Cardiac remodeling during and after renin–angiotensin system stimulation in Cyp1a1-Ren2 transgenic rats

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Abstract
This study investigated renin–angiotensin system (RAS)-induced cardiac remodeling and its reversibility in the presence and absence of high blood pressure (BP) in Cyp1a1-Ren2 transgenic inducible hypertensive rats (IHR). In IHR (pro)renin levels and BP can be dose-dependently titrated by oral administration of indole-3-carbinol (I3C). Young (four-weeks old) and adult (30-weeks old) IHR were fed I3C for four weeks (leading to systolic BP >200 mmHg). RAS-stimulation was stopped and animals were followed-up for a consecutive period. Cardiac function and geometry was determined echocardiographically and the hearts were excised for molecular and immunohistochemical analyses. Echocardiographic studies revealed that four weeks of RAS-stimulation incited a cardiac remodeling process characterized by increased left ventricular (LV) wall thickness, decreased LV volumes, and shortening of the left ventricle. Hypertrophic genes were highly upregulated, whereas in substantial activation a fibrotic response was absent. Four weeks after withdrawal of I3C, (pro)renin levels were normalized in all IHR. While in adult IHR BP returned to normal, hypertension was sustained in young IHR. Despite the latter, myocardial hypertrophy was fully regressed in both young and adult IHR. We conclude that (pro)renin-induced severe hypertension in IHR causes an age-independent fully reversible myocardial concentric hypertrophic remodeling, despite a continued elevated BP in young IHR.

Keywords
Renin–angiotensin system, Cyp1a1-Ren2 transgenic rat, cardiac remodeling, hypertrophy, fibrosis, heart failure

Introduction
High blood pressure (BP)-induced cardiac remodeling is an important risk factor for the development of heart failure. Cardiac remodeling consists of geometrical changes of the myocardium as well as structural alterations of the myocytes and their matricellular embedding.1 The renin–angiotensin system (RAS) has been established as an important modulator of cardiac remodeling mediating hypertrophy, fibrosis, as well as inflammation. To dissect the role of the various RAS components in the development of cardiac remodeling and heart failure, several transgenic models have been generated.2,3

Here we report on studies in inducible hypertensive rats (IHR), in which the mouse Renin-2 (mRen2) gene is placed under the transcriptional control of a cytochrome P-450 1a1 (Cyp1a1) promoter.4 In these IHR, (pro)renin levels and BP can be dose-dependently titrated by oral administration of an arylhydrocarbon agonist such as indole-3-carbinol (I3C).5 One of the advantages of the IHR model is that, by adding and withdrawing I3C from the diet, both the onset and the offset of RAS-dependent hypertension can be studied at predetermined moments in time. Using this approach it was shown that transient RAS-stimulation induced sustained hypertension in young, but not adult IHR.5,6 Most studies in IHR have been focusing on the kidney 5,7,8 and thus information on the cardiac remodeling processes in this model is limited.

IHR are related to the well-known homozygous transgenic hypertensive rat line (TGR) (mRen2)27 which expresses the mRen2 gene constitutively. These rats invariably develop severe hypertension, which is maintained throughout life.9

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malignant hypertension, cardiac hypertrophy and cardiac fibrosis. 

In approximately 50% of male TGR, systolic heart failure develops due to rapid dilatation and inflammation of the heart. The disruption of the myocardial matrix was found to be mediated by early activation of the matricellular protein thrombospondin-2 (TSP2). Activation of the mRen2 gene is underlying the cardiovascular pathology in TGR and IHR so one would expect that IHR develop a similar form of dilated cardiomyopathy when the heart is sufficiently stressed. While myocardial hypertrophy is evident in IHR, even after short-term (<2 weeks) stimulation, detailed information on cardiac function and structure is missing. A recent study reported a lack of cardiac fibrosis after prolonged mRen2 activation in IHR, but did not show data on cardiac function or heart failure. Furthermore, most studies used relatively young animals while a cardiac phenotype is usually more apparent in adult rats.

Therefore, the goal of the present study was to investigate if activation of mRen2 in both young and/or adult IHR leads to dilated cardiomyopathy and heart failure. Since BP remains elevated after transient treatment with I3C in young but not adult IHR, the regression of a potential cardiac phenotype after de-activation of the RAS can be compared in the presence and absence of sustained high BP. For this purpose we treated young (4-week old) and adult (30-week old) IHR during four weeks with I3C and observed their cardiac function and structure both during and after this treatment.

**Animals, materials and methods**

**Animals**

All experiments were approved by the Animal Ethics Committee of Maastricht University and were performed in accordance with institutional and international guidelines. Experiments were performed in male Cyp1a1-Ren2 transgenic rats (n=111), obtained from an internal breeding stock originally derived from animals supplied by the Center for Cardiovascular Science, University of Edinburgh, UK. These IHR harbor a genetically inserted construct for the transcription of mRen2 preceded by a Cyp1a1 promoter on the Y-chromosome. Cyp1a1 can be induced by adding an aryl hydrocarbon agonist to the diet, such as I3C, leading to an increase in (pro)renin levels and subsequently a BP increase. After withdrawal of I3C from the diet the production of mouse renin stops. In non-transgenic rats I3C does not affect plasma (pro)renin and angiotensin II (Ang II) levels.

Experiments were performed in three groups: (a) young (4-weeks old) rats on a 0.3% I3C diet; (b) adult (30-weeks old) rats on a 0.3% I3C diet; and (c) adult (30-weeks old) rats with 0.6% I3C in the diet. For each group a subset of IHR received normal rat chow (Sniff, Soest, Germany) and served as normotensive controls. All groups had free access to water and food ad libitum and were housed under controlled conditions of temperature (21°C) and light (12-hour light/dark cycle).

**Experimental set-up**

**Young rats 0.3% I3C (n=69)**

In a first set of experiments the development and regression of cardiac remodeling was studied in young IHR. Experiments were conducted in 4-week old IHR which were randomly assigned to either the I3C-treated group or the control group. I3C-treated IHR were fed a 0.3% I3C (Sigma-Aldrich, St. Louis, Missouri, USA) diet between 4–8 weeks of age to induce hypertension. Hereafter, I3C-treatment was stopped to investigate the potential regression of cardiac remodeling and heart failure. Rats were followed-up until 20 weeks of age. Systolic blood pressure (SBP) was determined by photoelectric/oscillometric tail-cuff. Approximately 10 consecutive measurements were recorded to establish a reliable SBP. Additionally, transthoracic echocardiography was performed at 4, 8, 12 and 20 weeks of age under light isoflurane anaesthesia to determine cardiac dimensions and function. At these time points subgroups of animals (n=5–12 per group) were sacrificed and blood and organs were harvested for further analysis.

**Adult rats 0.3% I3C (n=22)**

A second set of experiments was performed in adult IHR which were treated with the same dose of I3C as the young animals. At 30 weeks of age IHR received 0.3% I3C for four weeks (i.e. 30–34 weeks of age) and SBP was followed-up to 38 weeks of age by tail-cuff. In addition, echocardiography was performed at 30, 34 and 38 weeks of age. At 34 weeks of age and at the end of the experiment IHR (n=4–6 per group) were sacrificed and organs were collected for further analysis.

**Adult rats 0.6% I3C (n=20)**

A third set of experiments was performed in adult IHR which received 0.6% of I3C via the diet (between 30–34 weeks of age) to titrate BP to the same level as observed in the young I3C-treated rats. In contrast to the 0.3% I3C-treated adult IHR, SBP was followed-up to 40 weeks of age since at 38 weeks SBP in the 0.6% I3C-treated IHR was back at control levels for just two weeks. Again, cardiac dimensions and function were determined via echocardiography at 30, 34 and 38 weeks of age. A subset of I3C-treated IHR was sacrificed at the end of the I3C-treatment (i.e. 34 weeks of age), whereas the residual IHR and all control rats were sacrificed at 40 weeks of age. Since no differences were observed between the 34 and 38 week old control rats.
in our 0.3% I3C experiment, in the current experiment only one control group was sacrificed at the end of the experiment, i.e. at 40 weeks of age. Organs were collected for further analysis. This 0.6% group was primarily included to check the reversibility of a maximal BP increase in adult rats and was not fully phenotyped and analyzed.

The dosage of I3C was carefully chosen to induce maximal activation of the mRen2 gene. In studies with relatively young IHR\(^5,7\) 0.3% of I3C leads to malignant hypertension. Administering higher dosages of I3C to these animals results in high mortality and a serious drop-out. In adult IHR this phenotype becomes apparent at 0.6% I3C.

**Echocardiography**

Left ventricular (LV) dimensions and function were assessed under isoflurane anesthesia. B-mode echocardiographic recordings were made in midpapillary short-axis and parasternal long-axis using a Vevo2100 imaging platform (Visualsonics, Toronto, Canada). Data were derived from images in end diastole and peak systole and average values over at least three different cycles were used. In addition, M-Mode images were used to determine heart rate (HR). Parameters determined from short-axis images were LV internal diameter (LVID) and LV wall thickness (WT) at two sites. From these measurements fractional shortening (FS) was calculated as FS (%) = 100 x (LVID in diastole – LVID in systole)/LVID in diastole. From the long-axis images LV area (LVA), as well as the length of the LV lumen from base to apex (LVL) were determined. The end-diastolic volume (EDV) and end-systolic volume (ESV) were calculated from area and length measurements as \(8 \times (LVA)^2 / 3\pi \times LVL\) in diastole and systole, respectively. Furthermore, we calculated stroke volume (SV) as EDV–ESV, cardiac output (CO) as SV x HR and ejection fraction (EF) as 100 x(EDV–ESV)/EDV.

**Tissue preparation**

After sacrificing the rats by exsanguination via the abdominal aorta, hearts were excised, weighed and divided for further analysis. A cross-section of the heart was fixed in 4% formalin for histological examination and the remainder of the LV was snap-frozen for RNA extraction.

**Plasma measurements**

Blood was collected in two different cocktails. The first cocktail contained ethylenediaminetetraacetic acid (EDTA) and aprotinin for the determination of plasma renin and pro-renin activity by enzyme-kinetic assay, as described by Van Esch et al.\(^{16}\) A second cocktail consisted of EDTA, phenanthrolin, lisinopril, ethanol, neomycin sulfate and a renin inhibitor for the determination of plasma Ang II levels by radioimmunoassay as described before.\(^{16}\) All RAS-components were measured in a subset of plasma samples (\(n=4–6\) for each group). Samples of young IHR at the age of 8 and 12 weeks and adult IHR at the age of 34 and 38 weeks were analyzed to check whether the sustained elevation in BP in young IHR might have been due to prolonged elevation of circulating RAS-components.

**Gene expression analysis**

Total RNA was isolated from LV tissue by homogenization in TRIZOL reagent (Invitrogen, Carlsbad, California, USA). cDNA synthesis was performed using 1000 ng RNA according to the manufacturers’ protocol (iScript cDNA synthesis kit, Biorad Laboratories Inc., Hercules, California, USA). Gene expression was analyzed by quantitative polymerase chain reaction (PCR) on an iCycler Real-Time PCR detection system using the iQ SYBR-green supermix (Biorad Laboratories Inc.). Primers applied are presented in Table 1. Results were normalized to the housekeeping gene cyclophilin A and relative changes in expression levels were subsequently calculated using the qBase analyzer. Relative gene expression in the control rats was normalized for each time-point to compare them with age-matched I3C-treated IHR.

**Histochemistry and immunostaining**

**Myocyte size**

Myocyte cross sectional area (CSA) was determined on hematoxylin (Gurr, Searle Diagnostic, High Wycombe, Bucks, England) /eosin (Klinipath, Olen, Belgium) (H/E) stained sections by assessing the CSA of individual myocytes in 3–5 regions in the anterior wall and 3–5 regions in the septum using a computerized morphometric system. Since results did not differ between the two LV regions data were pooled as described before.\(^{17}\)

**Collagen deposition**

Interstitial and perivascular collagen was visualized using Sirius Red staining. Cardiac sections were incubated with 0.2% aqueous phosphomolybdic acid for 5 min and subsequently for 60 min with 0.1% Sirius Red F3BA (Polysciences, Northampton, United Kingdom) in saturated picric acid and washed for 2 min in 0.01 N HCl. Finally, tissues were rinsed in 70% ethanol and dehydrated in increasing concentrations of ethanol. Interstitial collagen was analyzed from 12 images (eight of left ventricle and four of right ventricle) taken from each heart by light microscopy at a magnification of 200×. Collagen density was determined as the percentage of Sirius Red stained tissue versus total tissue area using a computerized morphometric system (Leica Qwin 3.1) (see Supplementary Material, Figure S1(A)). Since no differences were found between the left ventricle and right ventricle, data were
pooled. Additionally, perivascular collagen was determined for all identified vessels in the cross-sections of the heart. Next to collagen area, the vascular lumen and tunica media area were determined and the amount of perivascular collagen was expressed as the area of vascular collagen divided by area of the media×100% (see Supplementary Material, Figure S1(B)).

**Macrofage infiltration**

A CD68-staining was performed to determine macrophage infiltration in the heart by immunohistochemical staining for CD68. First, slides were incubated for 30 min in 0.3% peroxide in phosphate buffered saline (PBS) to block endogenous peroxidase activity. Primary antibody (mouse-α-rat ED-1,18 1:50 in PBS with 0.1% BSA) was added and incubated overnight at 4°C. Hereafter, slides were incubated for 30 min with secondary antibody (rabbit-α-mouse-biotine, 1:400 in PBS, Sigma). Subsequently, AB complex and diaminobenzidine (Dako Denmark A/S, Glostrup, Denmark) was added to the slides to obtain staining of the macrophages. Finally, the tissues were counterstained with hematoxylin and rehydrated in increasing concentrations of ethanol. Between the different steps, slides were washed in PBS. Analysis of CD68-staining was done by light microscopy at a magnification of 200× by means of 12 images (eight of left ventricle and four of right ventricle) per rat. Brown staining was measured as percentage of total tissue area using Leica Qwin 3.1 software.

**Statistical analysis**

Data are expressed as mean ± standard error of the mean (SEM) and were analyzed by unpaired Student’s t test, one-way or two-way analysis of variance (ANOVA) with post-hoc Bonferroni correction where appropriate. Differences were considered statistically significant if p-values<0.05 (*p<0.05, †p<0.01 and ‡p<0.001).

**Results**

**Sustained hypertension in young I3C-treated IHR**

BP follow-up revealed sustained elevation in BP after I3C-treatment in young IHR. Adult IHR treated with a similar dose of I3C as the young rats also developed hypertension, however BP returned to control values within one week after stopping I3C-treatment. Giving a 0.6% I3C diet to adult IHR resulted in similar levels of SBP increase as in young 0.3% I3C-treated IHR. Nevertheless, stopping RAS stimulation in these adult rats also resulted in a return of SBP to control values (Figure 1). Upon I3C-treatment circulating RAS components were elevated. Young and adult I3C-treated IHR displayed increases in plasma pro-renin and renin activity when compared with normotensive controls. Furthermore, plasma Ang Ii concentrations were also increased in young and adult IHR (Table 2). Four weeks after withdrawal of I3C, circulating RAS components returned to control values both in the adult animals, in which BP was returned to normal, and the young I3C-treated IHR in which BP remained elevated. LV gene expression of the different RAS-components, (P)RR, rat and mouse renin, ACE: angiotensin converting enzyme; ANF: atrial natriuretic factor; βMHC: β-myosin heavy chain; CTGF: connective tissue growth factor; mRenin: mouse renin; NfκB: nuclear factor kappa B; (P)RR: (pro)renin receptor; rRenin: rat renin; TGFβ: transforming growth factor β; TSP2: thrombospondin-2.

**Cardiac remodeling in IHR**

Changes in cardiac geometry and function as determined by echocardiography are given in Figure 2 and Figure 3. As a consequence of four weeks of RAS-stimulation several cardiac changes occurred. The hearts of both young and
Figure 1. Systolic blood pressure in inducible hypertensive rats (IHR). (A) Systolic blood pressure (SBP) follow-up reveals sustained elevation in blood pressure (BP) after I3C-treatment in young IHR. (B) Adult IHR treated with the similar dose of I3C as the young rats also develop hypertension, however BP returns to control values within one week after stopping I3C-treatment. (C) Adult IHR treated with 0.6% I3C reached similar levels of SBP as young 0.3% I3C-treated IHR. Nevertheless, stopping renin–angiotensin system (RAS) stimulation in these adult rats also resulted in a return of SBP to control values. I3C, Indole-3-carbinol. *p < 0.05, †p < 0.01 and ‡p < 0.001. Figure adapted from Heijnen et al.5

Table 2. Plasma pro-renin and renin activity and angiotensin (Ang) II concentration.

|                  | Young IHR |                  | Adult IHR |                  |
|------------------|-----------|------------------|-----------|------------------|
|                  | 8 weeks   | 12 weeks         | 34 weeks  | 38 weeks         |
|                  | Control (n=4) | 0.3% I3C (n=6)  | Control (n=4) | 0.3% I3C (n=5)  |
| PR (ng Ang I ml⁻¹ hr⁻¹) | 30 (2–99) | 13,868‡ (2626–56,402) | 9 (3–24) | 11 (2–42)         |
| R (ng Ang I ml⁻¹ hr⁻¹)  | 26 (17–30) | 662‡ (173–3276)   | 26 (17–39) | 10† (7–13)        |
| Ang II (fmol ml⁻¹)     | 21 (11–65) | 52‡ (22–98)       | 52 (33–62) | 23 (13–42)        |
|                  | Control (n=4) | 0.3% I3C (n=5)  | Control (n=5) | 0.3% I3C (n=5)  |
| PR (ng Ang I ml⁻¹ hr⁻¹) | 27 (8–48) | 15,381‡ (10,429–30,548) | 6 (1–36) | 24 (17–37)        |
| R (ng Ang I ml⁻¹ hr⁻¹)  | 15 (10–20) | 1178‡ (571–1558)  | 24 (10–43) | 15 (9–22)         |
| Ang II (fmol ml⁻¹)     | 33 (19–41) | 191‡ (90–272)     | 51 (23–77) | 18† (12–28)       |

Data are presented as geometric mean and range. I3C: indole-3-carbinol; IHR: inducible hypertensive rats; PR: pro-renin; R: renin. *p < 0.05, †p < 0.01 and ‡p < 0.001.
adult I3C-treated IHR were significantly shorter when compared with control animals. In addition, the LV wall was thickened and the internal diameter was decreased. HR remained stable over time (see Supplementary Material, Figure S2), whereas EDV and SV were reduced leading to a decrease in CO. Despite the fall in CO, EF and FS remained at control levels. In summary, these echocardiographic data indicate that induction of malignant hypertension in IHR is associated with an inward concentric remodeling of the heart with significant reductions in cardiac output, but with compensated myocardial function. Changes observed in the young 0.3% I3C-treated IHR were similar to those in the adult 0.6% I3C-treated IHR. Four weeks after I3C was withdrawn from the food the hearts regained their length and EDV and SV were restored to values observed before induction. Furthermore, the remodeling process was associated with a return of wall thickness and CO.

LV hypertrophy

Figure 4 summarizes the effects on heart weight. When corrected for tibia length, heart weight gradually increased in the young IHR, whereas at all ages no significant difference was observed between I3C-stimulated and control IHR. Adult I3C-treated IHR, however, demonstrated a trend towards a decrease in heart weight upon RAS-stimulation, whereas four weeks later heart weight was significantly increased in both 0.3% and 0.6% I3C-treated groups. Heart weight normalized to 100 g body weight was

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Figure 2. Changes in cardiac geometry as determined by echocardiography. All presented parameters were determined in diastole. (A) Left ventricular (LV) wall thickness was significantly increased upon renin–angiotensin system (RAS) stimulation in young (i.e. eight weeks of age) and both adult (i.e. 34 weeks of age) IHR groups. In addition, (B) the internal diameter of the left ventricle was significantly decreased and (C) the heart demonstrated a reduction in LV length. All effects disappeared four weeks after stopping I3C-treatment. The n for each group is presented in the bars of Figure 2(A). I3C: indole-3-carbinol; LVID: left ventricular internal diameter; LVL: left ventricular length; WT: wall thickness. *p<0.05, †p<0.01 and ‡p<0.001.
significantly increased upon RAS-stimulation in young IHR (0.680±0.092 versus 0.350±0.014 in IHR versus control rats) and both adult IHR groups (0.3% I3C: 0.322±0.007 versus 0.262±0.005 and 0.6% I3C: 0.403±0.012 versus 0.236±0.004). Four weeks later these differences were reduced yet remained significantly higher in all groups when compared with control rats (young 0.3% I3C: 0.427±0.067 versus 0.346±0.030, adult 0.3% I3C: 0.291±0.013 versus 0.269±0.011 and adult 0.6% I3C: 0.278±0.009 versus 0.236±0.004). By 20 weeks of age the increase in heart...
weight had completely disappeared in the young transiently 13C-treated IHR (0.330±0.014 versus 0.318±0.041). The hypertrophic markers ANF and βMHC were markedly increased during 13C-treatment and returned to control values after withdrawal of the inducer. Strikingly, gene expression followed the same pattern as those observed for the heart weights corrected for body weight. In contrast, myocyte size did not increase between the 13C-treated and control IHR of the young group. In the adult groups only a small increase in myocyte size could be detected for the 0.6% 13C-treated IHR. Furthermore, the matricellular remodeling marker TSP2 expression was not altered due to the RAS-stimulation (Figure 5).

**Cardiac fibrosis**

Interstitial fibrosis scored on Sirius Red stained LV tissue sections revealed no significant differences among the young and adult rats (See Figure 6 and Supplementary Material, Figure 1(A)). Evaluation of perivascular collagen deposition revealed a significant increase in young 13C-treated IHR which persisted till 20 weeks of age. In contrast, adult 13C-treated IHR demonstrated a trend towards a decrease in perivascular collagen which disappeared four weeks later. Gene expression of several important fibrotic genes, i.e. transforming growth factor β (TGFβ) and col1α1, demonstrated almost no differences. However, connective tissue growth factor (CTGF), a precursor of fibrosis, was significantly increased upon RAS-stimulation in both young and adult IHR. This difference gradually disappeared in the young rats, whereas CTGF expression in the adult IHR was already at control levels four weeks later (Table 3).

**Cardiac inflammation**

Despite the increase in SBP and circulating RAS-components, no cardiac inflammation was detected. Macrophage influx was determined with CD68 immunohistochemistry and revealed no significant differences in young and adult 13C-treated IHR. However, adult rats demonstrated an increased presence of macrophages.
when compared with young IHR (see Supplementary Material, Figure S3). These data were confirmed at the gene expression level. Neither CD68, nor NFκB gene expression displayed any changes in the left ventricle between I3C-treated IHR and their age-matched controls (see Supplementary Material, Table S2).

**Figure 5.** Hypertrophy in inducible hypertensive rats (IHR). Gene expression of (A) and (B) hypertrophic markers atrial natriuretic factor (ANF) and β-myosin heavy chain (βMHC), and (C) the matrix integrity marker thrombospondin-2 (TSP2). Additionally, (D) myocyte size was scored on histological slides and presented as cross sectional area. The n for each group in (A), (B) and (C) is presented in the bars of Figure 5(A) and the n for each group in Figure 5(D) is given separately. I3C: indole-3-carbinol. *p<0.05, †p<0.01 and ‡p<0.001.
Additional results

Additional results belonging to this manuscript can be checked online in the Supplementary Material. This file consists of two tables and three figures. Table S1 demonstrates changes in LV RAS-related gene expression of (P) RR, rat renin, mouse renin, ACE and the AT1R. Table S2 addresses the effects on two inflammation related genes, i.e. CD68 and NF-κB. In addition, Figure S1 illustrates typical photomicrographs of the Sirius Red staining for the analysis of interstitial and perivascular collagen deposition in cardiac tissue of the IHR. Further, Figure S2 demonstrates that there was no change in heart rate in all groups with or without RAS-stimulation. Finally, the absence of changes in LV macrophage infiltration is presented in Figure S3.
Discussion

This study demonstrates that malignant hypertension induced by activation of the mRen2 gene in IHR resulted in concentric cardiac remodeling which was characterized by low cardiac output but preserved contractile function and a minimal inflammatory and fibrotic response. In addition, it was found that the hypertrophic phenotype was fully reversible and associated with a normalization of hypertrophic markers such as ANF and βMHC despite a sustained hypertensive response in the young IHR. The increase in hypertrophic markers did not lead to changes in myocyte size, but resulted in an increased wall thickness. An explanation for this phenomenon might be that, besides thickening of the wall, the heart also shortens leading to an inward conformational change. We believe that the hearts of the I3C-induced IHR are in a remodeling phase rather than suffering from hypertrophy. Additionally, the animals that receive I3C demonstrated a loss in bodyweight (BW). There might be an imbalance in anabolic and catabolic processes which may affect the growth of the myocytes. Hence, in these animals signaling of hypertrophic markers does not result in hypertrophy of the myocytes, but rather leads to a geometric change of the heart itself.

In both young and adult IHR, I3C was added in doses to increase afterload comparable to what has been observed in TGR. In spite of this, cardiac remodeling processes were found to be quite different. The mRen2 activation in IHR led to inward concentric myocardial remodeling. Despite the high afterload no signs of systolic failure or dilatation were observed and fractional shortening and ejection fraction were maintained. Furthermore, markers of matrixcellular activation such as TSP2 or matrix metalloproteases (latter not shown) were not altered. These findings are in sharp contrast with the cardiac phenotype observed in TGR, where activation of the mRen2 gene drives concentric and dilated cardiac remodeling.

Several factors may have contributed to the characteristic cardiac remodeling process in IHR:

Genetic background. It is important to mention that IHR are Fischer F344 rats, whereas the homozygous TGR are Sprague Dawley rats. Strain specificity of cardiovascular adaptations to mRen2 gene activation has been described before. It has been suggested that modifier loci close to the AT1 receptor and ACE gene control the malignancy of the hypertension. However, it is unknown if these genes do also determine the development of the different cardiac phenotypes. Genetic studies with IHR and TGR could shed light on the molecular mechanisms by which activation of the mRen2 gene drives concentric and dilated cardiac remodeling.

Site of gene activation. Besides strain effects, differences in the sites of mRen2 expression may have contributed to the divergent cardiac phenotypes. In IHR, the mRen2 gene is mainly expressed in the liver and small intestine but not in the heart. Consequently, the intracardiac kinetics and myocardial availability of (pro)renin and Ang II follow more closely human physiology, where cardiac renin originates from outside the heart. In contrast, in TGR, the mRen2 gene is expressed in many organs including the heart and local rather than systemic production of Ang II has been held responsible for the phenotype. Recently, Vanourkova et al. have shown that feeding IHR 0.3% I3C for a period of 10 days leads to a cardiac hypertrophic response that could be prevented by co-administration of the AT1 receptor blocker valsartan but not by triple therapy with reserpine, hydralazine and hydrochlorothiazide. Remarkably, while both treatments were equipotent in reducing BP, tissue Ang II levels and cardiac remodeling were blunted during AT1 receptor blockade but not triple therapy. This study in IHR confirms previous observations made in TGR that not afterload but Ang II signaling is the most important factor involved.

Timing of gene activation. Studies in spontaneously hypertensive rats have shown that transient inhibition of angiotensin signaling at early stages in life (from 4–8 weeks) blunts the development of hypertension and ameliorates cardiac hypertrophic remodeling into adult life. These data suggest that the phenotypic outcome of activation of the RAS pathway may be determined in a critical period during development. In the present study, we used four-week and 30-week old IHR. In the young IHR, four weeks of mRen2 activation leads to a strong elevation of BP which lasts well beyond the period of activation. The cardiac phenotype returns to normal after stopping the I3C treatment. In the adult IHR both BP and cardiac phenotype return to normal shortly after stopping the I3C treatment. In IHR, the mRen2 gene is only expressed upon I3C-treatment whereas in TGR mRen2 is constitutively expressed. Taken together, these data suggest that the timing of gene activation influences the reversibility of hypertension, but not the cardiac phenotype. Recent studies have shown that the long-lasting BP change in young IHR is due to irreversible renal damage following mRen2 activation.
Perspectives

In IHR, mRen2 gene-related malignant hypertension induces fully reversible myocardial concentric hypertrophy with characteristics of diastolic heart failure, whereas in the related TGR strain systolic heart failure develops. Despite marked LV concentric hypertrophy and reduced stroke volume, the absence of interstitial fibrosis and inflammation may make the IHR model not a true diastolic failure model but rather a model with benign reversible hypertrophy. On the other hand the clinical finding that patients with diastolic heart failure show depressed longitudinal but preserved circumferential myocardial deformation is in line with our observation that the heart of the IHR became shorter in length, but had increased wall thickness and slightly elevated fractional shortening. In addition, the absence of matricellular changes in this model may have facilitated the ability of the heart to fully regress to a normal phenotype after cessation of I3C. The IHR model constitutes a unique opportunity to study the molecular pathways of both induction and regression of myocardial remodeling. The role of Ang II as a direct mediator of LV remodeling has been studied extensively, leading to the conclusion that Ang II alone is not sufficient to induce diastolic heart failure, whereas in the related TGR strain systolic heart failure develops. Despite marked LV concentric hypertrophy and reduced stroke volume, the absence of interstitial fibrosis and inflammation may make the IHR model not a true diastolic failure model but rather a model with benign reversible hypertrophy. On the other hand the clinical finding that patients with diastolic heart failure show depressed longitudinal but preserved circumferential myocardial deformation is in line with our observation that the heart of the IHR became shorter in length, but had increased wall thickness and slightly elevated fractional shortening. In addition, the absence of matricellular changes in this model may have facilitated the ability of the heart to fully regress to a normal phenotype after cessation of I3C. The IHR model constitutes a unique opportunity to study the molecular pathways of both induction and regression of myocardial remodeling. The role of Ang II as a direct mediator of LV remodeling has been studied extensively, leading to the conclusion that Ang II alone is not sufficient to induce a remodeling response. Our current data are in line with these studies and indicate that differences in matricellular remodeling rather than pathological adaptations in myocyte function underlie the disparity in ventricular remodeling of IHR and TGR. A recent review by Kurdi and Booz puts these data in perspective, describing that Ang II needs additional factors, e.g. inflammation, oxidative stress or high sodium and aldosterone levels, to play an important role in pressure-induced remodeling of the left ventricle.

Acknowledgements

The authors wish to thank Helma van Essen, Jacques Debets and Gregorio Fazzi for expert technical assistance.

Conflict of interest

The authors declare that there are no conflicts of interest.

Funding

This work was performed within the framework of Dutch Top Institute Pharma, project ‘Renin–Angiotensin system blockade beyond Angiotensin II (T2-301)’ and NWO grant 91110016.

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