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Excess of Glucocorticoid Induces Cardiac Dysfunction via Activating Angiotensin II Pathway

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Key Words

Dexamethasone • Hypertension • Hypertrophy • Myocardium • Oxidative Stress

Abstract

Background: Glucocorticoid is widely used as an anti-inflammatory drug in various diseases however excess of it often causes cardiovascular complications. The present study was undertaken to understand the molecular mechanism of glucocorticoid-induced cardiac dysfunction. Methods: Rats were treated daily with synthetic glucocorticoid, dexamethasone with or without mifepristone or losartan up to 15 days. Hemodynamic parameters were measured by PV-loop method using Millar's instrument. Cardiac remodelling, fibrosis and oxidative stress were monitored after 15 days. Results: The systolic blood pressure was increased whereas the heart beat and cardiac output (n=6) were decreased by dexamethasone. Dexamethasone caused increase in the heart weight to body weight ratio (P<0.001, n=20), increased level of mRNA of atrial natriuretic peptide and an increased deposition of collagens in the extracellular matrix of the left ventricle which were inhibited by both mifepristone and losartan. The rate of oxygen consumption was decreased in association with increased levels of hypoxia inducible factor 1α, lipid peroxidation (P<0.01, n=3) and superoxide dismutase activity (P<0.01, n=3) in dexamethasone treated rat heart. All these changes were reversed by mifepristone and losartan. Conclusions: The excess of glucocorticoid induces cardiac remodelling and pathophysiolgical changes of the myocardium via angiotensin II signalling pathway.

Introduction

Glucocorticoid (GC) is commonly used as an anti-inflammatory drug but GC therapy is often limited by several adverse reactions because excess of it exhibits a variety of symptoms including complications of cardiovascular system [1-7]. Specifically, it was demonstrated that excess of GC induced hypertension and atherosclerosis [5]. Furthermore, a synthetic GC,

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dexamethasone (DEX) was shown to induce hypertension via the mediation of peroxisome proliferator activator receptor α (PPAR-α) which is known to be critically involved during cardiac hypertrophy and remodelling [2]. However, it is not known whether excess of GC causes myocardial remodelling in heart similar to other hypertrophic stimuli.

Remodelling of the myocardium is one of the most detrimental effects of chronic hypertension [8-10]. Due to an increased work load during chronic hypertension myocyte hypertrophy occurs resulting in ventricular enlargement which is initially beneficial to the cardiovascular system [11, 12]. However, in the long run, such compensatory enlargement of the left ventricle becomes maladaptive leading to the contractile dysfunction and heart failure [13]. Remodelling of the left ventricle during hypertrophic growth is associated with an enhanced collagen synthesis which results into interstitial fibrosis causing myocardial stiffness and contractile abnormality [14]. Because of increased fibrosis the adjoining myocytes may pose insufficient supply of oxygen which could result into myocardial hypoxia [15] and oxidative stress [16-18].

Usually, hypertension-induced LV remodelling is mediated via angiotensin II (Ang II). Inhibition of Ang II type 1 (AT1) receptor with angiotensin receptor blockers regresses LV remodelling and improves cardiac function in the diseased hearts suggesting that Ang II has a direct role in the development of cardiac hypertrophy, fibrosis and dysfunction [19, 20]. Both circulating as well as locally produced Ang II binds to AT1 receptor on cardiomyocytes and stimulates hypertrophic growth of the cells [21-23]. Furthermore, Ang II was shown to mediate the oxidative stress-induced transition to LV failure in rat [20]. Therefore, Ang II has a pivotal role in the progression of cardiac dysfunction and heart failure in a variety of pathological situations [24].

Although GC excess has been known to cause severe side effects in heart, the mechanisms involved in GC-induced cardiac malfunction were not investigated. Therefore, we examined whether LV remodelling and contractile abnormality are responsible for cardiovascular complications due to GC excess. Here we have demonstrated that treatment of rat with synthetic glucocorticoid, dexamethasone (DEX) resulted into extensive remodelling of the heart accompanied by fibrosis, hypoxia, and oxidative stress and decreased LV functions via Ang II pathway. These informations are entirely new in light of the molecular mechanism of cardiovascular malfunction due to excess of GC.

Materials and Methods

Animal

Male Sprague-Dawley (SD) rats were received from the Institute's animal facility. The animals were handled as per the guidelines of Institutional animal ethics committee in accordance with the committee for the purpose of control and supervision of experiment on animals, Ministry of Social Justice, Government of India.

Administration of rat with drugs

Male SD rats (220 g BW) were treated with DEX (35 µg/100g BW, orally once daily) for 15 days. To examine the effect of excess of GC, relatively high dose of DEX was used based on the reports that DEX induces cardiovascular disorders [3-5]. To examine the involvement of GR and Ang II in mediating the cardiovascular complications, the GR antagonist, mifepristone (MIF, 2 mg/100 g/BW, orally once daily) and the AT 1 receptor blocker, losartan (2 mg/100g/BW, orally once daily) were co-administered [3, 25] separately with DEX for similar periods. The rationale for terminating the studies at 15 days was that daily administration of DEX with the above dose beyond 15 days caused mortality of rats that increased gradually with the duration of the treatment [26].

Hemodynamic study

The rats were anesthetized with sodium pentobarbital (50 mg/kg BW) and heparine (500 units/kg BW). The right internal carotid artery was identified and ligated cranially. A miniaturized conductance catheter (SPR-838 Millar instruments, Houston, TX) was inserted into the carotid artery and then advanced into the left ventricle until stable PV loops were obtained [27]. Data were then acquired under steady state conditions. Using the pressure conductance data a range of functional parameters were then calculated (Millar analysis software PVAN 3.4). Each experiment was repeated with six animals.

Assessment of cardiac hypertrophy

The rats were weighed and sacrificed by cervical dislocation, and hearts were surgically removed and immersed in ice-cold 0.9% NaCl. The ventricles were collected, weighed and stored in -80°C for future use. The degree of hypertrophy was calculated in terms of heart (ventricular) weight/body weight (HW/BW) ratio.

Real time quantitative RT-PCR

Levels of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and AT 1 receptor mRNAs were measured by real time quantitative RT-PCR using the Dynamo™ SYBR® Green qPCR Kit (Finnzymes, Finland) and the iCycler real time detection system and software (Bio-Rad Laboratories, Hercules, CA, USA). For RT-PCR reactions (20 µl), equal amount (500 ng) of total RNA was used. The list of PCR primers were presented in Tab. 1. The level of expression was calculated as fold change compared to control using the Ct value after normalizing with the GAPDH as described earlier [26]. Experiment was repeated with samples from 6 different rats.

Gene	NCBI Genbank Accession No.	Forward primer (5'->3')	Reverse primer (5'->3')
ANP	NM012612	GTGTCCAACACAGATCTGAT GG	GCCAGCGAGCAGAGCCCTCA
BNP	M25297	TGGGAAGTCCTAGCCAGTCTC	GCCGATCCGGTCTATCTTCTG
AT1 Receptor	M87003	GAACATTCTGGGCTTCGTGT	TGACAAGCCTGCATGTGACT
GAPDH	XM216453	GCCATCAACGACCCCTTC	AGCCCCAGCCTTCTCCA

Table 1. List of primers.

Histological studies

After 15 days of treatment, hearts were collected and immediately fixed in 10% formalin and embedded in paraffin following routine procedure as described earlier [26]. Left ventricular sections (5 μM thick) were prepared and stained with hematoxylin-eosin (Sigma Chemical Co, Louis, MO, USA). The stained tissue sections were examined under Olympus BX51 (Olympus Corporation, Tokyo, Japan) microscope and images were captured with a digital camera attached to it. To measure the myocyte cross-sectional area, each HE stained LV section was observed under 400 x magnification using the same microscope and images were captured. Peripheral margin of 20-30 myocytes was manually traced and cross sectional area was measured [26, 28-29] using Olympus Micro Image (version 4.5) software.

Quantification of fibrosis by Confocal Microscopy

Left ventricular sections (5 μM thick) were stained with Sirius red (Direct Red 80; Sigma Chemical Co, Louis, MO, USA) and imaged with laser scanning confocal system (Leica TCS, SP2, Germany) and the stacked images through multiple slices were captured. The digitized images were then analysed using image analysis system (Image J, NIH Software, Bethesda, MI) and the total collagen area fraction of each image was measured and expressed as the % collagen volume as described earlier [26, 30].

Oxygen consumption study

Hearts were perfused with ice cold 0.9% saline. About 100 mg of tissue was obtained from the left ventricle. Then tissues were minced with sharp scissors into approximately 0.3-0.4 mm in diameter. Mitochondrial respiration was measured by monitoring oxygen (O_2) uptake as previously described [31] using an YSI 5300A biological oxygen monitor. The amount of O_2 consumption was calculated for each sample from the plotted graph.

Western Blot Analysis

The whole homogenate from left ventricular tissues was prepared as described earlier [26, 32]. Briefly, the left ventricle was homogenized in a buffer containing 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM PMSF, 1mM sodium orthovanadate, 1 μg/ml each of pepstatin A, leupeptin, and aprotinin. Equal amount of proteins (60 μg) was resolved in 10% SDS-PAGE for immunoblotting with antibodies for hypoxia inducible factor 1α (HIF-1α) and heat shock protein 70 (HSP-70) and actin (Santa Cruz Biotechnology Inc., CA).

Determination of lipid peroxidation in cardiac tissue

Left ventricular tissue (200 mg) was homogenized in ice-cold 0.9 saline (pH 7.0) with a Potter-Elvehjem glass homogenizer for 30 sec. The lipid peroxides in the homogenate was determined as thiobarbituric acid reactive substances (TBARS) by previously described method [33, 34]. Briefly, the homogenate was mixed with TBA-TCA (Thiobarbituric acid-Trichloro acetic acid) reagent with thorough shaking and boiled for 15 min in a boiling water bath. The samples were then cooled to room temperature. The absorbance of the pink chromogen present in the clear supernatant obtained after centrifugation was measured at 532 nm using a UV-VIS spectrophotometer. The amount of lipid peroxidation was calculated in terms of the level of TBARS.

Lactate Assay

Blood was collected directly from the heart of anesthetized animal and lactate concentration in the serum was measured using commercially available Enzy Chrom Lactate Assay Kit (Bioassay Systems, Hayward, CA, USA).

Measurement of superoxide dismutase activity

The activity of Cu,Zn-superoxide dismutase (Cu,Zn-SOD) was determined in rat heart following xanthine oxidase-cytochrome C method [35] with some modifications. Briefly, 500 mg ventricular tissue was homogenized in a buffer containing 0.2 mM EDTA, 0.25 M sucrose, 10 mM Tris-HCl, pH 7.8) in the presence of protease inhibitor cocktail following centrifugation at 1000 x g for 10 minutes to remove nuclear debris. The supernatant was collected and spun at 12000 x g for 10 minutes. The supernatant was collected and used for Cu,Zn-SOD assay. The SOD assay was performed spectrophotometrically at 550 nm with a xanthine/xanthine oxidase, a superoxide generating system, in the presence of cytochrome C. The SOD activity was expressed as units/mg protein. The experiment was repeated 3 times with different individual rats.

Catalase Assay

Frozen myocardium (0.2 gm) was homogenized in 0.5 ml of ice-cold phosphate buffer (0.05 M, pH 7.0). The homogenate was centrifuged at 12000 x g for 12 min. The supernatant was collected and 0.01 ml of absolute ethanol was added and incubated at 4°C for 30 min prior to the addition of 10% Triton X-100 to a final concentration of 1%. The addition of ethanol is thought to prevent peroxisomal H_2O_2 generation and thus protect catalase activity during extraction. The samples thus

Experiment	Control	DEX	DEX + MIF	DEX + LOS
Heart beat	333.86 ± 14.7	275.93 ± 5.41*	370.87 ± 4.86	370.73 ± 7.47
Pmax (mm Hg)	108.35 ± 4.6	147.93 ± 1.54*	86.45 ± 3.3	110.99 ± 5.68
Pmin (mm Hg)	20.08 ± 0.91	14.11 ± 0.51	14.02 ± 2.28	21.76 ± 0.14
CO	24053 ± 740	7997 ± 121*	22936 ± 2105	23011 ± 121
dP/dt max	5402 ± 665	4274 ± 34*	6441 ± 185	5102 ± 283
dP/dt min	5115 ± 529	3555 ± 195*	4193 ± 80	5680 ± 897

Table 2. Hemodynamic parameters of heart function. *P< 0.01 vs control, n=6.

Treatment	Control	DEX	DEX + MIF	DEX + LOS
HW (mg)	583 ± 3.1	723 ± 9.7*	588 ± 2.45	592 ± 2.88
BW (g)	205 ± 1.5	185 ± 1.6	200 ± 1.44	197 ± 1.3
HW/BW (mg/g)	2.84 ± 0.02	3.90±0.04*	2.94 ±0.02	3.0 ± 0.03

Table 3. Dexamethasone-induced cardiac hypertrophy is inhibited by mifepristone and losartan. *P< 0.001 vs control, n=20.

obtained, were then used to determine catalase activity as described earlier [36].

Statistical Analysis

The data are presented as the mean ± S.E.M. Most of the experiments were conducted independently at least with 3 animals. For real time PCR analysis, 6 rats were used in each group. Hypertrophy of heart was calculated based on 20 rats in each set of experiment. Data were evaluated by one-way analysis of variance using the software Microcal Origin 6.0 (Microcal Software Inc, MA, USA). A level of P<0.05 was considered the threshold for statistical significance between the control and various experimental groups.

Results

Dexamethasone causes cardiac dysfunction via angiotensin II pathway

As shown in Tab. 2, the systolic blood pressure was significantly (P>0.01, n=6) increased in DEX treated rat (Pmax, 147.93 ± 1.54 mm Hg) compared to that of control (Pmax, 108.35 ± 4.6 mm Hg). The heart beat and cardiac output (CO) were significantly reduced in DEX treated rat. The parameter of diastolic function (dP/dt min) was significantly reduced by DEX compared to control. Both MIF and losartan significantly restored DEX-induced alteration of the hemodynamic parameters.

Involvement of Ang II in glucocorticoid-induced myocardial remodelling

Rats were administered with DEX for different duration up to 15 days. There was a gross enlargement of the myocardium after 15 days of treatment with DEX (Fig. 1A). Co-administration with either GR antagonist, MIF or AT 1 receptor blocker, losartan, clearly inhibited DEX-induced enlargement of the myocardium (Fig. 1A). The absolute weight of heart (combined left and right ventricle) was 24% greater than control (Tab. 3). The HW/BW ratio was gradually increased with the duration of treatment (Fig. 1B) which was significantly higher after 15 days of treatment (P<0.001, n=20). Cardiac hypertrophy did not occur when MIF or losartan separately co-administered with DEX.

To further confirm the involvement of Ang II for the induction of cardiac hypertrophy, the expression of hypertrophic marker, ANP and BNP was also examined. As shown in Fig. 1C, the expression of ANP mRNA was increased with the duration of DEX treatments attaining a 4-fold induction (4.22 ± 0.11, P<0.01, n=6) over control after 15 days. Similarly, there was a time dependent induction of BNP expression (Fig. 1D) which was about 6 fold higher after 15 days of treatment (P<0.01, n=6). These increases were prevented by treatment with MIF or losartan (Figs. 1C, 1D). Treatment of rat separately either with MIF or losartan alone did not have any effect on HW/BW ratio (data not shown). Therefore, the effect of MIF or losartan alone on the other parameters was not shown in the subsequent experiments.

Histology of the left ventricle

The left ventricular sections showed cellular hypertrophy in DEX treated rat (Fig. 2A). The cross sectional area of the myocytes was significantly

Fig. 1. Involvement of angiotensin pathway in dexamethasone-induced cardiac hypertrophy. Representative image showing enlarged ventricular portion after 15 days of treatment with either vehicle (CON), dexamethasone (DEX) or dexamethasone with mifepristone (DEX+MIF) or losartan (DEX+LOS). B. The degree of hypertrophy (HW/BW) of rats treated with DEX for 5 days (d5), 10 days (d10) and 15 days (d15) are shown along with regression when co-treated with either mifepristone (MIF) or losartan (LOS). This data represents mean ± SEM for 20 rats in each group. *P<0.001 vs control (n=20). Real time RT-PCR showing ANP (C) and BNP (D) gene expressions as fold change over control after normalizing with GAPDH mRNA expression in left ventricle of similar experimental group. Data represents mean ± SEM from 6 animals, in duplicates. *P<0.01 vs control (n=6).

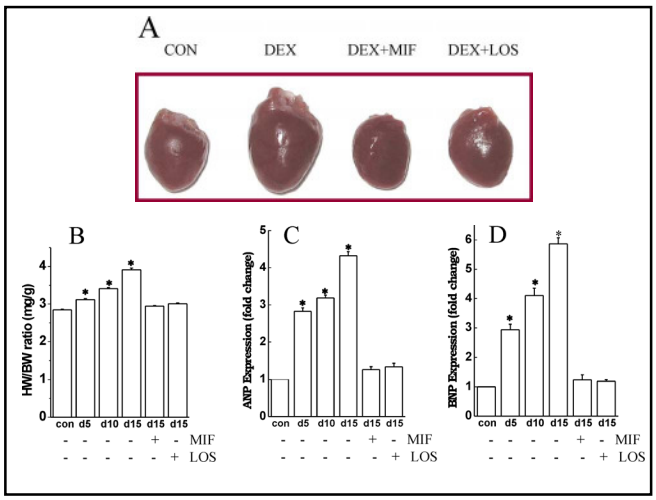
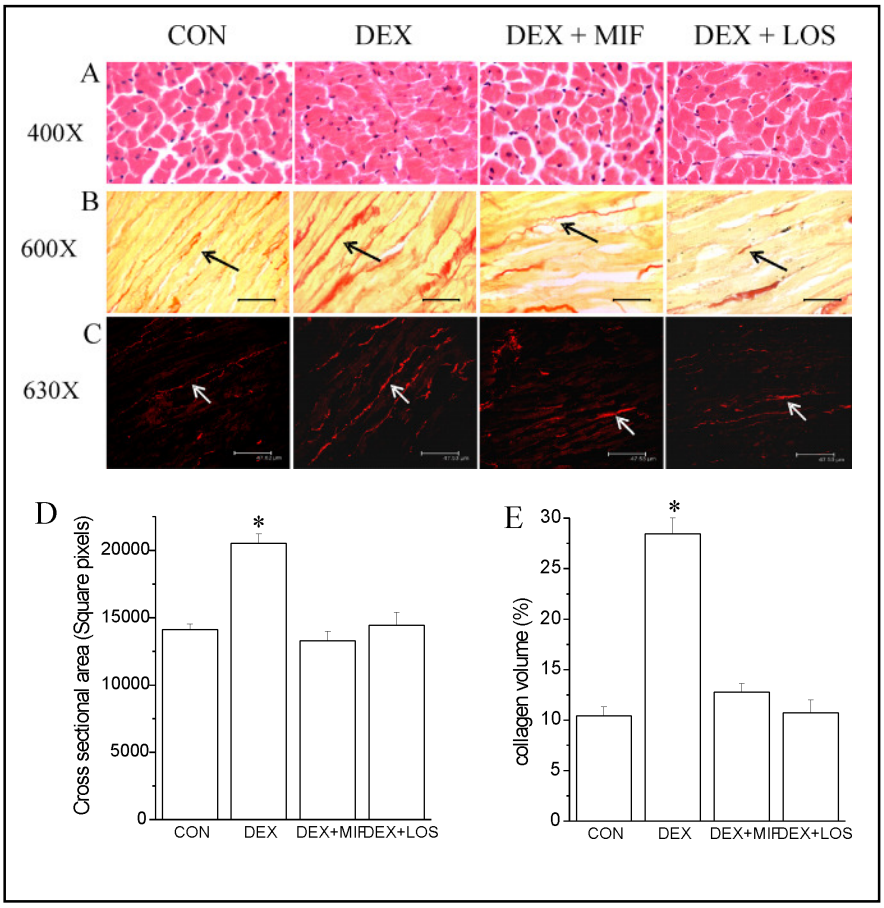


Fig. 2. Ventricular remodelling and fibrosis. Representative images (400 X magnification) of hematoxyline-eosine stained cross sections (A) through left ventricles of rats treated with vehicle (CON) or dexamethasone (DEX) or dexamethasone with mifepristone (DEX+MIF) or dexamethasone with losartan (DEX+LOS) for 15 days. (B) Images (600 X magnification) shown are the left ventricular tissue sections stained with Sirius red. Red color stretches are collagen depositions. (C) The similar images were captured by laser scanning confocal microscope for quantification of fibrosis. Arrow heads indicate collagen fibers. (D) Myocyte cross-sectional area calculated from the hematoxyline and eosin-stained sections as shown in (A). Each bar represents myocyte cross-sectional areas in mean ± SEM of square pixels of 3 images from 3 individual rats (* P<0.01 vs control, n=3). (E) Histogram showing % collagen volume in the left ventricular tissues. Data indicate mean ± SEM % collagen volume obtained from 3 images from each of 3 different rats.



increased in DEX treated rat heart compared to control (Fig. 2D). When MIF or LOS were co-administered separately with DEX, the cross sectional area of the myocytes was similar to that of control tissue (Fig. 2D). As revealed by picrosirius red staining, there was an increased deposition of collagens in the ventricular sections in DEX treated rat compared to those of control (Figs. 2B, 2C). The volume of total collagens was also increased significantly (P<0.01, n=3) in DEX treated rat heart which was decreased to the normal level in the presence of MIF or losartan (Fig. 2E) indicating that the development of cardiac fibrosis in DEX treated rat was mediated both via GR and AT 1 receptor.

Dexamethasone induces the expression of AT 1 receptor genes

Since inhibition of Ang II by losartan inhibited DEX-induced cardiac hypertrophy and remodeling, the mRNA level of AT 1 receptor in the LV was measured by real time quantitative RT-PCR using gene specific primers. The level of AT1 receptor (Fig. 3) mRNA were increased significantly (P<0.01, n=6) by DEX which was blocked when co-administered with MIF. In contrast, co-treatment with losartan did not inhibit DEX-induced expression of AT1 receptor.

Effect of GC excess on cellular respiration in heart

Oxygen consumption is one of the important parameters of mitochondrial respiration and heart function. Hence, oxygen consumption in cardiac tissue was measured after 15 days with DEX in the absence or presence of either MIF or losartan. As shown in Fig. 4, the oxygen consumption was significantly (P<0.01, n=3) decreased in DEX treated rat heart which was restored to the level of control tissue when co-administered either with MIF or losartan. The result suggested that DEX considerably affect mitochondrial respiration in heart via the Ang II mediated signalling pathway.

Induction of hypoxia-inducible factor by dexamethasone

To examine whether cellular hypoxia induces the expression of hypoxia-inducible factor, LV homogenates were subjected to western blotting analysis with anti-HIF-1α antibody. As shown in Fig. 5, treatment of rat with DEX enhanced the level of HIF 1α in cardiac tissue which was inhibited by MIF as well as losartan.

Dexamethasone induces oxidative stress in heart

Since oxidative stress is known to affect cardiovascular functions directly in a variety of pathological conditions and glucocorticoid directly influences mitochondrial function, we examined whether oxidative stress is generated in ventricular tissue due to the treatment with DEX. As shown in Fig. 6, the level of lipid peroxidation was significantly (P<0.01) increased by DEX which was inhibited by MIF and losartan.

To examine the involvement of mitochondria and oxidative stress, we examined the level of HSP-70 expression in LV homogenates and lactate level in serum. As shown in Fig. 7A, the expression of HSP-70 was increased by DEX. The level of lactate was also increased significantly (P<0.01, n=3) in DEX treated rat compared to control. DEX-induced HSP -70 expression was reversed partially whereas lactate level was restored significantly by MIF and LOS (Figs. 7A, 7B).

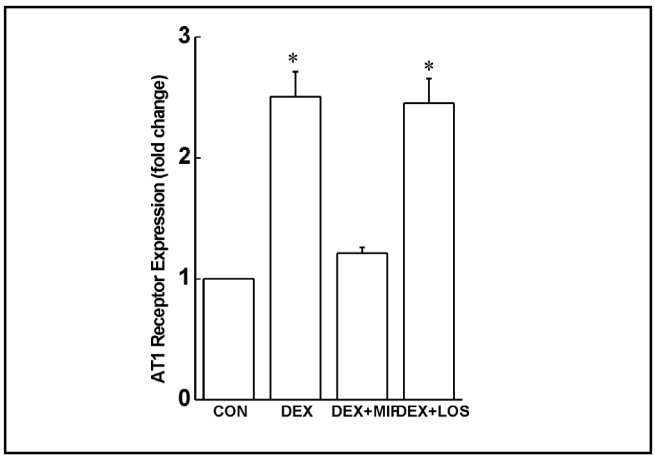


Fig. 3. Effect of DEX on AT1 receptor mRNA levels in left ventricle. Real time quantitative RT PCR analysis showing the expression of AT1 receptor gene in the left ventricular tissue of rats treated with vehicle (CON) or dexamethasone (DEX) or DEX in combination with either mifepristone (DEX+MIF) or losartan (DEX+LOS) for 15 days. Data represents mean ± SEM from 6 animals, in duplicates. *P<0.01 vs control (n=6).

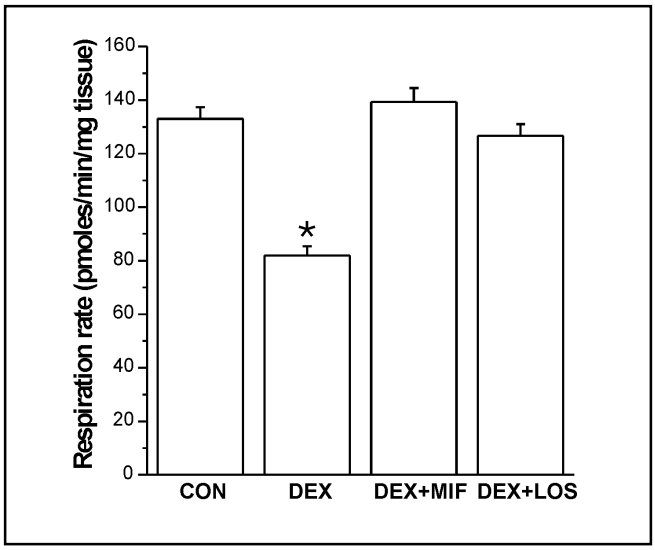


Fig. 4. Oxygen consumption. Rate of respiration was measured using ventricular tissues of rat treated either with vehicle (CON) or dexamethasone (DEX) or dexamethasone with mifepristone (DEX+MIF) or dexamethasone with losartan (DEX+LOS) for 15 days. Ventricular tissue was minced to very small pieces and the rate of oxygen uptake was measured as described in Materials and Methods. Each bar represents mean ± SEM of 3 separate observations (n=3). *Significantly different at P <0.01 vs control.

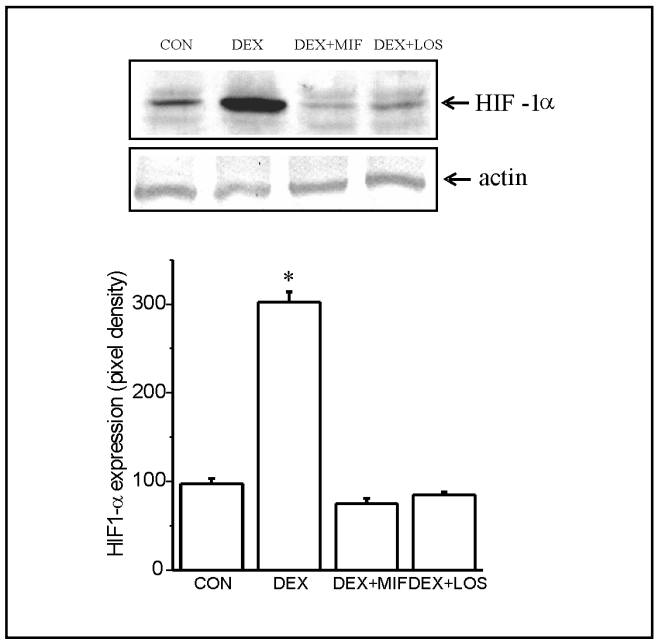


Fig. 5. Monitoring marker for hypoxia. Expression of HIF-1α in the LV homogenates of rat treated with vehicle (CON) or dexamethasone treated alone (DEX) or in combination with either mifepristone (DEX+MIF) or losartan (DEX+LOS). The equal amount of protein (70 µg) was loaded onto the polyacrylamide gel and the immunoblotting analysis was conducted with anti-HIF-1α antibody. Histogram showing mean pixel intensities (arbitrary units) of 3 immunoblots performed with different individual rats (n=3). *Significantly different compared to control at $P<0.01$.

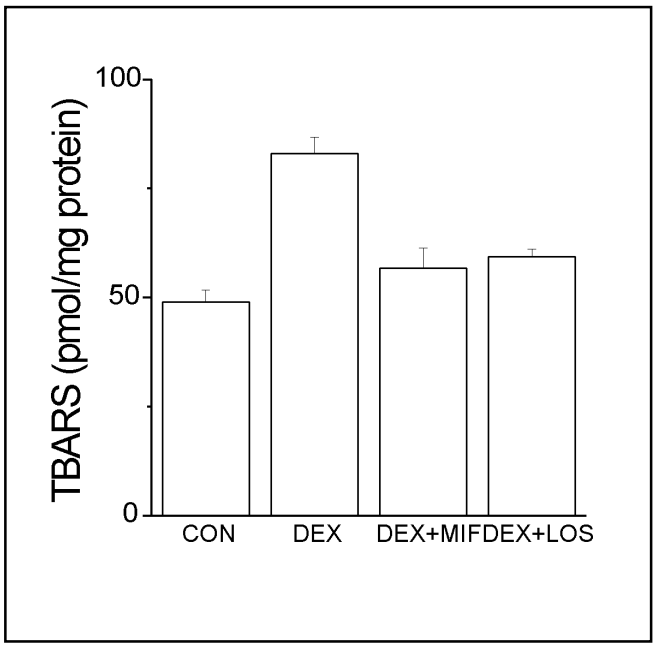
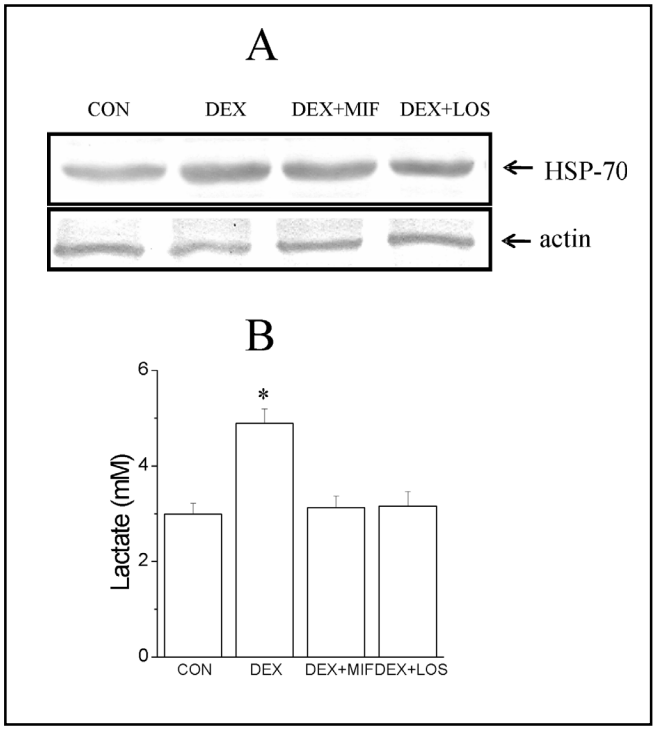


Fig. 6. Lipid peroxidation in rat heart tissues. LV homogenates from rats treated with vehicle (CON) or dexaemthasone (DEX) alone or dexamethasone in combination with either mifepristone (DEX+MIF) or losartan (DEX+LOS) were subjected to estimation of lipid peroxidation. Lipid peroxidation is expressed as pmoles TBARS/mg protein. Data represents mean ± SEM of 3 animals (n=3) and *indicates significantly different compared to control at $P<0.01$.

Fig. 7. DEX induces the expression of HSP-70 in LV and lactate in serum. Immunoblotting analysis to examine the expression of heat shock protein (HSP-70) in the ventricular tissue homogenate (50 µg) of rat treated with vehicle (CON) or dexamethasone alone (DEX) or in combination with either mifepristone (DEX+MIF) or losartan (DEX+LOS). B. The serum collected from similar experimental groups was subjected to lactate estimation as described in the "Materials and Methods". Each bar represents mean ± SEM of 3 separate animals in each experimental group. *indicates significantly different compared to control at $P<0.01$.



To examine the status of endogenous antioxidants, the activities of SOD and catalase were examined in LV homogenates. The activity of SOD was significantly ($P<0.01$, n=3) increased whereas the catalase activity was significantly ($P<0.01$, n=3) decreased by DEX compared to control (Figs. 8A, 8B). These changes were significantly prevented by MIF and losartan.

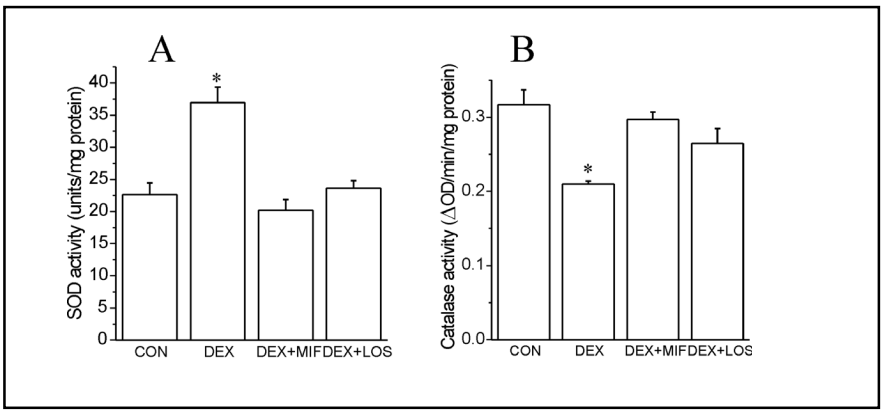


Fig. 8. Effect of mifepristone and losartan on superoxide dismutase and catalase in dexamethasone-treated rat heart tissue. Activities of Cu,Zn-SOD (A) and catalase (B) in LV tissues of rats treated with vehicle (CON), dexamethasone (DEX) or dexamethasone along with mifepristone (DEX+MIF) or losartan (DEX+LOS) for 15 days. The results are mean ± SEM of 3 separate animals (n=3) in each experimental group.*P<0.01 control vs DEX.

Discussion

The present study demonstrates one possible mechanism of cardiac toxicity due to an excess of GC administration. The molecular basis of GC-induced cardiac malfunction could be attributed to extensive myocardial remodeling, hypoxia and oxidative stress. Importantly, all these effects are mediated via the AT1 receptor suggesting that Ang II pathway is critically involved in GC-induced pathophysiological changes of the myocardium.

Application of glucocorticoid for a prolonged period may cause gross enlargement of the ventricle as shown in the present study (Figs. 1A, 1B). Consistent with our earlier report [26], GC-induced enlargement of the myocardium is accompanied by increased level of ANP and BNP mRNAs and massive remodelling of the extracellular matrix (ECM) as shown in Figs. 1 and 2. Since GC primarily acts via GR which belongs to nuclear hormone receptor family [37], GR antagonist MIF, significantly blocks hypertrophy and expression of ANP and BNP (Fig. 1). It is likely that activation of the Ang II pathway by DEX leads to cardiac hypertrophy since losartan ameliorated such changes. This is further supported by increased expression of AT1 receptor mRNAs by DEX (Fig. 3).

Extracellular matrix plays an important role in regulating the function of cardiomyocytes besides imparting the architectural support to the adjoining cells [38]. Increased deposition of collagen in the ECM leads to fibrosis which cause contractile abnormality and reduced heart beat [14]. In the present study also, DEX causes increased collagen deposition which might be responsible for reduced heart beat (Table 2). Fibrosis is also directly responsible for reduced blood flow to the heart due to increased distance between blood vessels and the cardiomyocytes [15]. Therefore, oxygen consumption is reduced as shown in Fig. 4. This is further supported by increased level of HIF 1α in DEX treated rat heart (Fig. 5) which is a marker of hypoxia in cell [39].

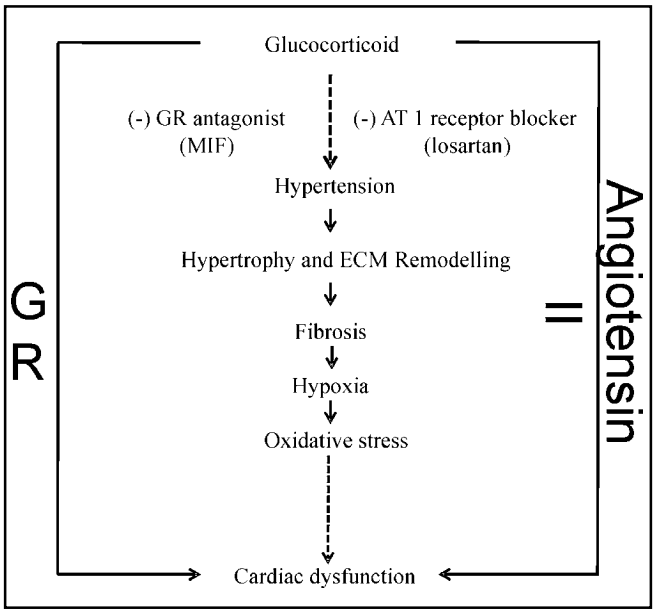


Fig. 9. Schematic representation depicting possible mechanism of glucocorticoid-induced cardiac dysfunction. Glucocorticoid induces hypertension, cardiac hypertrophy, ECM remodeling or fibrosis. Fibrosis may lead to low oxygen availability to the cardiomyocytes resulting in hypoxic condition and oxidative stress. In overall, glucocorticoid via GR may induce pathological changes which possibly cause reduced cardiac output and LV dysfunction where Ang II pathway is critically involved. Solid line indicates direct involvement and dashed lines indicate involvement of other intermediates.

Sufficient oxygen supply to cardiac muscle is crucial for survival of mammals due to its central role as an electron acceptor in the mitochondrial respiratory chain driving ATP synthesis. Mitochondria have been suggested

as the primary oxygen sensor and increased reactive oxygen species (ROS) production originating from the mitochondrial respiratory chain has been observed under hypoxia [17, 40]. Since excessive generation of ROS is known to cause increased lipid peroxidation in cell, in the present study, increased level of lipid peroxidation probably indicates a higher level of ROS in LV under hypoxic condition (Fig. 6). As one of the markers of oxidative stress, HSP-70 is increased in DEX-induced hypertrophied heart which is further supported by increased production of lactate in blood (Fig. 7). This is consistent with reduced cellular respiration and a high level of HIF-1α (Fig. 5). Moreover, in the present study, SOD activity is increased (Fig. 8A) as a compensatory response to protect the heart from ROS mediated damage. However, a concomitant decrease in catalase activity (Fig. 8B) is indicative of an accumulation of H_2O_2, a potentially toxic ROS capable of inflicting oxidative damage. Amelioration of the deleterious effects of DEX by losartan supports the idea that hypertension is associated with an elevation of ROS and frequently also with an impairment of endogenous antioxidant mechanisms [41]. A decrease in cellular respiration and an increase in oxidative stress may be directly responsible for poor heart function in many pathophysiological situations.

In the present study also, the heart function is drastically affected which is evident from reduced cardiac output and remarkable alteration in diastolic parameter (Table 2). The systolic parameter was also reduced significantly by DEX, but not to the extent of diastolic dysfunction, which might be due to the overall impairment of the LV. Though the hemodynamic parameters in the present study were measured in different settings of the heart beats, in overall, these alterations reflect the actual condition per se due to DEX treatment.

Angiotensin II may exert its numerous effects in modulating cardiovascular physiology and pathology by inducing signalling pathways in vascular smooth muscle cells, endothelial cells, and cardiac fibroblasts, and by affecting their interaction with the extracellular matrix. These cascades of events, in addition to other abnormalities, ultimately lead to cardiac dysfunction [24]. Similarly, it appears that GC (via induction of Ang II pathway) evokes signaling events in the cardiac fibroblasts and myocytes leading to cardiac fibrosis, decreased cellular respiration, increased oxidative stress which could be attributed to cardiac malfunction (Fig. 9). However, the present study does not conclusively demonstrate whether all these events are in direct consequences of glucocorticoid-induced fibrosis. Some of these events might be due to secondary effects of glucocorticoid. On the other hand, nitric oxide system has also been implicated in glucocorticoid induced hypertension [42, 43]. Therefore, the involvement of pathways other than AT 1 receptor in glucocorticoid-mediated hypertension and oxidative stress may also be possible [42-44]. In overall, it is likely that excessive use of this anti-inflammatory drug could evoke Ang II pathway leading to cardiotoxicity in the long run.

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