated with L2286
SPB	systolic blood pressure
TBS	TRIS-buffered saline
TGF-β	transforming growth factor-β
TL	lenght of right tibia
WKY	Wistar-Kyoto rat

Introduction

Hypertension is a major public health problem both in middle-aged and in elderly people. It is both a complex disease and an important risk factor for other cardiovascular outcomes, such as sudden cardiac death, stroke, myocardial infarction, heart failure, and renal diseases. Unfortunately, the control of arterial hypertension is far from optimal and has improved only minimally over the last decades. Side effects of antihypertensive drugs, complaints due to their blood pressure lowering effect and inadequate compliance are the key factors in the background of inadequate control of hypertension. Moreover lowering blood pressure to the optimal range can be harmful in elderly patients. In order to optimize management of hypertension, some recent efforts focus on protecting the heart and the vasculature from hypertension induced remodeling with or without lowering the blood pressure.

Experimental model of chronic hypertension

SHR have been widely used as a model for hypertensive heart disease and hypertension induced vascular remodeling. The SHR was originally introduced by Okamoto and Aoki as a model of genetic hypertension. The progression of hypertrophy and impaired cardiac function in the SHR is similar to the clinical course of patients with hypertension. Persistent hypertension develops in the SHR after approximately 6 weeks of age. Following a relatively long period of stable hypertension and compensated hypertrophy, at approximately 18 months of age, animals begin to develop evidence of impaired function (tachypnea, labored respiration).

The development of vascular remodeling is an early and important consequence of hypertension. Vascular remodeling is mainly characterized by vascular smooth muscle cell hypertrophy and increased production of extracellular matrix. Remodeling is initially an adaptive process that evolves in response to long-term pressure overload, but finally it can contribute to the development of hypertensive target organ damages.

Cardiovascular effects of PARP inhibition

It is known that activation of poly(ADP-ribose) polymerase enzyme (PARP) plays an important role in the development of postinfarction as well as long-term hypertension induced heart failure. The poly(ADP-ribose) polymerase (PARP) enzyme becomes activated in response to DNA single-strand breaks that can be excessive as a response to free radicals and oxidative cell damage. PARP is an energy-consuming enzyme that transfers ADP-ribose units to nuclear proteins. As a result of this process, the intracellular NAD^+ and ATP levels decrease remarkably resulting in cell dysfunction and cell death via the necrotic route. PARP activation can induce ROS production, calcium elevation, and activates JNK, p38 MAP kinase and RIP1 which can destabilize mitochondrial

membrane system leading to the release pro-apoptotic proteins from the mitochondrial inner membrane space, like Cytochrome C, AIF and endonuclease G. In addition, PARP activation can activate NF-kappaB and AP-1 transcription factors which can contribute to cardiovascular remodeling.

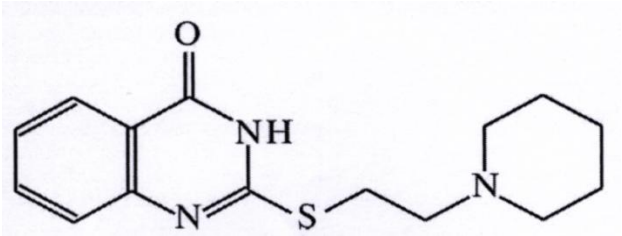

Figure 1. Chemical structure of L-2286 (2-[(2-Piperidine-1-ylethyl)thio]quinazolin-4(3H)-one).

It has been shown previously that our experimental agent, an isoquinoline derivative PARP-inhibitor, L-2286 (Fig. 1) had beneficial effects against oxidative cell damage, ischemia-reperfusion injury and against the development of postinfarction or long-term high blood pressure-induced heart failure. Although the molecule have a slight scavenger characteristic, its forementioned effects were mediated mainly by influencing the Akt-1/GSK-3β, MAPK and PKC signal transduction factors.

Importance of bradykinin B1 receptor antagonism

An important factor in the background of the inadequate hypertension control is the drug interactions between antihypertensive agents and several non-cardiovascular drugs e.g. analgetics, such as NSAIDs.

NSAIDs are the most widely used analgetics nowadays. Unfortunately all of them (except low dose of aspirin) increase markedly the cardiovascular mortality and morbidity. Therefore we considered important to monitor the cardiovascular effects of a novel analgetic agent, bradykinin B1 receptor antagonist. According to several previous works bradykinin B1 receptor antagonists may have beneficial effects in some cardiovascular diseases.

Kinins are biologically active peptides that exert a broad spectrum of physiological effects, including vasodilation, inflammation, and pain induction. The biological effects of kinins are mediated through the stimulation of bradykinin B1 and B2 receptors. The B2 receptor is constitutively expressed and is activated by intact kinins, bradykinin, and kallidin. This receptor is believed to play an important role in mediating the beneficial effects of ACE-inhibitors, but it is also involved in the acute phases of inflammation. However the B1 receptor is activated by the carboxypeptidase metabolites of kinins, des-Arg9-BK and des- Arg10-kallidin. The B1 receptor is normally weakly expressed, but it is upregulated in the presence of cytokines and endotoxins or during tissue injury. The B1 receptor participates in chronic inflammation and pain; thus, bradykinin B1 receptor antagonists are a potentially novel approach for treating these conditions without having deleterious cardiovascular effects.

Aims of the study

Our present study aimed to clarify whether pharmacological PARP-inhibitior L-2286 has protective effect in an SHR model against the development of the early stage of hypertensive cardiac remodeling.

-The aim of this work was to provide evidence for new molecular mechanisms of the cardioprotective effect of PARP inhibition.

-We estimated its effect on cardiac fibrosis.

-We tested whether PARP inhibition had beneficial effect on signal transduction pathways taking part in cardiac remodeling.

In the second experiment we investigated the effects of the bradykinin B1 receptor antagonist test substance, FGY-1153 on the development of hypertensive organ damages in spontaneously hypertensive rats (SHR).

- We tried to examine the effect of bradykinin B1 receptor antagonism on body weight, food consumption and blood pressure.

- We tried to examine the effect of bradykinin B1 receptor antagonism on hypertension induced cardiovascular remodeling (intima media thickness, interstitial fibrosis, LVHT).

- We tested whether bradykinin B1 receptor antagonist had beneficial effect on signal transduction pathways taking part in cardiovascular remodeling.

Effect of PARP inhibition on gravimetric parameters of spontaneously hypertensive rats

Body weights did not differ significantly among the three groups (WKY: 71.01±0.11 g, SHR-C: 72.03±2.36 g, SHR-L: 69.92±3.21 g, 6-week-old rats) at the beginning of our study. However, at the end of the 24-week-long treatment period, body weights of WKY group were significantly higher than those of SHR-C and SHR-L groups (WKY: 392.7±14.01 g, SHR-C: 323.8±11.27 g, SHR-L: 321.9±6.84 g, $p<0.01$ WKY vs. SHR groups, 30-week-old rats). The degree of myocardial hypertrophy was determined by ventricular weight to body weight ratio (WV/BW, mg/g). This parameter was significantly increased in SHR groups compared to the WKY group (WV/BW: WKY: 2.95±0.17, SHR-C: 4.48±0.12, SHR-L: 3.85±0.15, $p<0.05$ WKY vs. SHR groups). Similar results were obtained in case of weights of ventricles (WV, WKY: 1.16±0.17 g, SHR-C: 1.45±0.18 g, SHR-L: 1.24±0.24 g, $p<0.05$ WKY vs. SHR groups). The WV and WV/BW ratios were significantly decreased by L-2286 treatment ($p<0.05$ SHR-L vs. SHR-C). The lung wet weight-to-dry weight ratio was not elevated significantly in SHR-C and SHR-L compared to WKY groups (Table 1). All these results indicate the presence of cardiac hypertrophy without congestive heart failure in the SHR-C group that was ameliorated in the SHR-L group.

	WKY	SHR-C	SHR-L
BW^{6w} (g)	71.01±1.89	72.02±2.36	69.9±3.21
BW (g)	393±14.01	323.8±11.27[a]	321.86±6.8[a,c]
WV (g)	1.16±0.17	1.45±0.18[b]	1.24±0.24[b,c]
WV/BW (mg/g)	2.95±0.17	4.48±0.12[b]	3.85±0.15[b,c]
Lung wet weight/dry weight	4.84±0.92	4.79±0.84	4.77±0.99
p-BNP (ng/ml)	2.19±0.011	2.33±0.034	2.31±0.031

Table 1. Effect of L-2286 treatment on gravimetric parameters and on plasma BNP in SHR. WKY: normotensive age-matched control rats, n=7, SHR-C: SHR age-matched control rats, n=8, SHR-L: SHR treated with L-2286 for 24 weeks, n=9. BW^{6w}: body weight of 6-week-old rats, BW: body weight, WV: weights of ventricles, BNP: plasma b-type natriuretic peptide. Values are means±S.E.M. [a]<0.01 (vs. WKY group), [b]<0.05 (vs. WKY group), [c]<0.05 (vs. SHR-C).

L-2286 treatment did not influence the levels of plasma BNP and blood pressure

Slightly elevated plasma BNP levels were found both in SHR-C and SHR-L groups (not significant vs. WKY group). Although plasma BNP level was a little higher in SHR-C group than in SHR-L group, this difference was also not statistically significant (Table 1). In both SHR groups,

blood pressure was significantly elevated compared to the WKY group ($p<0.05$). L-2286 treatment did not decrease significantly the elevated blood pressure (Table 3).

L-2286 decreased the interstitial collagen deposition in the myocardium

Histological analysis revealed slight interstitial collagen deposition in the WKY group. Chronic high blood pressure caused significantly higher collagen deposition in SHR-C rats that was significantly diminished ($p<0.05$) in the SHR-L group (Fig. 2).

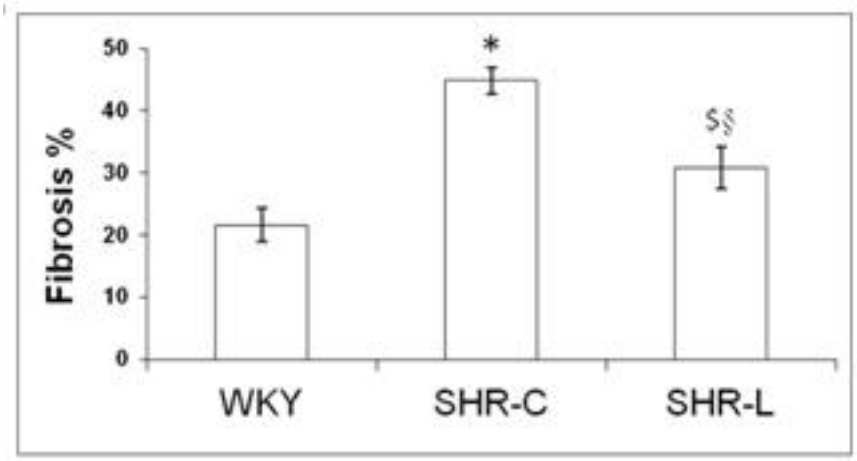

Figure 2. L-2286 treatment decreased the deposition of interstitial collagen. WKY: normotensive age-matched control rats. SHR-C: 30 week-old spontaneously hypertensive rats, SHR-L: 30 week-old spontaneously hypertensive rats treated with L-2286 for 24 week. Denzitometric evaluation of the sections is shown. $^{*}p<0.01$ vs. WKY, $^{\S}p<0.05$ vs. WKY, $^{\$}p<0.05$ vs. SHR-C.

PARP inhibition decreased the left ventricular hypertrophy in spontaneously hypertensive rats

At the beginning of the study the echocardiographic parameters of the three groups did not differ significantly from each other (Table 2). At the age of 30 weeks there was no significant difference in LV systolic functions (EF and FS) between the WKY and SHR groups. Heart rate did not differ significantly during the anesthesia among the groups. LVESV and LVEDV were increased significantly in SHRs ($p<0.05$ WKY vs. SHR-C and SHR-L), and these unfavorable alterations were not reduced by L-2286 treatment. The thickness of the septum, and the posterior wall and the relative wall thickness were also increased in SHR groups (indicating the presence of ventricular hypertrophy) comparing to the WKY group ($p<0.05$), and these parameters could be significantly reduced by the administration of L-2286 ($p<0.05$ SHR-C vs. SHR-L group) (Table 3).

	WKY	SHR-C	SHR-L
EF (%)^{6w}	67.26±0.525	68.4±1.77	68.23±1.81
FS^{6w}	38.63±4.47	38.03±5.52	39.35±4.15
LVEDV^{6w} (ml)	147.27±13.88	149.56±16.78	149.11±14.43
LVESV^{6w} (ml)	46.63±4.47	48.03±5.52	47.35±5.45
Septum^{6w} (mm)	1.2±0.07	1.18±0.05	1.17±0.12
PW^{6w} (mm)	1.19±0.07	1.16±0.067	1.14±0.04
LV mass^{6w} (uncorrected) (mg)	**344.14±35.49**	**351.66±36.23**	**354.77±33.23**

Table 2. Echocardiographic parameters in 6 weeks old SHRs. WKY: normotensive age-matched control rats, n=7, SHR-C: SHR age-matched control rats, n=8, SHR-L: n=9, SHR treated with L-2286 for 24 weeks.EF^{6w}: ejection fraction, FS^{6w}: fractional shortening, LVEDV^{6w}: left ventricular (LV) end-diastolic volume, LVESV^{6w}: LV end-systolic volume, Septum^{6w}: thickness of septum, PW^{6w}: thickness of posterior wall, LV mass^{6w}: weights of LVs. ±S.E.M.

	WKY	SHR-C	SHR-L
SAP^{30w}, (mmHg)	129±7	192±9^{a}	186±5^{a}
DAP^{30w}, (mmHg)	89±5	127±8^{a}	125±4^{a}
MAP^{30w}, (mmHg)	103±7	149±5^{a}	146±7^{a}
EF (%)^{30w}	69.1±2.4	68.72±2.1	69.01±3.2
FS^{30w}	39.8±1.9	39.04±1.85	40.57±2.66
LVEDV^{30w} (ml)	279.18±18.18	335.87±10.36^{a}	326.94±9.18^{a}
LVESV^{30w} (ml)	85.77±8.56	96.85±10.36^{a}	99.81±11.85^{a}
Septum^{30w} (mm)	1.43±0.04	1.93±0.04^{a}	1.79±0.05^{a,b}
PW^{30w} (mm)	1.54±0.08	2.15±0.12^{a}	1.87±0.03^{a,b}
RWT^{30w}	0.38±0.05	0.504±0.02^{a}	0.445±0.012^{a,b}
LV mass^{30w} (uncorrected) (mg)	1002.81±59.5	1370.35±79.87^{a}	1121.13±53.23^{a,b}
LV mass^{30}/BW^{30} (mg/g)	2.73±0.7	4.23±0.8^{a}	3.70±0.3^{a,b}

Table 3. L-2286 treatment moderately decreased the echocardiographic signs of LVHT in 30 weeks old SHRs. WKY: normotensive age-matched control rats, n=7, SHR-C: SHR age-matched control rats, n=8, SHR-L: n=9, SHR treated with L-2286 for 24 weeks.EF^{30w}: ejection fraction, F^{30w}: fractional shortening, LVEDV^{30w}: left ventricular (LV) end-diastolic volume, LVESV^{30w}: LV end-systolic volume, Septum^{30w}: thickness of septum, PW^{30w}: thickness of posterior wall, RWT^{30w}: relative wall thickness, LV mass^{30w}: weights of LVs. SAP, DAP, MAP^{30w}: systolic, diastolic and mean arterial blood pressure at 30-week-old age (n=3 from each group). Values are mean±S.E.M. $^{a}p<0.05$ (vs. WKY group), $^{b}p<0.05$ (vs. SHR-C group), $^{c}<0.05$ (vs. SHR-C).

Effect of L-2286 treatment on poly-ADP-ribosylation as well as on the phosphorylation state of Akt-1^{Ser473}/GSK-3β^{Ser9} and FKHR^{Ser256}

Akt-1^{Ser473} was moderately phosphorylated in WKY group. In SHR-C group, the phosphorylation of Akt-1^{Ser473} was more pronounced ($p<0.01$ vs. WKY). Moreover, in SHR-L rats the L-2286 treatment caused further elevation in Akt-1^{Ser473} phosphorylation ($p<0.01$ vs. WKY and SHR-C groups). The same result was obtained in the case of GSK-3β^{Ser9} phosphorylation.

To detect the effectivity of L-2286, the ADP-ribosylation of the samples were analysed by Western-blot. The lowest degree of ADP-ribosylation was present in SHR-L group, and the most pronounced ADP-ribosylation was seen in SHR-C group ($p<0.05$ vs. WKY) . Another target protein of Akt-1^{Ser473} (besides GSK3β^{Ser9}) is FKHR^{Ser256}. Consistently with the result of Akt-1^{Ser473} phosphorylation, the strongest phosphorylation (therefore inhibition) could be observed in SHR-L group ($p<0.01$ vs. SHR-C and WKY). The lowest phosphorylation and therefore the highest activity of FKHR was seen in SHR-C group ($p<0.05$ vs. WKY).

Effect of L-2286 on the amount of Hsp72 and 90

There was no significant difference among the three groups in the level of Hsp72. On the other hand, the level of Hsp90 was elevated in SHR-L group compared to WKY and SHR-C groups ($p<0.01$ SHR-L vs. WKY or SHR-C groups), and the lowest amount of this protein was present in WKY samples.

Effect of L-2286 administration on MAPKs

Phosphorylation of p38-MAPK$^{Thr180\text{-}Gly\text{-}Tyr182}$, ERK 1/2$^{Thr183\text{-}Tyr185}$ and JNK was the lowest in the WKY group compared to SHR-C and SHR-L groups (p38-MAPK$^{Thr180\text{-}Gly\text{-}Tyr182}$: $p<0.01$ vs. SHR groups, ERK 1/2: $p<0.05$ vs. SHR groups, JNK: $p<0.05$ vs. SHR groups). In the case of p38-MAPK$^{Thr180\text{-}Gly\text{-}Tyr182}$ and JNK, their phosphorylation was elevated in both SHR-C and SHR-L groups, but there were no significant differences between the two SHR groups.

Phosphorylation of ERK 1/2$^{Thr183\text{-}Tyr185}$ was increased significantly in SHR-C and SHR-L groups. L-2286 treatment did not alter significantly the phosphorylation in SHR-L group compared to the SHR-C group.

Influence of L-2286 treatment on the phosphorylation state of several PKC isoforms

The overall (pan) phosphorylation of PKC (pan βII^{Ser660}) was low in the WKY group and became significantly higher in SHR-C and SHR-L groups ($p<0.01$ WKY vs. SHR groups). Administration of L-2286 could not affect the phosphorylation state of PKC pan βII Ser^{660} in SHR-L group compared to the SHR-C group.

The lowest phosphorylation could be observed in the WKY group in case of PKC $\alpha/\beta II^{Thr638/641}$, δ^{Thr505}, $\zeta/\lambda^{Thr410/403}$ and ε^{Ser729} ($p<0.01$ vs. SHR groups). As PKC ζ antibody, we used a combined antibody (i.e. PKC ζ/λ $Thr^{410/403}$), which did not discriminate between PKC ζ and λ; PKC λ being structurally highly homologous to PKC ζ in the COOH-terminal end of the molecule. L-2286 treatment decreased significantly the phosphorylation of PKC $\alpha/\beta II^{Thr638/641}$ and ζ, while it could increase the phosphorylation of ε^{Ser729} (PKC $\alpha/\beta II^{Thr638/641}$, ζ , ε^{Ser729}: $p<0.01$, SHR-L vs. SHR-C). In the case of PKC δ^{Thr505} there was no significant difference between the SHR groups.

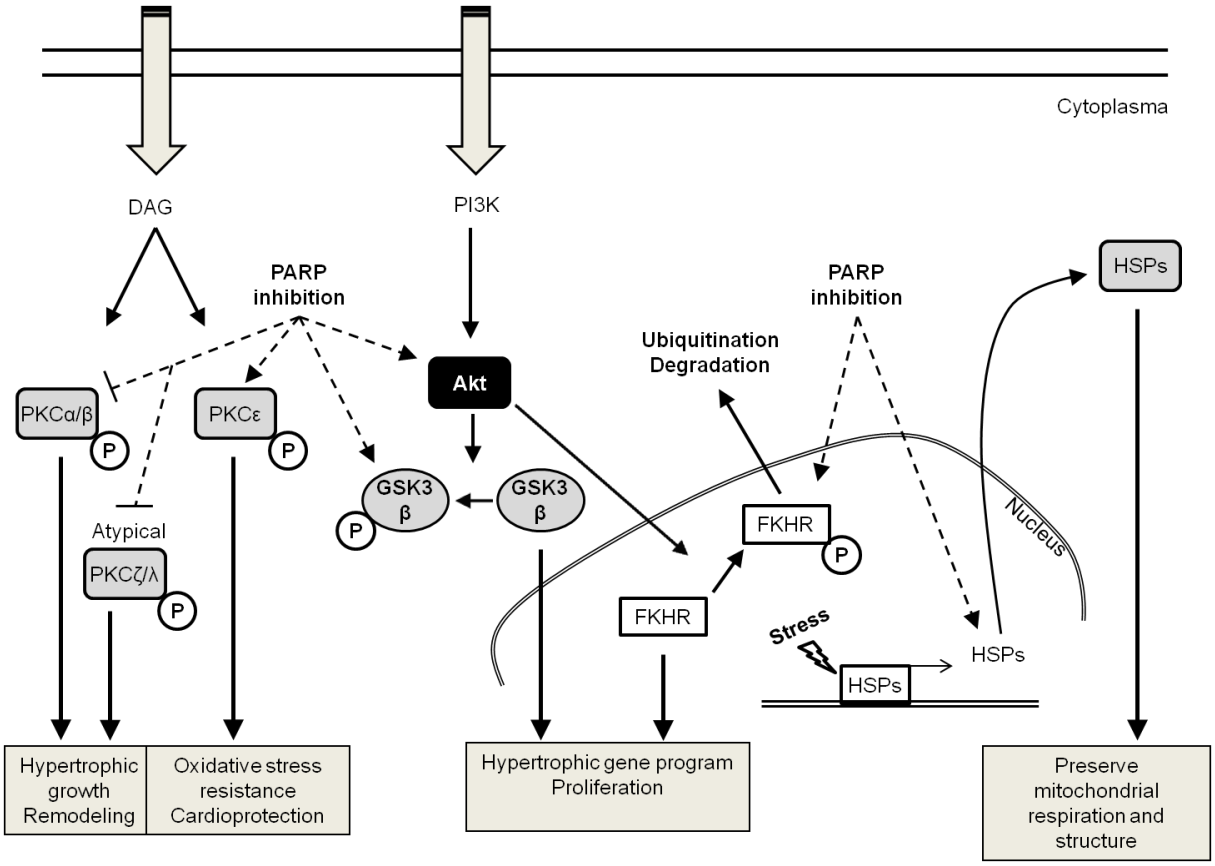

Figure 3. Summary of pathway alterations due to L-2286 treatment.

Effect of FGY-1153 on body weight

Body weights were measured and recorded once weekly during the treatment period. There were no significant differences between the three groups (Fig. 4).

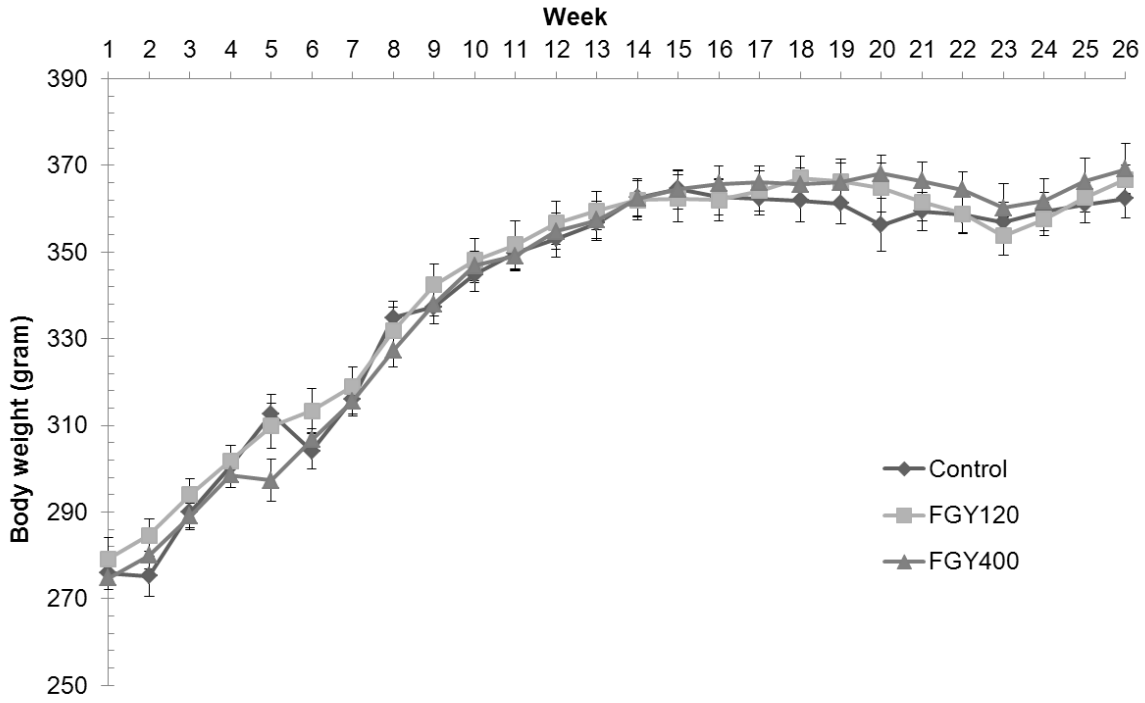

Fig. 4. Effect of FGY-1153 on body weight during the treatment period. Data are presented as mean±S.E.M. One-way ANOVA analysis conducted for each week did not reveal statistically significant differences between groups.

Effect of FGY-1153 on food consumption

The quantity of food consumed by each cage of animals was measured and recorded once weekly during the treatment period. There were no overt differences between the food consumptions of the three groups throughout the study. (Fig. 5).

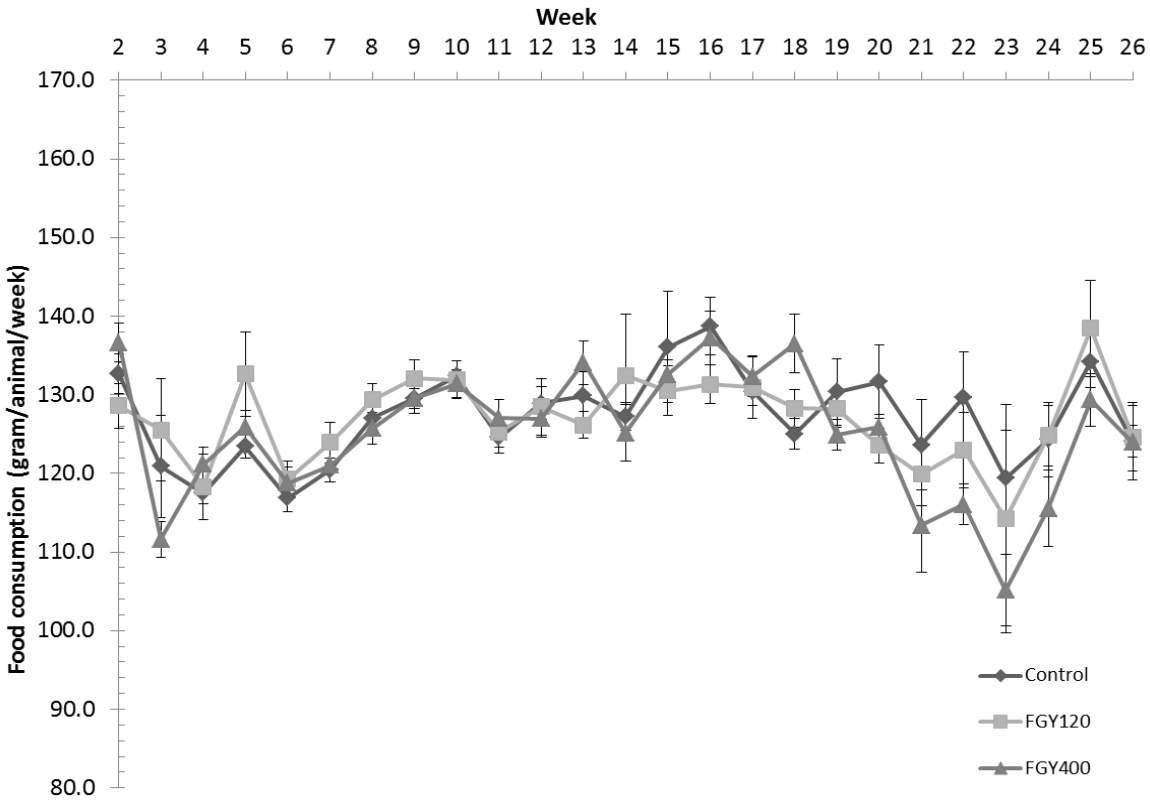

Fig. 5. Effect of FGY-1153 on food consumption during the treatment period. Data are presented as mean±S.E.M. Data were analysed with one-way ANOVA. No statistically significant differences were found at any time points between the groups.

Effect of FGY-1153 on blood pressure

At the beginning of the study there was no significant difference between the mean arterial blood pressure of the three groups (Control: 178.71±3.488 mm Hg, FGY120 group: 172.47±3.810 mm Hg, FGY400 group: 174.53±2.303 mm Hg, p=0.374). The FGY-1153 treatment seemed to have no significant effect on blood pressure parameters either at Week 13 or and Week 26. Mean arterial blood pressure values did not differ significantly between the groups at Week 13 (Control: 215.93±6.114 mm Hg, FGY120 group: 212.73±5.682 mm Hg, FGY400 group: 228.80±4.488 mm Hg, p=0.096) and at Week 26 (Control: 256.36±8.039 mm Hg, FGY120 group: 256.80±7.693 mm Hg, FGY400 group: 275.33±3.067 mm Hg, p=0.078).

Nevertheless, a non-significant trend of higher blood pressure in the FGY400 group compared to the other two groups was apparent.

Effect of FGY-1153 on echocardiographic parameters

Compared to the parameters measured at the beginning of the study, the septum and posterior wall thicknesses increased in all groups during the treatment period. However treatment with both low dose and high dose FGY-1153 significantly attenuated the elevation of these parameters indicating that the treatment with FGY-1153 reduced the hypertension induced left ventricular hypertrophy.

LVIDs and LVESV were also increased in all groups during the study, the elevation of these parameters were however significantly attenuated in the FGY120 group, but not in the FGY400 group. Left ventricular systolic function - expressed as ejection fraction (EF%) - showed a decreasing tendency in both the Control group and the FGY400 group by the end of the study compared to the initial parameters. In comparison with the Control group these changes were however significantly attenuated in the FGY120 group, indicating that the low dose FGY-1153 treatment prevented the hypertension induced decrease in systolic left ventricular function. The E/E' ratio showed an increasing tendency during the study in the Control group, while this parameter was significantly decreased in both of the FGY120 and FGY400 groups. It may indicate that FGY-1153 treatment could attenuate the diastolic dysfunction seen in SHR rats.

In the last column of Table 4 the typical values of structural and functional parameters of age-matched normotensive animals (WKY group) can be seen.

	SHR Week 0	**Control** Week 26	**FGY120** Week 26	**FGY400** Week 26	**WKY** age-matched
Septum (mm)	1.66 ± 0.01	2.09 ± 0.04	1.90 ± 0.04**	1.88 ± 0.02**	1.67 ± 0.07**
Post. Wall(mm)	1.58 ± 0.02	1.94 ± 0.02	1.82 ± 0.01*	1.81 ± 0.04*	1.644 ± 0.11*
LVIDd	7.28 ± 0.07	8.28 ± 0.08	7.94±0.09*	7.98 ± 0.11	8.00 ± 0.25
LVIDs	4.40 ± 0.07	5.38 ± 0.09	4.85 ± 0.08**	5.12 ± 0.14	4.52 ± 0.12**
LVEDV (ml)	280.49 ± 6.06	373.54 ± 8.11	340.79 ± 9.25*	344.72 ± 10.78	349.85± 24.66
LVESV (ml)	88.72 ± 3.33	141.56 ± 5.89	111.69 ± 4.15**	127.31 ± 8.66	97.07 ± 5.54**
EF (%)	68.48 ± 0.75	62.16 ± 1.24	67.10 ± 1.33*	63.36 ± 1.37	71.67 ± 0.87**
E/E'	35.16 ± 1.54	42.17 ± 5.26	30.05 ± 0.86*	26.50 ± 2.77*	30.00 ± 2.26
RWT	0.447 ± 0.004	0.485 ± 0.014	0.469 ± 0.007	0.464 ± 0.011	0.413 ± 0.01**

Table 4. Evaluation of echocardiographic parameters. Data of all animals are presented in the first column (SHR Week 0, N=21) at the beginning of the study and data from the three groups (Control, FGY120, FGY400 Week 26) (N=7 in each groups) are indicated at the end of the treatment period. Last column represents the data of age-matched normotensive animals (WKY, N=7). Values are expressed as mean±S.E.M. Comparisons between WKY and Control groups were made by independent samples t-test. Data of Control and Treatment groups were analysed with one-way ANOVA followed by Dunnett's post-hoc test. (*p<0.05, **p<0.01 vs. Control).

Effect of FGY-1153 on the interstitial fibrosis of heart and great vessels

The ANOVA analysis of interstitial fibrosis in SHR heart samples revealed no statistically significant difference between Control and Treatment groups (p=0.783). The collagen content however in WKY hearts was significantly lower (p=0.025) compared to the hypertensive Control group (Mean area fractions ± SEM: WKY: 0.390±0.021; Control: 0.657±0.069; FGY120: 0.636±0.088; FGY400: 0.582±0.041;). A statistically non-significant increase of vascular collagen could be observed in carotid arteries and aortas in Control group compared to WKY. No significant differences could be found between Control, FGY120 and FGY400 groups (Mean area fractions ± SEM: Aorta: WKY: 1.084±0.112 (p=0.536 vs. Control); Control: 1.378±0.414; FGY120: 1.239±0.526; FGY400: 1.458±0.324, (p=0.936); Carotid arteries: WKY: 4.860±0.532 (p=0.229 vs Control); Control: 5.994±0.660; FGY120: 5.745±1.465; FGY400: 5.158±1.097; (p=0.866), data not shown).

Effect of FGY-1153 on the intima-media thickness of great vessels

In comparison with the Control group, the intima-media thickness (IMT) of aorta was not altered significantly (p=0.718) neither in the FGY120 nor in the FGY400 groups. IMT was however significantly smaller in WKY group (Fig. 6) (p=0.0012 vs. Control).

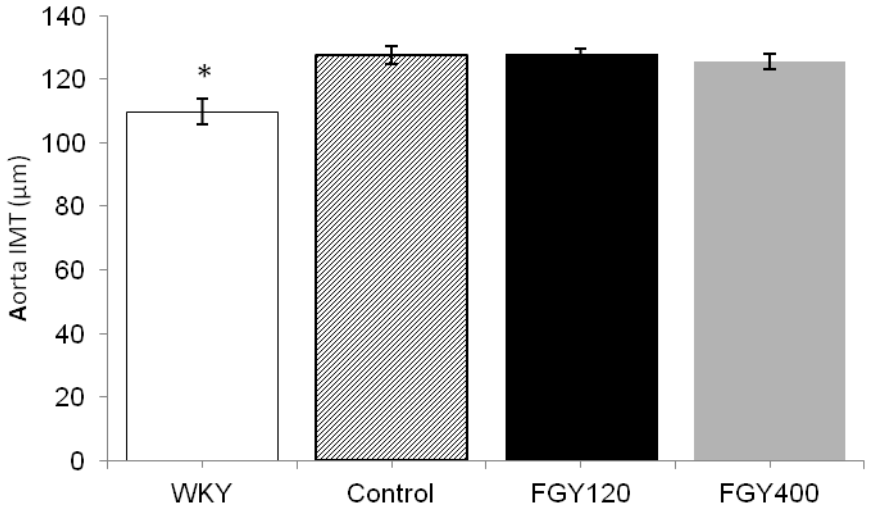

Fig. 6. Effect of FGY-1153 on the aortic intima-media thickness. (*p<0.05 vs. Control group).

In comparison with the Control group, the intima-media thickness of carotid vessels was slightly decreased in both the FGY120 and FGY400 groups. However the alterations were not significant (p=0.149). The IMT of carotid arteries was the lowest in the WKY group (Fig. 7) (p=0.031 vs. Control).

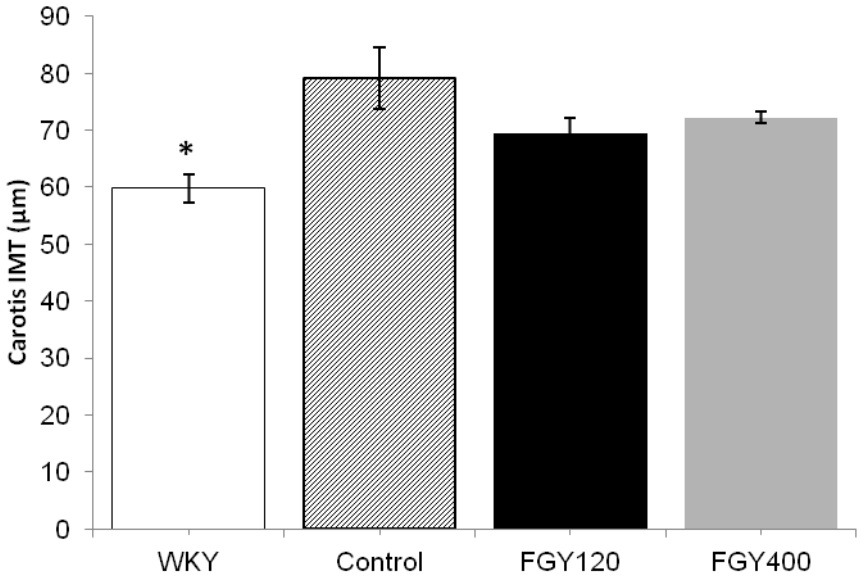

Fig. 7. Effect of FGY-1153 treatment on intima-media thickness of carotid vessels. ($^{*}p<0.05$ vs. Control group).

Effect of FGY-1153 on the TGFβ/SMAD2 signaling pathway in heart and great vessels

Western blot analysis of heart samples.

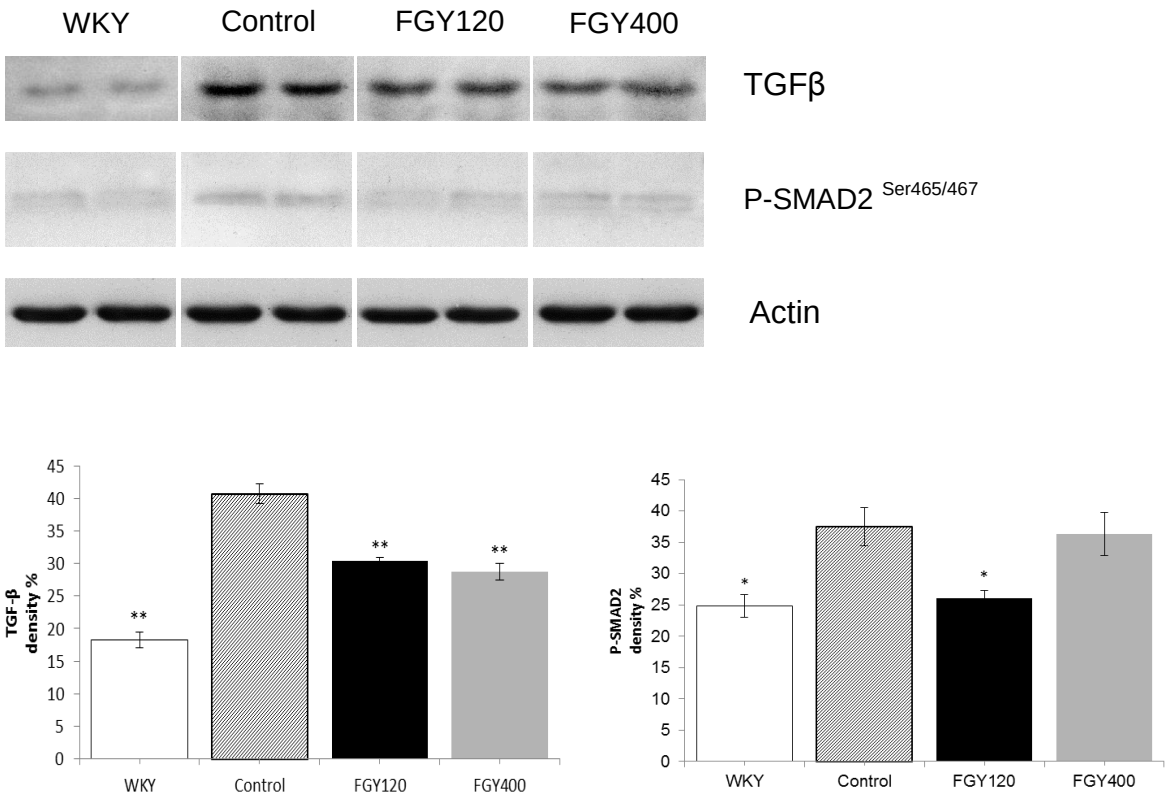

Fig. 8. The effect of FGY-1153 on the TGFβ/SMAD2 signaling pathway in heart samples. Western blot analysis showed that FGY-1153 treatment inhibited the cardiac expression of TGFβ and the phosphorylation of the SMAD2 protein in the FGY120 group, however the high dose treatment had no effect on the phosphorylation of SMAD2 in the FGY400 group. Actin is shown as loading control. Representative immunoblots from four experiments and densitometric evaluation are demonstrated. Data are presented as mean±S.E.M. Data were analysed with one-way ANOVA followed by Dunett's post-hoc test. *$p<0.05$, **$p<0.01$ vs. Control

Western blot analysis of aorta samples.

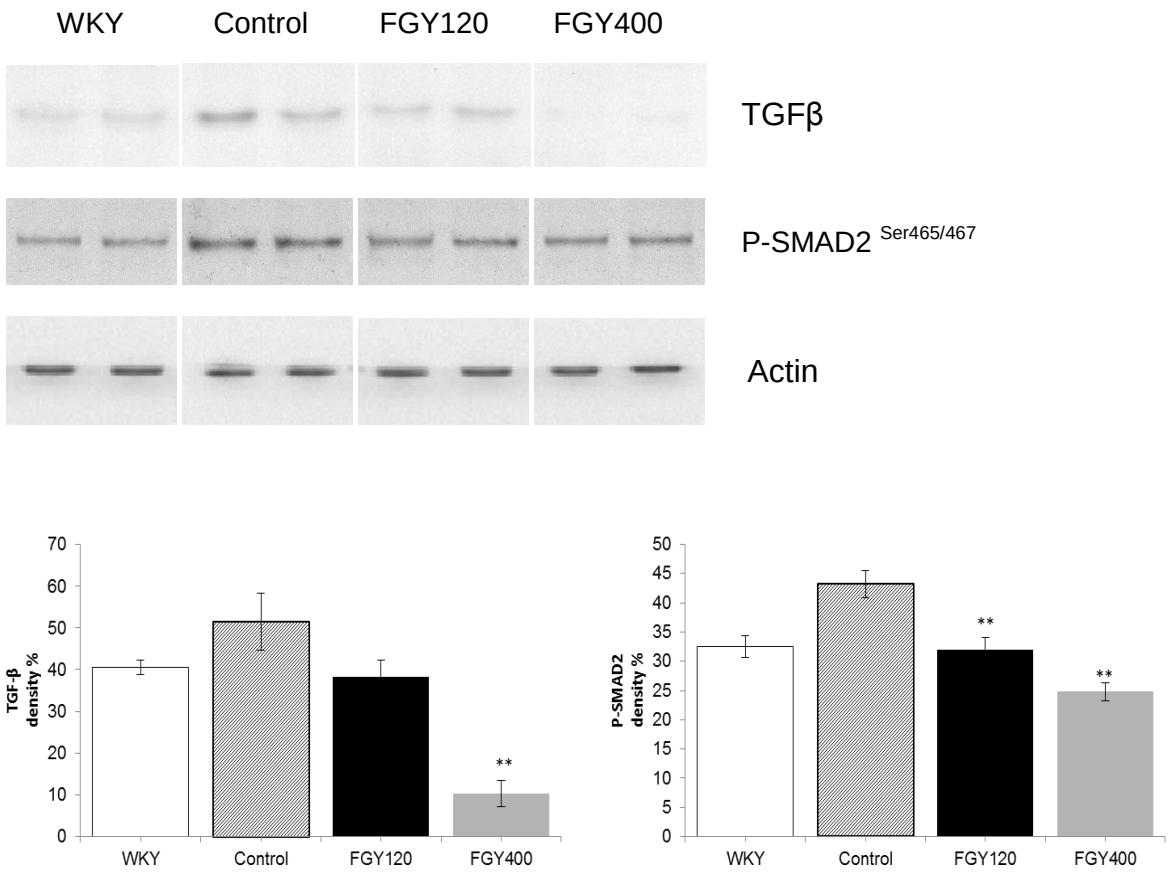

Fig. 9. The effect of FGY-1153 on the TGFβ/SMAD2 signaling pathway in the aortic wall. Western blot analysis showed that low dose FGY-1153 treatment had no significant effect on the TGFβ expression, however the high dose treatment significantly inhibited the expression of TGFβ in the FGY400 group in comparison with both the Control and FGY120 group. The phosphorylation of the SMAD2 protein was significantly decreased in both the FGY120 and in FGY400 aortic samples. Actin is shown as loading control. Representative immunoblots from four experiments and densitometric evaluation are demonstrated. Data are presented as mean±S.E.M. Data were analysed with one-way ANOVA followed by Dunett's post-hoc test. *$p<0.05$, **$p<0.01$ vs. Control

Western blot analysis of carotis samples.

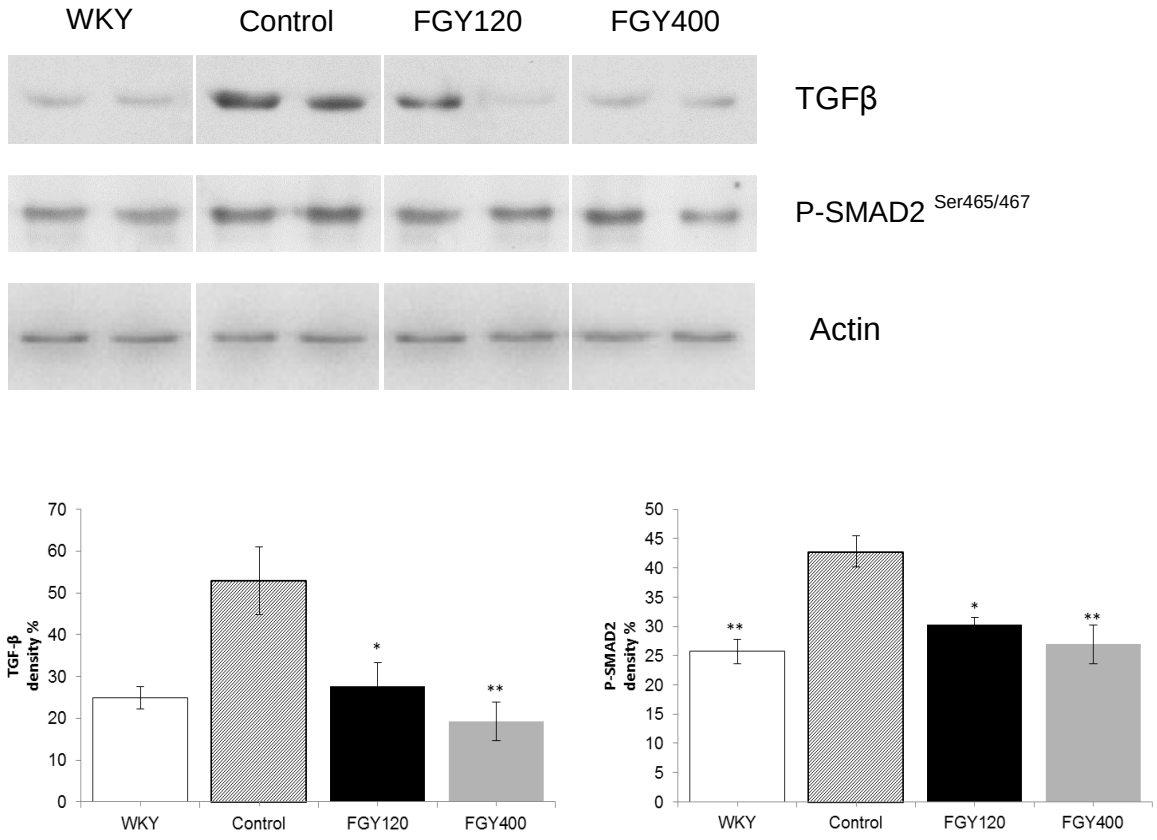

Fig. 10. The effect of FGY-1153 on the TGFβ/SMAD2 signaling pathway in the carotid arteries. Western blot analysis showed that both TGFβ expression and SMAD2 phosphorylation levels were significantly higher in Control group relative to WKY. Both high and low dose FGY-1153 treatment significantly inhibited the expression of TGFβ. The phosphorylation of the SMAD2 protein was significantly decreased in both the FGY120 and in FGY400 groups in carotid tissues. Actin is shown as loading control. Representative immunoblots from four experiments and densitometric evaluation are demonstrated. Data are presented as mean±S.E.M. Data were analysed by independent samples t-test between WKY and Control groups. Comparisons of Control and Treatment groups were made by one-way ANOVA followed by Dunnett's post-hoc test. *$p<0.05$, **$p<0.01$ vs. Control.

Effect of FGY-1153 on the phosphorylation of Akt/GSK-3β signaling cascade in heart and great vessels

Western blot analysis of heart samples

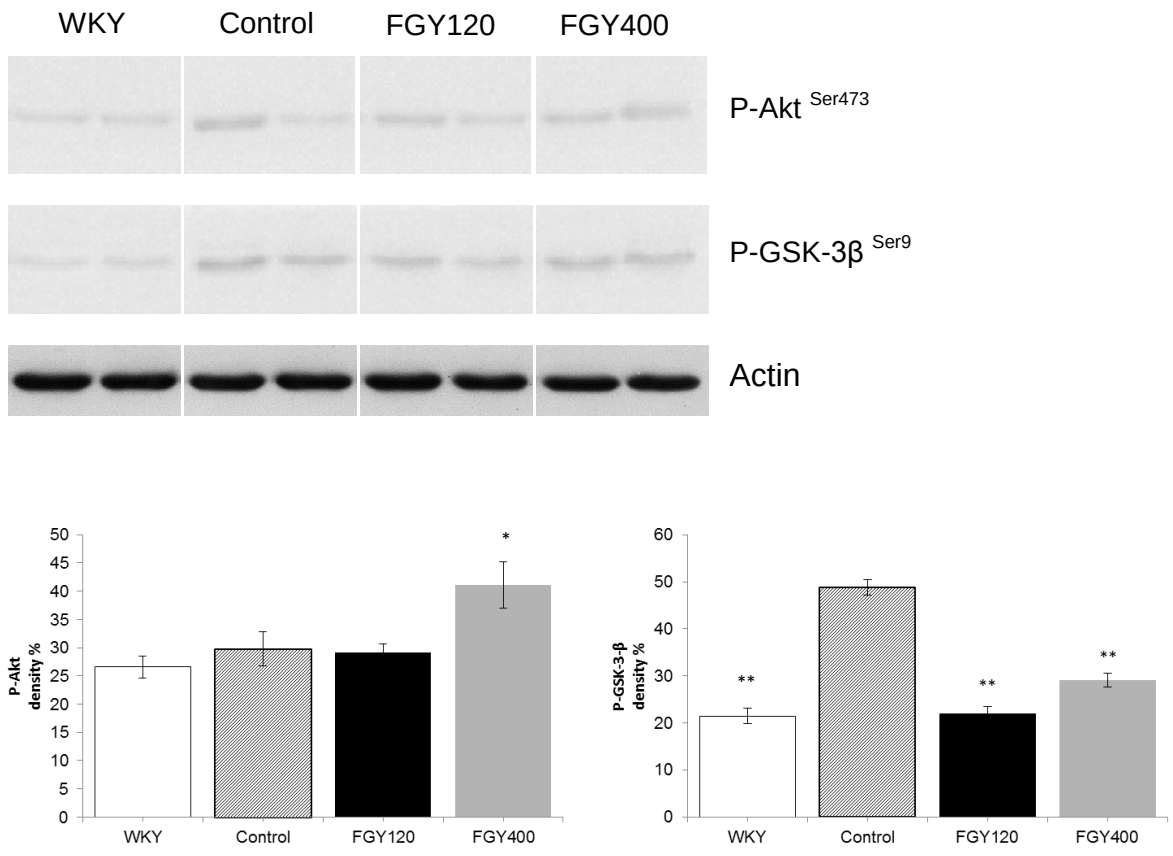

Fig. 11. The effect of FGY-1153 on the Akt/GSK-3β signaling cascade in heart samples. Western blot analysis showed GSK-3β phosphorylation to be significantly lower in WKY group relative to Control. High dose of FGY-1153 treatment significantly elevated phosphorylation of Akt protein, and both low dose and high dose treatment significantly attenuated the GSK-3β phosphorylation. Actin is shown as loading control. Representative immunoblots from four experiments and densitometric evaluation are demonstrated. Data are presented as mean±S.E.M. Data were analysed by independent samples t-test between WKY and Control groups. Comparisons of Control and Treatment groups were made by one-way ANOVA followed by Dunnett's post-hoc test. *$p<0.05$, **$p<0.01$ vs. Control.

Western blot analysis of aorta samples.

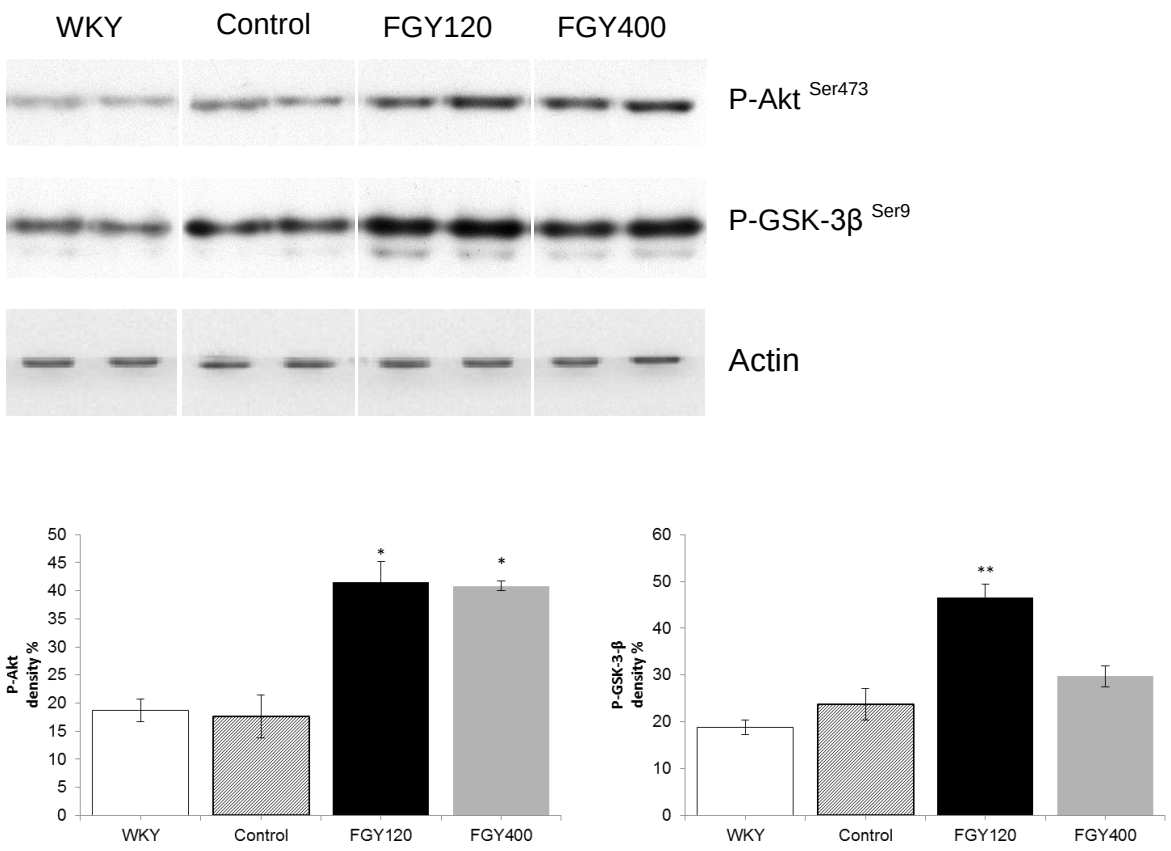

Fig. 12. The effect of FGY-1153 on the Akt/GSK-3β signaling cascade in aortic wall. Western blot analysis showed that FGY-1153 treatment significantly promoted the phosphorylation of Akt protein in the aortic tissues of both FGY120 and FGY400 groups, and the GSK-3β phosphorylation in the FGY120 group. However the high dose treatment had no effect on the GSK-3β phosphorylation in the FGY400 group. Actin is shown as loading control. Representative immunoblots from four experiments and densitometric evaluation are demonstrated. Data are presented as mean±S.E.M. Data were analysed by independent samples t-test between WKY and Control groups. Comparisons of Control and Treatment groups on GSK-3β data were made by one-way ANOVA followed by Dunnett's post-hoc test. On Akt data one-way ANOVA with Welch correction were conducted followed by Dunnett T3 post hoc test. *p<0.05, **p<0.01 vs. Control.

Western blot analysis of carotis samples.

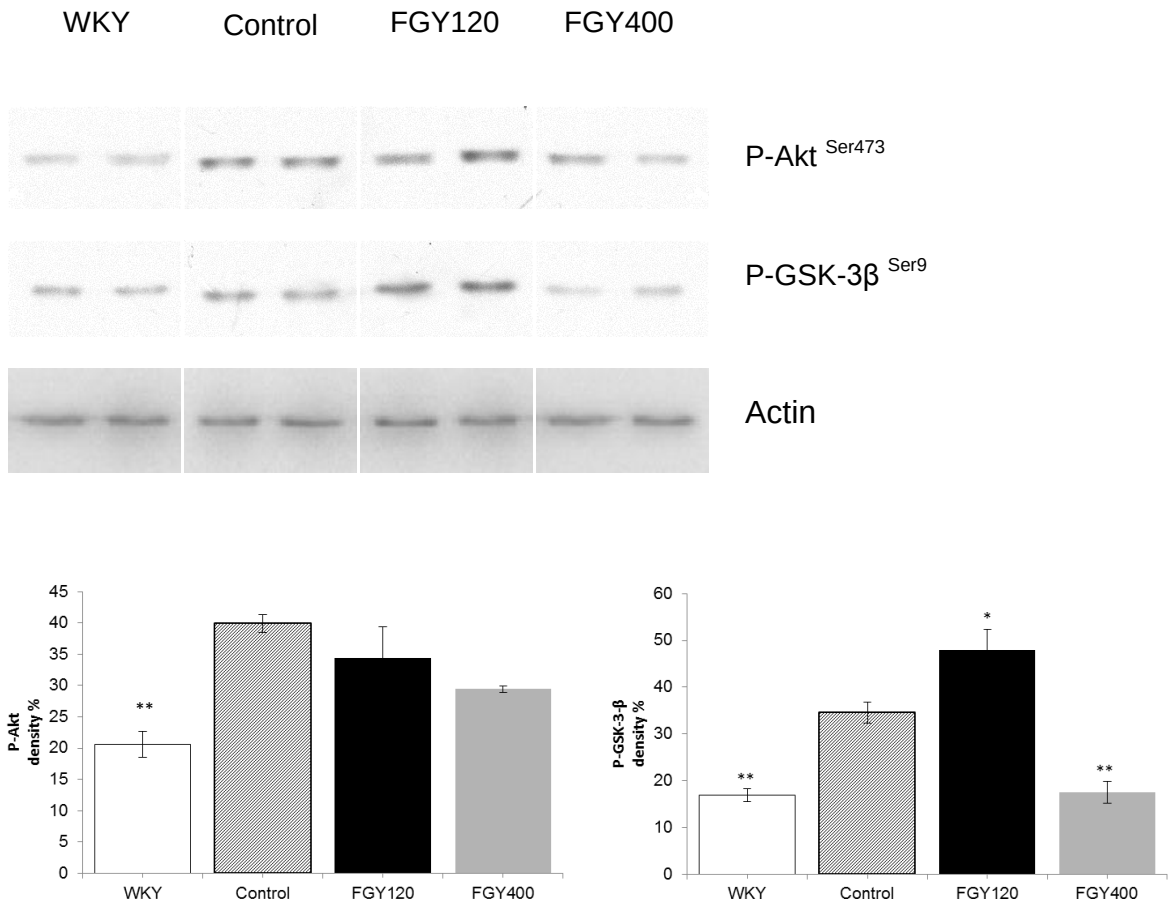

Fig. 13. The effect of FGY-1153 on the Akt/GK-3β signaling cascade in carotid vessels. Western blot analysis showed that phosphorylation level of both Akt and GSK-3β proteins were significantly higher in Control group relative to WKY. Low dose FGY-1153 treatment promoted while high dose treatment decreased the phosphorylation of GSK-3β protein, however it did not significantly influenced the Akt-1 phosphorylation in the carotid tissues of SHR rats. Actin is shown as loading control. Representative immunoblots from four experiments and densitometric evaluation are demonstrated. Data are presented as mean±S.E.M. Data were analysed by independent samples t-test between WKY and Control groups. Comparisons of Control and Treatment groups were made by one-way ANOVA followed by Dunnett's post-hoc test. *p<0.05, **p<0.01 vs. Control.

CONCLUSIONS

In our study, we examined the effect of a PARP inhibitor (L-2286) in SHR at the stage of the development of LV hypertrophy. L-2286 exerted a beneficial effect on the progression of myocardial hypertrophy (thickness of PW and septum, RWT) and myocardial fibrosis. In the background of these changes, we did not observe any blood pressure lowering effect of PARP-inhibition. According to our results, PARP-inhibition can exert this antihypertrophic effect due to the activation of several prosurvival (especially Akt-1/GSK-3β, FKHR, PKCε and Hsp90) and the inhibition of prohypertrophic (PKC- α/βII, - ζ/λ) protein kinases.

In conclusion, pharmacological inhibition of PARP-1 enzyme exerted significant protection against hypertensive cardiac remodeling in spite of the lack of having any antihypertensive effect. Therefore PARP can be a promising therapeutic target to prevent hypertensive cardiac complications even in those patients who do not reach the target blood pressure because of complaints or because of side effects caused by antihypertensive drug therapy. Our previous [36] and present results can introduce a new concept into the treatment of essential hypertension, namely to lower blood pressure to a more tolerable level and to prevent target organ damages by PARP-inhibition.

The long-term administration of the bradykinin B1 receptor antagonist compound FGY-1153 did not have any deleterious effects in SHR rats. Moreover we could observe some protective effect against the development of hypertensive cardiovascular remodeling despite that FGY-1153 did not have any antihypertensive effect. Inhibition of the TGF-β-Smad signaling may be the main underlying mechanism in the background of the cardiovascular protective effect.

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