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ABSTRACT

Cardiac failure is a common feature in the evolution of cardiac disease. Among the determinants of cardiac failure, the renin–angiotensin–aldosterone system has a central role, and antagonism of the mineralocorticoid receptor (MR) has been proposed as a therapeutic strategy. In this study, we questioned the role of the MR, not of aldosterone, on heart function, using an inducible and cardiac-specific transgenic mouse model. We have generated a conditional knock-down model by expressing solely in the heart an antisense mRNA directed against the murine MR, a transcription factor with unknown targets in cardiomyocytes. Within 2–3 mo, mice developed severe heart failure and cardiac fibrosis in the absence of hypertension or chronic hyperaldosteronism. Moreover, cardiac failure and fibrosis were fully reversible when MR antisense mRNA expression was subsequently suppressed.

Cardiac failure is a major health problem with increasing incidence with aging of the population. Cardiac fibrosis is a marker of cardiac failure and a crucial determinant of myocardial heterogeneity, increasing diastolic stiffness, systolic dysfunction, and the propensity for reentry arrhythmias (1). Animal models are necessary to investigate the mechanisms of appearance and regression of cardiac remodeling and to improve therapeutic strategies. In this paper, we report on a conditional mouse transgenic model in which cardiac fibrosis can be induced and reversed. This was achieved by regulating the expression of the mineralocorticoid receptor (MR) in cardiomyocytes.

Studies in both experimental animals and humans have suggested that aldosterone excess may have deleterious effects on cardiac function (2). Recently, the Randomized Aldactone Evaluation Study (RALES) showed that treatment of patients experiencing severe heart failure with spironolactone, an antagonist of the aldosterone receptor (mineralocorticoid receptor) used in the treatment of hypertension, improved both morbidity and mortality (3). These findings have resulted in the recommendation of spironolactone use in the treatment of severe heart failure (4). In RALES, the mechanisms and cellular targets involved in the beneficial effect of spironolactone action are largely unknown. Because MR is expressed in both cardiomyocytes (5) and the kidney (6), it has been difficult to separate the direct effects of signaling through the cardiac MR and indirect effects resulting from actions of the drug on renal MR. MR is a ligand-dependent transcription factor of the steroid receptor superfamily (7). In the kidney, aldosterone binding to MR increases sodium reabsorption and potassium

excretion (6). Inactivating mutations of MR result in chronic renal salt wasting, and activating mutation of MR causes hypertension, underscoring the essential role of this pathway in sodium balance and the control of blood pressure (8,9). In the heart, however, the role of MR in physiologic and pathologic situations has not been defined. A MR knockout mouse model is available (10), but these animals die in the first week of life from renal salt wasting, precluding the direct assessment of the role of myocardial MR on cardiac function in adult animals. The goal of this study was to evaluate the consequences of changes in MR expression in the heart, an approach clearly distinct from *in vivo* modifications of aldosterone, the MR ligand. To address the physiopathological role of MR in the heart independently of its renal effects, a targeted approach is required. We generated a conditional down-expression model by cardiac-restricted expression of a murine MR antisense mRNA. This strategy allowed temporal control of the expression of the endogenous MR specifically in cardiomyocytes and resulted in heart failure and cardiac remodeling, which were reversible when antisense MR mRNA expression was turned off.

EXPERIMENTAL PROCEDURES

Transgenic Mice. A 316-bp (from +13 to +328 bp, according to the rat MR cDNA sequence) cDNA fragment (MR1) encoding part of the mouse mineralocorticoid receptor (mMR) was cloned by reverse transcription–PCR from heart mRNA by using sense (5′-CATgTCgACggCTACCACAgTCTCCCTgAAgg-3′) and antisense (5′-gTAgACgTCggTATATATTTgggTACCTgAC-3′) oligonucleotide primers (European Molecular Biology Laboratory accession no. AJ 311855). The 316-bp MR1 cDNA was cloned in the antisense orientation in the *Sal*I/*Pst*I sites located between the tetO promoter and the simian virus 40 polyadenylation signal of the bidirectional tet-inducible pBI3 vector (11), allowing coexpression of the LacZ reporter gene and MR1 antisense mRNA. Six founder transgenic mice were obtained by pronuclear microinjections of a 6.8-kb AseI–AseI fragment into (B6D2)F2 fertilized eggs in the Service d'Expérimentation Animale et de Transgenèse (SEAT, Villejuif, France). Two independent MR-AS mouse strains (MR-AS17 and MR-AS27) were used for phenotypic analysis. Double-transgenic (DT) mice were obtained by crossing the MR-AS mice with the α-MHCtTA transactivator mouse strain kindly provided by G. I. Fishman, Mount Sinai School of Medicine, New York (12). MR-AS and αMHC-tTA monotransgenic littermates were not different from wild-type mice (not shown) and were used as controls. Because male and female exhibited the same phenotype, the data were pooled for controls as well as for DT. A control transgenic mouse model (LacZ20 line) was generated by using a construct similar to the one used to generate MR-AS transgenic mice, except that the MR antisense cassette was omitted.

When required, Dox (2 mg/ml) plus Sucrose (2%) to mask the bitter taste of Dox or 20 mg/kg/d spironolactone (Aldactone, Amersham Pharmacia) were added to the drinking water. To adjust daily dosage of spironolactone, mice were weighed, and water intake was measured.

RNase Protection Assay. The 316-bp MR1 cDNA fragment was subcloned in pGEM-T Easy plasmid (Promega) and used for the detection of MR antisense mRNA expression by RNase protection assay by using 10 μg of total RNA, as described (13). The full length of the protected fragment was 303 bp. Sense (5′-AAgAgCCCTATCATCTgTCATgAgAA-3′) and antisense (5′- ggACTggAgACTggAgATTTTACACTgC-3′) oligonucleotide primers were used for the cloning of another mMR cDNA fragment (MR2) (from +543 to + 863 bp, according to the rat MR cDNA sequence) (European Molecular Biology Laboratory accession no. AJ 311856). The MR2 cDNA fragment was then subcloned in pT-Adv plasmid

(CLONTECH) and used to assay the mouse endogenous MR by RNase protection assay by using 10 μg of total cardiac RNA or for *in situ* hybridization studies. The length of the protected fragment was 320 bp. Glyceraldehyde-3-phosphodeshydrogenase (GAPDH) was used as an internal standard (protected fragment, 164 bp) and cohybridized during the assay. Radioactivity was quantified with an InstantImager (Packard).

Morphological Analysis. Histological analysis was performed blindly on paraffin sections on hearts obtained from five DT mice and five control littermates at 2 mo of age. Hearts were fixed in 3.7% formalin solution, cut at the midventricular level, embedded in paraffin, and cut (eight sections of 5 μm per heart). Hemalun–eosin-stained sections were examined at ×250. Fragmentation of DNA was studied at the single-cell level by using the terminal transferase-mediated dUTP-biotin nick end labeling, as described (14). Scoring was performed on five criteria: disarray, myocytes and nuclear hypertrophy, cellular infiltrate, fibrosis (score: 0, absent; 1, few; 2, major), and apoptosis (score: 0, absent; 1, present).

Collagen Morphometry. Hearts were put in Tissue-Tek (Sakura, The Netherlands) and frozen at −155°C in liquid nitrogen-cooled isopentane. Equatorial cryostat sections containing both left and right ventricle (LV and RV) were stained with the collagen-specific Sirius red stain (0.5% in saturated picric acid). Quantification of fibrosis was performed blindly on 10 sections, on 8–10 fields per section, located in midventricular regions, not adjacent to cavities. Interstitial collagen volume fraction (i.e., the ratio of interstitial collagen surface area to total ventricular surface area, as a percentage) was determined in both LV and RV, as described (15).

Echocardiographic Analysis. Echocardiography was performed on lightly anaesthetized adult mice (isoflurane Abbot, in oxygen) (1- to 3-mo-old, body weight 15–30 g) with a Toshiba Power Vision 6000 (SSA 370A) equipped with a linear 8–14 MHz transducer. Body temperature was maintained by a heating pad. LV dimensions were obtained from long axis view by two-demensional-guided M-mode imaging. Outflow velocities were obtained by Doppler sampling from apical four-chamber view for measurement of the ejection time (ET). LV ejection fraction (EF) was calculated as follows: $EF = (LVEDD^3\text{-}LVESD^3/LVEDD^3$ where LVEDD is the LV end diastolic diameter and LVESD is LV end systolic diameter (16). The mean velocity of circumferential fiber shortening (Vcfc) was corrected for heart rate and calculated as follows: Vcfc = SF/ETc where SF is shortening fraction obtained as SF = (LVEDD-LVESD)/LVEDD, and ETc is the ejection time corrected for heart rate (as ET divided by square root of R-R interval) (17).

Arterial Blood Pressure. Mean systolic arterial blood pressure was measured via a catheter implanted under anesthesia in the femoral artery and connected to a pressure transducer.

Corticosteroid Hormones and 11β-Hydroxysteroid Deshydrogenase Measurements. Plasma corticoisteroid hormones were measured by RIA. The activity of the cardiac 11β-hydroxycorticosteroid deshydrogenase type II was assessed as described (5).

Statistical Analysis. Data are expressed as mean ± SE. Groups were compared by using the unpaired Student's test or ANOVA by using STATVIEW software (Abacus Concepts, Berkeley, CA); *, control vs. DT mice, and #, treated vs. untreated mice when $P < 0.05$.

RESULTS AND DISCUSSION

Murine MR Cardiac Expression Can Be Tightly Controlled in a Conditional Transgenic Animal Model. The tetracycline-dependent regulatory system permits tight control of gene expression in a wide range of cell type both *in vitro* and *in vivo* (18). We have generated transgenic mice that allow cardiac and specific inducible expression of an antisense mRNA of the murine MR (MR-AS).

The target for the antisense segment was chosen in the N-terminal portion of MR, close to the translation initiation site, where it is proposed that antisense mRNAs are more efficient because of both transcriptional and posttranscriptional effects (19). Importantly, the antisense segment is located in the region that is the most divergent portion comparing steroid receptor family members and presents no significant sequence homology with other genes including all other steroid receptors. To restrict expression of the MR antisense mRNA in the heart, we used the well-characterized αMHC-tTA transactivator mouse strain, which allows expression of the tet-OFF transactivator in cardiomyocytes only (20). Mating monotransgenic MR-AS and αMHC-tTA mice resulted in the generation of αMHC-tTA/MR-AS DT mice in which Dox-controlled MR antisense mRNA expression is restricted to cardiomyocytes (Fig. 1*a*). RNase protection assays indicated that MR antisense mRNA at the expected full size (303 bp) was expressed in DT mice (Fig. 1*b*) and that steady-state expression of endogenous MR mRNA was decreased by ≈50% in the whole heart of 1-mo-old DT mice, as compared to monotransgenic littermates used as controls (Fig. 1*c*), whereas it was unchanged in the kidney (Fig. 1*c*). *In situ* hybridization showed that the reduction in MR mRNA occurred homogeneously in the myocardium (not shown). Substantial efforts to measure MR expression at the protein level (Western blot) were made by using several anti-MR-Abs; unfortunately, no specific signal could be obtained in mouse tissues (including kidney). The administration of Dox for 10-d- to 1-mo-old animals abolished antisense MR mRNA expression (Fig. 1*b*) and allowed endogenous MR mRNA to return to control levels (Fig. 1*c*), indicating that endogenous MR expression can be tightly controlled in this conditional model.

In contrast to MR expression, cardiac glucocorticoid receptor expression was not affected by MR-AS expression at either the RNA or protein levels (not shown). The activity of the type II 11β-hydroxysteroid dehydrogenase (HSD2), which protects MR from illicit occupancy by glucocorticoid hormones (21), was similar in control and double-transgenic mice (fmol of 3H-11-dehydrocorticosterone formed/2 h/mg protein: 243 ± 40, $n = 5$ vs. 247 ± 18, $n = 5$). Plasma aldosterone (pg/ml, controls: 291 ± 44, $n = 15$; DT: 258 ± 63, $n = 12$) and corticosterone concentrations (ng/ml, controls: 134 ± 20, $n = 15$; DT: 101 ± 17, $n = 12$) were not significantly different comparing DT and control animals. Therefore, our model results in a reduction of MR expression in heart without modifying circulating corticosteroid hormone levels or the expression of glucocorticoid receptor and HSD2.

Down-Expression of Endogenous MR mRNA Leads to Cardiac Remodeling and Severe Heart Failure. DT mice had evidence of severe heart disease (rapid weight gain, s.c. edema, pleural effusion, and ascite) leading eventually to death (Fig. 2*a*). Phenotypic analyses were mainly performed by using DT27 mice because most of the DT17 mice died within 12 wk of birth (Fig. 2*b*). DT mice (3-mo-old) had cardiomegaly (Fig. 2*c*) and increased heart/body weight ratio (Fig. 2*d*), which was already highly significant in 2-mo-old mice (Fig. 2*e*). Cardiac function was evaluated by noninvasive transthoracic Doppler echocardiography. DT mice had evidence of dilated cardiopathy (25% increase in LVEDD) and severe myocardial hypokinesis (30% decrease in EF and 35% decrease in Vcfc corrected for heart

rate) (Table1). Mean arterial blood pressure in anesthetized nonfailing DT was not different from that of control littermates (mmHg, controls: 107.7 ± 1.9, $n = 5$; DT, 99.4 ± 8.1, $n = 4$).

Hypertrophied myocytes with large hyperchromatic nuclei and myocardial fiber disarray were observed in all DT mice and in both LVs and RVs (Fig. 3*a*). Apoptosis and a mononuclear infiltrate with only sparse interstitial mononuclear cells were very low in all of the observed cases. There was no necrosis, and all of the vessels observed were normal. Histological semiquantitative analysis showed that all of the 2-mo-old DT hearts present several alterations, as expressed by the mean of summed scores per animal (control = 0.2 ± 0.2; DT = 5.8 ± 1.5; $n = 5$; $P < 0.01$). Histopathological score was higher in more hypertrophied hearts. Multifocal disarray and myocyte hypertrophy are absent in control mice (Fig. 3*a*). Taken together, the histological lesions were similar to those observed in human hypertrophic cardiomyopathy. Marked interstitial fibrosis, without perivascular fibrosis, was evidenced on collagen staining in both ventricles (Fig. 3*b* and *c*). Fibrosis was not restricted to focal areas but diffuse over sections (on RV as well as LV) and was observed as early as 4 wk after birth and major in 2-mo-old animals (Fig. 3*d*).

Phenotypical analysis of a control transgenic mouse model (LacZ20) in which transgene expression in the heart, as estimated by LacZ reporter gene expression, was similar to that in DT27, indicated that the cardiac phenotype observed in αMHC-tTA/MR-AS DT was not due to nonspecific effect of transgene expression in cardiomyocytes. The heart/body weight ratio and functional parameters were similar in controls and DT-LacZ20 mice (heart/body weight: controls, 4.9 ± 0.02; DT, 5.07 ± 0.2, $n = 5$ NS; ejection fraction: controls, 84 ± 1.5%; DT, 83 ± 2%, $n = 5$).

The use of the tet system permitted the evaluation of double-transgenic mice that do not express the MR antisense mRNA. When Dox was administered to the mother during gestation and pursued in offspring after birth, the development of cardiac abnormalities was completely prevented: (*i*) survival at 12 wk was similar in Dox-treated DT17 mice and control littermates, in contrast to the reduced survival seen in nontreated DT mice (Fig. 2*c*); (*ii*) functional echographic parameters (Table1), heart/body weight ratio (Fig. 2*d*), and cardiac collagen deposit (Fig. 3*d*) were not different between Dox-treated DT mice and control littermates (treated or not with Dox). Taken together, these data indicate that the specific expression of a MR antisense mRNA in heart is associated with a cardiopathy, characterized by progressive myocardial disarray, hypertrophy with interstitial fibrosis, and ultimately severe heart failure.

Cardiac fibrosis appearing after chronic aldosterone loading in rats has been described (15, 22,23), but the relative importance of the direct effects of aldosterone on cardiac myocytes cannot be inferred from this model, because the effects of aldosterone in other target organs could be involved in cardiac alterations. This animal model is complex and requires uninephrectomy, high salt intake, plus aldosterone infusion (15, 22,23). In mice, cardiac fibrosis also was documented in the deoxycorticosterone (DOCA)-salt model (24,25). Mineralocorticoid administration resulted in an increase in MR occupancy in all MR-expressing cells, particularly renal cells, leading to sodium retention and kaliuresis. The pathogenesis of the cardiomyopathy in these models is not clear. Cardiac hypertrophy and fibrosis also are observed in other rat models such as sterone-prone spontaneously hypertensive rats (SP-SHRs), L-NAME/Angiotensin II treatment (26,27), where aldosterone does not play a major role. In the aldosterone–salt model, cardiac fibrosis has been attributed to a combination of aldosterone-induced cardiac effects (like cardiac AT1 receptor up-regulation), extracardiac (renal) effects, and a critical but unexplained role of salt (2,28).

Moreover, inflammatory cells were present in the cardiac fibrosis obtained after experimental hyperaldosteronism (15), which is not the case here. Transgenic mice with overexpression of human MR in heart and kidney exhibit mild cardiopathy without cardiac fibrosis (29). These observations raise further questions about the role of the cardiac MR in the aldosterone–salt model.

Spironolactone, a MR Antagonist, Potentiates the Effects of mMR-AS Expression in Cardiomyocytes. The Randomized Aldactone Evaluation Study clinical trial suggested a deleterious effect of aldosterone in heart failure. Indeed, spironolactone treatment of patients with severe heart failure (in addition to angiotensin converting enzyme inhibitors and other drugs) resulted in an improvement in morbidity and in a spectacular reduction in mortality (3). The mechanism(s) of this benefit is not known but may depend on MR antagonism in the kidney, resulting in the limitation of potassium loss, and may play an important role in the restoration of plasma and/or intracellular potassium levels, thereby decreasing hyperexcitability of cardiomyocytes (30). We used our conditional mouse model to evaluate whether 20 mg/kg/d spironolactone administration at 1 mo of age (at a time when no major phenotypic alteration was detectable) affected the DT mice. After 2 mo of spironolactone treatment, DT27 mice had more severe cardiac failure than untreated DT mice. Significant increases in heart/body weight ratio (Fig. 4*a*) and interstitial cardiac fibrosis (Fig. 4*b*) were observed in spironolactone-treated as compared to nontreated DT mice. Spironolactone has no effect when administered to control mice (Fig. 4 *a* and *b*). Therefore, spironolactone treatment, which reduces transactivation activity of the remaining endogenous MR, potentiated the effect of MR antisense mRNA expression.

Heart Failure and Cardiac Remodeling Are Reversible When mMR Antisense mRNA Expression Is Turned Off. A specific advantage of our inducible model over conditional gene inactivation using the Cre-Lox system is the unique opportunity to manipulate cardiac MR expression with time (through up- or down-regulation of MR antisense mRNA expression), thereby permitting the assessment of the kinetics of onset or resolution of the resulting abnormalities. Administration of Dox to a DT mouse with decompensated heart failure (major weight gain and dyspnea) resulted in rapid clinical improvement and weight loss (Fig. 5*a*). After initiation of Dox treatment to 2-mo-old DT mice that had cardiac alterations, physiological testing by echocardiography showed progressive improvement in systolic LV function (Fig. 5 *b* and *c*). The heart/body weight ratio (Fig. 5*d*) returned to control values within 1 mo of Dox administration. Strikingly, interstitial cardiac fibrosis also regressed (Fig. 5*e*), indicating that abnormal extracellular matrix deposition was reversible in this model. Another model of cardiac fibrosis has been recently described with conditional expression of a modified Gi-coupled receptor (Ro1) (31) but, in this model, fibrosis was not reversible on transgene expression shut-off. Differential gene expression analysis comparing different time points before and during the onset or resolution of the cardiopathy observed in our model should permit identification of altered signaling cascades in mice expressing the MR antisense mRNA, allowing the characterization of molecular events involved in the development of the disease. It would be interesting to determine whether interstitial fibrosis is a secondary or primary event in the development of the cardiopathy, and which cardiomyocyte functions are affected.

In summary, we have designed a transgenic mouse model that allows the specific and reversible inhibition of expression of MR in cardiomyocytes. A targeted approach, as used in this study, is critical to determine the role of the transgene in heart independent of its function in other cell types. This model allows analysis of the specific contribution of MR in heart, and

therefore differs from previous models, in which MR expression has been altered constitutively in multiple organs by overexpression (29) or gene inactivation (10), leading to both local and systemic effects. Here we observe that decreased cardiac MR without hyperaldosteronism dramatically affects cardiac structure and function in mice, leading to cardiac hypertrophy, ventricular dysfunction, interstitial fibrosis, and heart failure. These findings raise new questions about the role of the MR in the heart and offer a flexible model of cardiac fibrosis as a tool for drug efficiency testing

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ABBREVIATIONS

DT : double-transgenic
LV and RV : left and right ventricle
EF : ejection fraction
MR : mineralocorticoid receptor
mMR : mouse MR
MR-AS : antisense mRNA of the murine MR
ET : ejection time
EDD : end diastolic diameter
Vcfc : mean velocity of circumferential fiber shortening

FOOTNOTES

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FIGURES AND TABLES

Figure 1

Inducible expression of MR-AS RNA allows cardiac specific and Dox-controlled down-expression of endogenous MR RNA expression. (*a*) To obtain cardiac-specific doxycycline-controlled transgene expression, a α-MHC tTA transgenic mouse line (transactivator mouse line), expressing the tTA transactivator under the control of the cardiac-specific α-MHC promoter, was mated with MR-AS transgenic mice (MR-AS mouse line), leading to double transgenic mice with inducible and cardiac-restricted expression of an antisense mRNA of the murine MR and LacZ as a reporter gene. (*b*) MR antisense mRNA (RNase protection assay) is expressed in 1-mo-old DT27 mice, whereas no expression is detectable in mono-transgenic littermates used as controls. Expression is shut down when DT mice received Dox for 10 d. (*c*) MR antisense mRNA expression resulted in a decrease of ≈50% of the cardiac endogenous MR mRNA in 1-mo-old DT27 (black bars; $n = 5$) as compared to controls (open bars; $n = 4$); 10-d Dox treatment restored MR mRNA levels (black bars: +Dox) close to control values ($n = 4$). By contrast, renal expression of MR was not modified. *, Control vs. DT mice and #, Dox-treated vs. untreated mice, when $P < 0.05$.

Figure 2

Expression of mMR-AS mRNA in the heart causes dilated cardiomyopathy. (*a*) Control mouse (4-mo-old) (Cont) and 4-mo-old DT27 mouse with decompensated heart failure (DT). (*b*) Survival of mice expressing the MR-AS mRNA in the heart. More than 70% of DT17 (■, $n = 14$) and ≈25% of DT27 (▲, $n = 16$) males died by 12 wk, whereas control littermates (○, $n = 20$) did not. None of the Dox-treated DT17 (□, $n = 7$) died within 12 wk. (*c*) Heart from a control mouse (Cont) and a DT27 mouse with a major cardiac hypertrophy (DT). (*d*) Heart/body weight ratio of 3-mo-old control mice (open bars) and DT27 mice (black bars), treated or not with Dox from day 8 postcoitum. In DT mice, MR antisense mRNA expression induces an increase in heart/body weight ratio, which could be prevented by turning down transgene expression with Dox treatment ($n = 11$ for each group).*, Control vs. DT mice and #, Dox-treated vs. untreated mice when $P < 0.05$. (*e*) Time course of cardiac phenotype in control or DT27 mice. Mice were analyzed at 4, 8, and 12 wk of age. Increase of heart/body weight ratio was observed in 2-mo-old DT mice (▲), whereas it was unchanged in control mice (△) ($n = 6–10$ in each group; *, $P < 0.05$).

Figure 3

The cardiopathy induced by MR antisense mRNA expression is associated with cardiac remodeling and extensive interstitial fibrosis. (*a*) Histological analysis showed that DT heart (DT) is pathologic with large hyperchromatic nuclei (*Inset*), myocardial fiber disarray and myocyte hypertrophy that are absent in the control mouse (*Left*, cont) with (×1,000). (*b*) Sirius red-stained sections of myocardium of a DT (DT) and a control mouse (Cont). The interstitial fibrosis observed in DT is diffuse over the section (on RV as well as LV) (×200). (*c*) Interstitial cardiac fibrosis in 3-mo-old DT27 mice (black bars, $n = 11$), as compared to untreated mice (open bars, $n = 11$), is prevented when mice are treated with Dox from day 8 postcoitum. *, Control vs. DT mice and #, Dox-treated vs. untreated mice, when $P < 0.05$. (*d*) Quantification of cardiac fibrosis in control (△) and DT27 (▲) mice reveals major interstitial fibrosis in 2- and 3-mo-old animals with a mild increase in interstitial fibrosis in 1-mo-old DT mice ($n = 6–10$ for each group). *, Control vs. DT mice and #, Dox-treated vs. untreated mice, when $P < 0.05$).

Figure 4

Spironolactone, an MR antagonist, has synergistic effects with MR-AS mRNA expression on cardiac phenotype. Control mice (open bars) or DT27 mice (black bars) were treated or not with 20 mg/kg/d spironolactone (spiro) for 8 wk after weaning. Spironolactone-treated DT mice ($n = 9$) showed an increase in the heart/body weight ratio (*a*) and the interstitial fibrosis (*b*), as compared to nontreated DT mice ($n = 20$). Spironolactone had no effect in control mice ($n = 15$), as compared to nontreated control mice ($n = 20$). *, Control vs. DT mice and #, spiro-treated vs. untreated mice, when $P < 0.05$.

Figure 5

The cardiopathy is reversible on Dox administration. (*a*) A 4-mo-old DT27 with decompensated heart failure (*Right*, DT, day 0) was treated with Dox. Rapid improvement of edema was observed. Within 10 d, the mouse recovered a weight similar to that of the control littermate (*Left*, control). (*b* and *c*) Dox administration to 2-mo-old DT mice (black bars) allowed improvement of the EF (*b*) and of the Vcfc (*c*) as early as 1 wk after Dox administration (open bars: control animals). Differences between treated and nontreated DT animals are significant after 3 wk of Dox administration ($n = 5–7$ in each group). #, Dox-treated vs. untreated DT mice, when $P < 0.05$. (*d* and *e*) A 1-mo Dox administration to 2-mo-old DT mice (8 wk + reversal, black bar) allows complete normalization of the heart/body weight ratio (*d*) and the reversal of the interstitial fibrosis (*e*) observed in nontreated DT mice (12 wk, black bar), to levels of control mice (open bars) ($n = 7–10$ in each group).*, Control vs. DT mice and #, Dox-treated vs. untreated mice, when $P < 0.05$.

Table 1

Functional and echocardiographic cardiac parameters in control littermates and DT27 mice

	Control littermates, $n = 28$	**DT27, $n = 14$**	**DT27 + Dox, $n = 10$**
Heart rate, bpm	419 ± 9.3	404 ± 11	452 ± 12
LV EDD, mm	4.4 ± 0.06	5.01 ± 0.2*	4.05 ± 0.09
EF, %	78 ± 1.8	69 ± 4.3*	81 ± 2

	Control littermates, *n* = 28	**DT27, *n* = 14**	**DT27 + Dox, *n* = 10**
Vcfc, circ/s	2.64 ± 0.08	2.20 ± 0.18*	2.71 ± 0.15

Dilated cardiopathy was evidenced in 3-mo-old DT mice when compared to control littermates. It is characterized by LV dilation (as estimated with LVEDD), decrease in EF, and decrease in Vcfc. The administration of Dox starting during embryogenesis and pursued until 3 mo of age prevented the disease (*, $P < 0.05$, DT vs. controls).