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Original article

Antihypertensive and cardioprotective effects of different monotherapies and combination therapies in young spontaneously hypertensive rats – A pilot study

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ABSTRACT

Spontaneously hypertensive rats (SHR) are an established animal model for antihypertensive treatment. The aim of this pilot study was a systematic search for two lines of antihypertensive treatment – a monotherapy and a combination of two drugs – to be applied in a future study on old SHR. Originally, representatives of three drug classes recommended for antihypertensive therapy in humans should be applied, namely captopril (CAP) as an antagonist of the renin-angiotensin-aldosterone system, nifedipine (NIF) as calcium channel blocker and propranolol (PROP) as β -adrenergic blocker. As we observed that PROP had been poorly ingested, all groups with PROP therapy were excluded from the study. CAP ($60 \text{ mg kg}^{-1} \text{ d}^{-1}$), NIF ($10 \text{ mg kg}^{-1} \text{ d}^{-1}$) or both were administered orally to seven-week-old SHR over 3 weeks. A further group of SHR received no treatment (SHR/CTRL). Age-matched normotensive Wistar-Kyoto rats served as normotensive controls. We examined the effect of the antihypertensive therapies on systolic blood pressure, heart weight and on histological and biochemical markers of cardiac hypertrophy and fibrosis.

CAP proved to be the most effective treatment reducing blood pressure and relative heart weight significantly compared to SHR/CTRL without reaching normotensive values. Beginning cardiac fibrosis observed in SHR/CTRL was completely abrogated with CAP treatment. Similar effects were achieved with a combination of CAP and NIF. CAP as monotherapy and CAP + NIF as combination therapy were chosen for the forthcoming study on old SHR.

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1. Introduction

Arterial hypertension is number one risk factor for morbidity and mortality of cardiovascular diseases and the leading cause of death in industrialized countries. In 2018, the global prevalence of hypertension has been estimated to be around 40% (Mazur et al., 2019). Untreated or insufficiently treated arterial hypertension leads to constant pressure load of the left ventricle (LV) and

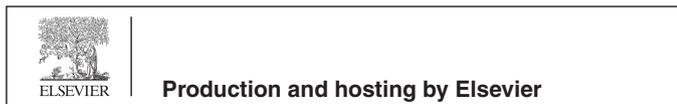
first results in cardiac hypertrophy and may progress to cardiac remodeling, fibrosis, dilatation and in final stages to heart failure. The earlier in the course of pathophysiological development an efficient antihypertensive therapy is started, the more successful it can be in preventing complications (Demirci et al., 2005; Zicha et al., 2008).

Spontaneously hypertensive rats (SHR) are a common animal model for studying causes and development of hypertension as their prehypertensive stage and their clinical complication pattern is similar to that of humans (Boluyt and Bing, 2000). Consequently, they also serve as a model for antihypertensive treatment. For antihypertensive therapy in humans, drugs antagonizing the renin-angiotensin-aldosterone-system (RAAS) and calcium channel blockers are highly recommended. In addition, β -blockers should be used when a specific cardiac indication is given (Rochlani et al., 2017; Thomopoulos et al., 2015; Williams et al., 2018). In SHR,

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upregulation of RAAS-related key genes and increased intracellular calcium concentration belong to the main factors responsible for the increased blood pressure (BP) (Frank et al., 2002; Williamson et al., 2017). Counteracting these factors by antagonizing the RAAS and reducing intracellular calcium concentration induced significant antihypertensive effects and, in addition, cardioprotective and further beneficial effects by reducing end organ damage (Camilion de Hurtado et al., 2002; Demirci et al., 2005; Gan et al., 2018; Rocha et al., 2010; Rodrigo et al., 1997; Xie et al., 2008). If started very early in life (between 4 and 10 weeks of age), those drugs reduced blood pressure (BP) even to normotension (Zicha et al., 2008) and prevented cardiovascular complications (Demirci et al., 2005; Hale et al., 2012; Rocha et al., 2010). Treatment with β -blockers, particularly with propranolol, also decreased BP, reduced sympathetic vasomotor tone and inhibited cardiac fibrosis in SHR (Su et al., 1999; Takeda and Buñag, 1980).

One of the major problems in the treatment of human hypertension is the fact that hypertension is often diagnosed late in life when the BP has been elevated for many years and cardiovascular complications may have already developed. A therapy started in this stage of disease is probably less effective than a treatment started in the very early stage of hypertension. For this reason, we have planned a study on old SHR to investigate the efficiency of an antihypertensive therapy started late in life. The present study was designed as a pilot study to systematically probe antihypertensive drugs in young SHR to identify the most efficient treatment lines, namely a monotherapy and combination of two drugs, for the subsequent study in old SHR. Originally, a RAAS antagonist (captopril, an inhibitor of angiotensin-converting enzyme (ACE)), a calcium channel blocker (nifedipine, a dihydropyridine) and a β -blocker (propranolol) were planned to be administered to young SHR. We assessed the efficiency of the drugs in lowering BP and reducing cardiovascular sequelae of hypertension, in particular, LV hypertrophy and remodeling. We investigated BP, heart weight (HW), biochemical markers of cardiac hypertrophy and cardiac fibrosis as well as cardiac histology.

2. Materials and methods

2.1. Animal model

The experiments were performed on 42 male SHR and 8 male Wistar-Kyoto rats (WKY), all supplied by Charles River, Sulzfeld, Germany. The animals were delivered to the Experimental Centre of the Faculty of Medicine at the University of Leipzig at the age of 3–4 weeks and stayed there for about two weeks. They were fed a standard pellet diet (Altromin C100, Altromin GmbH, Lage, Germany) and had free access to tap water. All animal protocols were approved by the state agency in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and with the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes” (Council of Europe, 1986).

2.2. Experimental design

At the age of 5 weeks, the animals came into the Carl-Ludwig-Institute for Physiology where the experiments were performed. The study phase was preceded by a 2-week adaptation period for accustoming the animals both to drug-free tablets and to the procedure of BP measuring. The experiments started in the 7th week of life and lasted 3 weeks. The initial body weight (BW) of the SHR and WKY at the beginning of the experiment, was 185.7 ± 6.0 g and 188.9 ± 3.9 g, respectively. WKY served as normotensive controls (further referred to as WKY/CTRL). The SHR were divided

randomly into 7 groups ($n = 6$ per group). Three groups were planned for monotherapy, three for combination therapy and one as untreated control group. However, we observed that PROP had been poorly ingested. Therefore, all groups with PROP therapy were excluded from the study. Finally, 24 SHR remained in the study. Eighteen SHR received monotherapy with captopril (CAP, $60 \text{ mg kg}^{-1} \text{ d}^{-1}$; Axxora, Lörrach, Germany) or nifedipine (NIF, $10 \text{ mg kg}^{-1} \text{ d}^{-1}$; Sigma-Aldrich, Deisenhofen, Germany), or a combination therapy with CAP $60 + \text{NIF } 10 \text{ mg kg}^{-1} \text{ d}^{-1}$. These drug doses had been chosen as proven effective in significantly reducing BP in SHR (Camilion de Hurtado et al., 2002; Rodrigo et al., 1997; Xie et al., 2008). The drugs were added to commercially available rodent sweets (Vitakraft-Werke, Wührmann & Sohn GmbH & Co. KG, Bremen, Germany). The sweets were given into the cages for oral uptake along with chow once daily between 9 and 10 a.m. for 3 weeks. The treated groups are further referred to as SHR/CAP, SHR/NIF and SHR/CAP + NIF. The last SHR group that served as untreated control group (SHR/CTRL) and the WKY/CTRL group received drug-free sweets as placebo. During the experimental time, non-invasive BP measurements were performed weekly (see below). At the end of the experimental period, animals were sacrificed, and their hearts were removed for further analyses.

2.3. Non-invasive blood pressure measurement

In all animals, systolic blood pressure (SBP) was measured non-invasively in the awake state using the tail-cuff-method (TSE Blood Pressure Monitor, Series 209002, TSE Systems GmbH, Bad Homburg, Germany). The animals were placed on a heated plate (36°C) and were held loosely by the experimenter's hand, which allowed them to move relatively freely. This method is less stressful than the use of a conventional restraint box, and it was tolerated much better by the animals. After six to eight preliminary tests, animals usually became familiarized with the procedure and remained quiet during the measurements. To ensure the reproducibility of the results, the mean was calculated from two to three tests with each test containing 10 single readings. All animals from the same group were measured the same day.

2.4. Hemodynamic measurements

Heart catheterization was performed 1–3 days after the last SBP measurement. Animals were anesthetized with thiopental (Trapanal® 80 mg kg^{-1} , i.p.). They were tracheotomized, and a polyethylene cannula was placed in the trachea. The right ventricle (RV) and left ventricle (LV) were catheterized with Millar® (Millar Instruments, Houston, TX) ultraminiature catheter pressure transducers to measure RV and LV systolic pressures (RVSP, LVSP). After withdrawal of the LV catheter tip into the aorta, diastolic aortic pressure (DAP) was measured.

2.5. Further analyses on heart tissue

After hemodynamic measurements, hearts were excised and weighed. Heart weight (HW) normalized to BW (HW/BW) was determined as a measure of cardiac hypertrophy. Then, the apex was separated and fixated in formalin for histological examination. Pieces of the LV were frozen and stored at -80°C for biochemical analyses.

2.6. Ribonuclease Protection assay

A ribonuclease protection assay was performed for determination of mRNA expression of atrial natriuretic peptide (ANP), Transforming Growth Factor- β_1 (TGF- β_1), TGF- β_2 and TGF- β_3 , matrix metalloproteinase 2 (MMP-2), tissue inhibitor of

metalloproteinases 2 (TIMP-2) and collagen types I (Coll I) and III (Coll III) in the LV.

Total RNA isolation was performed according to a method of Chomczynski and Sacchi (1987) using TRIZOL (Invitrogen GmbH, Karlsruhe, Deutschland) according to the manufacturer's protocol. The isolated RNA was hybridized with template sets extracellular matrix (ECM)-3 and rTGF- β and labelled with RiboQuant® In vitro Transcription Kit (Pharmingen, Hamburg, Germany) and [α -³²P]-UTP as described by the manufacturer.

For investigation of TGF- β mRNA 2.5 μ g of total RNA and for ECM (ANP, Coll I, Coll III, MMP-2, TIMP-2) 5 μ g of total RNA was used for hybridization. After overnight hybridization, the unprotected probes were digested with RNases. The protected radioactive RNA was displayed on a denaturing polyacrylamide gel. Densitometric evaluation was performed using the Molecular Imager (BioRad, München, Germany). mRNA expression was semi-quantitatively determined in relation to glyceraldehyde-3-phosphate dehydrogenase (GAP-DH)-mRNA that was obtained from the same sample as the mRNA of interest.

2.7. Histological investigation of the heart

For histological evaluation of cardiac fibrosis, the apex was embedded in paraffin, and 8–9 μ m-thick sections were stained with hematoxylin–eosin and Masson's trichrome. Histological section planes of rat hearts perpendicular to the heart axis were prepared. To evaluate the histological findings, the sections were examined with Axioscope microscope (Carl Zeiss, Oberkochen, Germany), digitized using AxioCam MRc 5 (Carl Zeiss, Oberkochen, Germany) and then evaluated using Photoshop CS6 image processing program. The histological degree of fibrosis was assessed by a score ranging from 0 to 3 (0 = no signs of fibrosis; 0.5 = marginal perivascular fibrosis and/or marginal interstitial fibrosis; 1 = perivascular fibrosis + mild interstitial fibrosis; 2 = perivascular fibrosis + moderate interstitial fibrosis; 3 = fibrosis of the entire heart).

2.8. Statistical analysis

The results of the biochemical analyses are demonstrated as medians with 25% and 75% percentiles. SBP, HW/BW, hemodynamic parameters and quantitative evaluations from histology are presented as means \pm SEM.

Using the program SIGMAPLOT (Systat Software GmbH, Erkrath, Germany) multiple-sample comparisons were performed. These initially included a one-way variance analysis (ANOVA) or a rank-based ANOVA according to Kruskal and Wallis. If ANOVA indicated the existence of significant differences between the groups, a multiple range test with the criterion of least significant differences (Fisher's LSD) or a multiple comparison according to Dunn's method followed. Differences of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Course of the experiment

At the beginning of the adaptation period, animals were anxious and difficult to handle, in particular during the SBP measurement procedure. During the two-week adaptation period, the animals became familiar with their environment, with the experimenters, with the drug-free tablets and with the SBP measurement procedure, although there were differences among the animals. In general, we observed that SBP results were more consistent and more reliable when the animals were not restrained but only

gently held by the experimenter's hand. As the animals were sensitive to noise and sudden movements, we took care to calmly handle the animals and to avoid external interference as far as possible. When the drug-free rodent sweets were given into the cages, part of them was eaten immediately, and another large part within the next 6 h. During the experimental period, tablets with drugs were eaten in a similar way.

3.2. Systolic blood pressure

Baseline SBP values of SHR groups ranged between 154 and 187 mmHg. In contrast, SBP of WKY/CTRL was 115 mmHg at the beginning of the measurements and was significantly lower than baseline SHR/CTRL value ($p < 0.001$). SBP in WKY/CTRL increased only slightly to 123.1 \pm 0.9 mmHg. In the SHR/CTRL group, SBP continued to rise over time up to 201.7 \pm 6.5 mmHg ($p < 0.001$ compared to WKY/CTRL). Treatment with CAP significantly decreased SBP (148.3 \pm 2.5 mmHg, $p = 0.008$ compared to SHR/CTRL). In the SHR/NIF and SHR/CAP + NIF groups, SBP showed a marked decline during the first two weeks but a partial re-increase afterwards. The final SBP in SHR/NIF was lower than at baseline but not significantly lower than in SHR/CTRL, while in SHR/CAP + NIF, final SBP was in the baseline range but significantly below the time-correspondent SHR/CTRL value (Fig. 1).

3.3. Results of heart catheterization

LVSP of SHR/CTRL was with 187.5 \pm 7.0 mmHg significantly above the WKY/CTRL value (141.0 \pm 4.3 mmHg, $p = 0.003$). DAP showed similar differences (155.2 \pm 5.1 vs 115.0 \pm 4.4 mmHg, $p < 0.001$). All types of treatment did not significantly decrease LVSP and DAP below the level of SHR/CTRL, however, there were no significant differences to WKY/CTRL (Fig. 2). RVSP was similar in all groups ranging between 24.7 \pm 2.3 (SHR/NIF) and 34.7 \pm 3.3 mmHg (SHR/CTRL) ($p = 0.13$, data not shown).

3.4. Cardiac hypertrophy

3.4.1. Heart weight

At the end of the experiment, HW/BW in SHR/CTRL rats was significantly increased (3.5 \pm 0.07) compared to normotensive WKY/CTRL (2.7 \pm 0.04). CAP and CAP + NIF therapies significantly reduced HW/BW but did not reach normotensive levels (Table 1).

3.4.2. ANP mRNA-expression

The expression of ANP mRNA, a marker of cardiac hypertrophy, was higher in SHR/CTRL than in WKY/CTRL (11.0 vs. 8.1% of GAP-

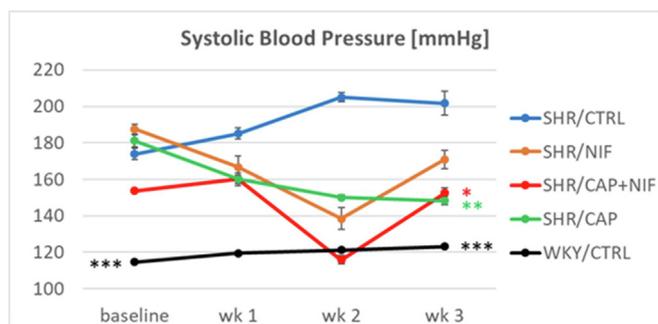


Fig. 1. Changes of systolic blood pressure (in mmHg; mean \pm SEM) over time. Measurements were performed at the beginning of the experimental period (baseline) and at the end of each experimental week (wk 1–3). Asterisks mark significant differences vs. time-correspondent SHR/CTRL: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

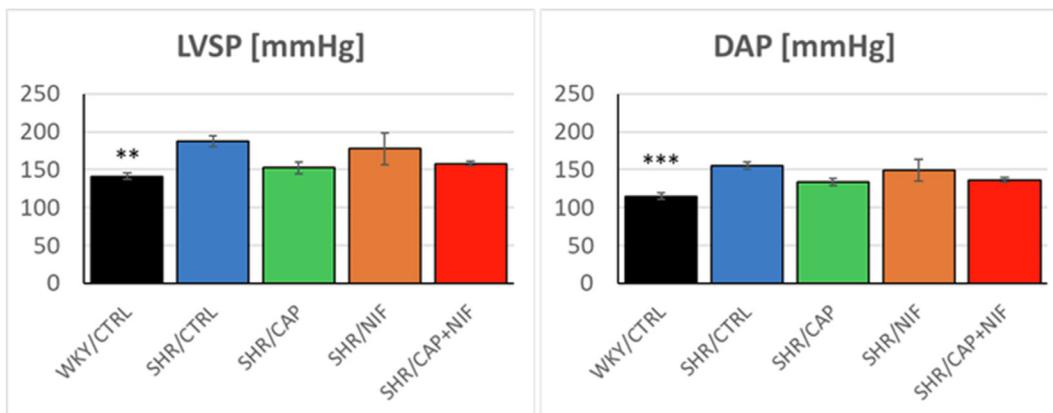


Fig. 2. Results of heart catheterization at the end of the experimental period. LVSP: left ventricular systolic pressure; DAP: diastolic aortic pressure. Values are given as mean ± SEM. Asterisks mark significant differences vs. SHR/CTRL: ** p < 0.01; *** p < 0.001.

Table 1
Markers of cardiac hypertrophy in young rats at the end of experiment (age 10 wks).

Group	HW/BW	ANP mRNA [% GAP-DH]
WKY/CTRL	2.71 ± 0.04 ***	8.1 [4.3/22.1]
SHR/CTRL	3.50 ± 0.07 ###	11.0 [7.5/20.3]
SHR/CAP	3.10 ± 0.08 ***, ###	6.1 [5.4/7.6]
SHR/NIF	3.40 ± 0.05 ###	20.8 [10.4/30.8]
SHR/CAP + NIF	3.06 ± 0.06 ***, ###	4.6 [3.7/6.7]

Heart weight (HW, means ± SEM), mRNA expression of atrial natriuretic peptide (ANP) in LV (median [25th /75th percentile]). Significance marks: *** p < 0.001 vs SHR/CTRL; ### p < 0.001 vs WKY/CTRL

DH expression, respectively). ANP expression varied widely among the groups. The lowest values were achieved by treatment with CAP and CAP + NIF (6.1% and 4.6%, respectively), but these differences were not significant (Table 1).

3.5. Cardiac fibrosis

3.5.1. Biochemical markers of cardiac remodeling

There were no significant differences in LV mRNA expression of TGF-β isoforms, MMP-2, TIMP-2, Coll I and Coll III between WKY/CTRL and SHR/CTRL rats. Neither did any of the therapies induce significant changes in the mRNA expression of these markers (Table 2).

3.5.2. Histological manifestation of cardiac fibrosis

In the hearts of SHR/CTRL rats, we observed histological signs of a beginning cardiac fibrosis (p < 0.01 compared to WKY/CTRL; Table 3). The hearts showed slight to moderate perivascular fibrosis and slight multifocal fine-spotted interstitial fibrosis (Fig. 3a). All treatments significantly reduced the degree of fibrosis. Treatment with CAP completely prevented fibrosis development. These

Table 2
Markers of cardiac remodeling at the end of experiment (age 10 wks).

Group	TGF-β1	TGF-β2	TGF-β3	MMP-2	TIMP-2	Coll I	Coll III
WKY/CTRL	1.74 [1.14/3.04]	0.11 [0.07/0.13]	0.95 [0.63/1.40]	5.08 [4.03/6.42]	10.05 [7.15/14.82]	18.8 [13.4/24.1]	28.1 [24.0/30.8]
SHR/CTRL	1.37 [1.34/1.54]	0.16 [0.14/0.18]	0.64 [0.54/0.67]	3.92 [3.57/4.69]	8.01 [6.95/10.20]	16.3 [9.6/16.6]	20.9 [20.1/22.4]
SHR/CAP	1.06 [0.85/1.40]	0.14 [0.09/0.22]	0.55 [0.44/0.87]	3.45 [3.42/4.45]	7.52 [5.73/11.43]	10.3 [9.6/12.9]	18.2 [15.6/19.2]
SHR/NIF	1.16 [1.14/1.18]	0.17 [0.13/0.17]	0.60 [0.55/0.61]	3.86 [3.61/4.34]	9.05 [7.33/10.75]	18.3 [17.2/21.1]	25.9 [21.8/30.1]
SHR/CAP + NIF	1.15 [1.02/1.56]	0.26 [0.15/0.38]	0.62 [0.52/1.09]	3.79 [2.82/6.07]	9.27 [5.76/11.19]	17.6 [9.5/25.7]	24.3 [18.9/35.4]

mRNA expression of isoforms of transforming growth factor (TGF)-β, matrix metalloproteinase (MMP)-2, tissue inhibitor of MMP (TIMP)-2 and collagen type I (Coll I) and type III (Coll III) in LV (in % of GAP-DH mRNA expression). Data is given as medians [25th/75th percentile].

Table 3
Degree of cardiac fibrosis.

Group	Degree of cardiac fibrosis
WKY/CTRL	0.50 ± 0.1 **
SHR/CTRL	1.47 ± 0.1
SHR/CAP	0.00 ± 0.0 ***
SHR/NIF	0.48 ± 0.1 **
SHR/CAP + NIF	0.59 ± 0.2 **

Data are given as means ± SEM. Asterisks mark significant differences to SHR/CTRL: ** p < 0.01; *** p < 0.001

animals presented only discrete perivascular fibrotic spots without pathologic value (Fig. 3b). In the SHR/CAP + NIF group, little perivascular or interstitial fibrosis with low pathological value was observed (Fig. 3c).

4. Discussion

Treatment with CAP proved to be the most efficient therapy. With CAP both as monotherapy and in combination with NIF, significant BP reduction as well as prevention of cardiac hypertrophy and fibrosis were achieved.

4.1. Development of hypertension and antihypertensive treatment in young SHR

SHR, which serve as an animal model for human essential hypertension, develop hypertension at an early age. Young SHR in their prehypertensive state (age 3–4 wk) present SBP of 171 ± 5 mmHg (Demirci et al., 2005). Until the age of 11 wk, it rises to 219 ± 14 mmHg (Hale et al., 2012). In contrast, SBP of young and adult WKY rats ranges between 137 and 140 mmHg (Demirci et al., 2005). The baseline SBP values observed in the present study are in

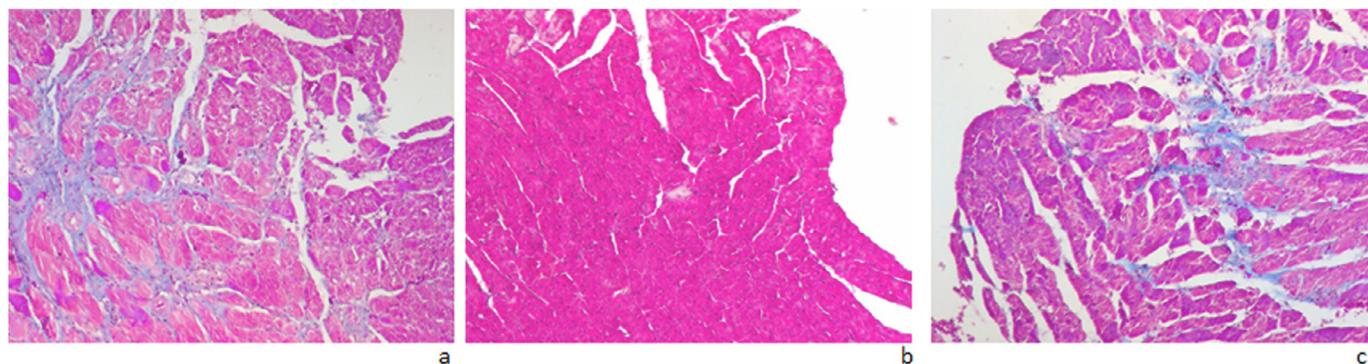


Fig. 3. Hearts of SHR/CTRL and treated SHR in trichrome staining (10-fold magnification). a: SHR/CTRL, moderate perivascular fibrosis; b: SHR/CAP, no fibrosis; c: SHR/CAP + NIF, slight interstitial fibrosis.

line with these reports. In 7-week-old SHR, SBP was about 50% higher than in normotensive WKY of the same age. During the following 3 weeks, SBP in untreated SHR/CTRL increased significantly to almost 170% of WKY/CTRL (Fig. 1). Three weeks of treatment with different antihypertensive drugs exerted heterogeneous effects on SBP. Treatments with CAP induced the strongest reduction of SBP. However, even the most effective antihypertensive therapy with CAP did not reduce SBP to normotensive levels. We assume that a higher CAP dose and a longer treatment period such as used by Paulis and Zicha and their co-workers (Paulis et al., 2007; Zicha et al., 2008) might have decreased SBP even further.

The degree of BP reduction is associated with the age of treatment onset and the duration of treatment. Antihypertensive treatment in SHR during their first 10 weeks of life significantly reduced SBP. With captopril therapy over 6 weeks, even normotensive levels were achieved (Paulis et al., 2007). With the same treatment started at the age of 30 weeks, SBP remained about 40% above normotensive level (Hojna et al., 2007). A direct comparison of different treatment intervals (2.-6., 6.-10. and 2.-10. week of life) showed that the antihypertensive effect was stronger with the prolonged treatment period (Harrap et al., 1990). We suggest that a longer treatment period in our study might have enhanced the antihypertensive effect of CAP therapy. Moreover, even after treatment withdrawal, SBP remained lower than in untreated SHR (Harrap et al., 1990; Kost et al., 1995; Zicha et al. 2008). Of note, all of these 3 studies used ACE inhibitors.

Nifedipine also decreased SBP in a dose-dependent manner in SHR, but the BP response to nifedipine was reduced after chronic captopril treatment (Paulis et al., 2007). This might account for the better and consistent antihypertensive effects of CAP compared to CAP + NIF. The final SBP of SHR/CAP and SHR/CAP + NIF was about 50 mmHg lower than that of SHR/CTRL. With NIF, final SBP was only 30 mmHg lower than in SHR/CTRL. Dihydropyridines such as nifedipine can trigger reflex sympathetic activation (van der Lee et al., 1998; Ruzicka et al., 2004; Parker et al., 2020). This effect was much more pronounced with short-acting compared to extended release nifedipine (Parker et al., 2020) and was only observed in young but not in old hypertensive patients (Ruzicka et al., 2004). We assume that the reduced antihypertensive effect in the NIF group might result from sympathetic activation. Of note, a significant SBP decrease was observed in awake SHR after long-term treatment with nifedipine given orally in the same dose as we used (from 195 in untreated to 181 mmHg in treated animals; Xie et al., 2008).

In addition, some external stress might have affected the animals. We observed that some animals were more afraid of the experimenters than others. These anxious animals were more difficult to handle and mostly presented elevated BP values. A study

in SHR showed that nifedipine was not able to reverse the BP increase induced by acute stress (Hosono et al. 1995). Narcosis and heart catheterization also cause stress to the animals. This may explain, at least in parts, the high systolic and diastolic pressures found in hemodynamic measurements in the SHR/NIF group (Fig. 2).

4.2. Development of cardiac hypertrophy and remodeling in young SHR and treatment effects

In this early stage of hypertension, SHR develop functional and morphological vascular changes (Anishchenko et al., 2015; Bencze et al., 2016), which are associated with vascular hypertrophy and lead to cardiac pressure overload due to increasing vascular resistance (Waleska and Marcelo, 2011). The development of cardiac hypertrophy in SHR starts between four (Kokubo et al., 2005) and twelve weeks of age (van Empel and De Windt, 2004). In the context of hypertrophy, fetal genes such as ANP are re-expressed in the cardiac ventricles (Day et al., 1987; Du, 2007). In young SHR at 1–2 months of age, cardiac ventricle weight was about 30% higher than in age-matched WKY. However, ANP mRNA expression in LV was equal in young SHR and WKY. At 21 months of age, ventricle weight of SHR had increased further to about 170%, and ANP mRNA reached 3.7-fold values compared to age-matched WKY (Kinnunen et al., 1991). We found a similar relation of HW/BW between our young SHR and WKY. Likewise, LV ANP expression was higher in SHR/CTRL and even in some treated SHR groups compared to WKY/CTRL but this difference was not significant (Table 1). As hypertension progresses, hypertrophy and structural changes in the SHR heart develop. Differences between SHR and WKY already occur at 1 month of age and manifest between the 6th and 24th months of life (Engelmann et al., 1987). Although collagen deposition in the hearts of 8-week-old SHR was higher than in normotensive controls (Perrucci et al., 2018), significant cardiac collagen deposition occurred in week 24 and further worsened until week 28 (Huang et al., 2020). Our histological findings of cardiac fibrosis in the hearts of our young SHR (Fig. 3, Table 3) correspond to these results. However, even in old SHR, if no heart failure was present, mRNA expression of ECM markers such as collagens and TGF- β 1 in the LV was not significantly elevated compared to age-matched WKY. Only in old SHR with heart failure, these markers were significantly upregulated (Boluyt et al., 1994). This may explain that mRNA expression of various ECM markers in the LV of our young SHR did not show significant differences to age-matched WKY/CTRL (Table 2).

Early start of antihypertensive therapy does not only reduce BP but has also cardioprotective effects, which have been documented very well (Demirci et al., 2005; Hale et al., 2012; Rocha

et al., 2010). This is also confirmed by the present results: Three weeks of antihypertensive treatment in young SHR not only decreased SBP but also reduced the development of cardiac hypertrophy and fibrosis. This was mainly achieved by CAP, which completely prevented the development of cardiac fibrosis and significantly decreased HW/BW. Combination with NIF induced similar effects indicating a potent antihypertrophic efficacy of these medications.

These results are in full accordance with previous findings demonstrating that RAAS antagonists have superior cardioprotective effects compared to other therapies, in particular, to calcium channel blockers (Ziegelhöffer-Mihalovicova et al., 2006). RAAS antagonists play an important role in lowering HW, preventing cardiac hypertrophy and mitigating structural remodeling of the cardiovascular system and terminal organ damage in young SHR (Demirci et al., 2005, Hale et al., 2012; Huang et al., 2020; Rocha et al., 2010). Nifedipine-induced sympathetic activation (van der Lee et al., 1998; Ruzicka et al., 2004; Parker et al., 2020) might account for the lower antifibrotic effect of NIF compared to CAP.

4.3. Limitations of the study

The original intention was to test three types of antihypertensive drugs. Due to the poor ingestion of PROP, we excluded these groups from further evaluation. Some preliminary tests showed that drug administration by gavage induced massive stress to the rats. Therefore, we decided to administer the tablets along with chow. Application of drugs as separate tablets allowed to check the ingestion of the drugs.

Despite similar experimental conditions, we observed that some animals were more afraid of the experimenters than others. These anxious animals were more difficult to handle and mostly presented elevated BP values. This might account for the variation of SBP values.

4.4. Conclusion

The positive antihypertensive and, even more importantly, cardioprotective effects of CAP both as monotherapy and combination therapy were the main reasons for choosing CAP and CAP + NIF for the treatment of old SHR in the planned future study.

Ethics Statements

This manuscript does not contain clinical studies or patient data. All animal protocols were approved by the state agency in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and with the “European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes”.

Authors' Contribution Statement

BR, HGZ, CH and JB contributed conception and design of the study; CH, JB and BR performed the animal experiments including blood pressure measurements. CH prepared the histological slices; CH, KS and PG evaluated and took photographs from the histological slices. CH performed the molecular biological analyses; CH and AS evaluated the molecular biological data. CH and BR performed the statistics. CH, JB and PG created figures and tables. CH and BR drafted the original manuscript; AS and HGZ reviewed and edited the manuscript. All authors contributed to the final version of the manuscript, read and approved the submitted version.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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