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ORIGINAL ARTICLE

Atorvastatin improves cardiac function and remodeling in chronic non-ischemic heart failure: A clinical and pre-clinical study

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KEYWORDS

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Abstract *Aims:* The aim was to evaluate the cardio-protective effect of atorvastatin in combination with standard chronic heart failure (CHF) therapy that might improve cardiac function, remodeling, and further delay the progression of CHF in patients and rats.

Methods and results: CHF patients (n = 20 per group) with left ventricular ejection fraction (LV-EF) <45% were randomized into: standard anti-failure treatment alone (controls) and standard anti-failure treatment plus atorvastatin (40 mg/day) for 6 weeks. After 6 weeks, the patients were assessed using echocardiography. Laboratory evaluation for lipid profiles, high sensitive C-reactive protein (hs-CRP), cardiac troponin-T (cTnT) and malondialdehyde (MDA) were performed in all patients. In parallel, rats (n = 10 per group) received treatment for 4 weeks and were divided as follows: saline treated (control, 1 ml intraperitoneal, IP), doxorubicin treated (2.5 mg/kg, IP), atorvastatin–doxorubicin treated (10 mg/kg, orally), and digoxin–doxorubicin treated (0.02 mg/kg, orally). The same laboratory analysis including histopathology of heart tissues was performed on the rats.

In patients, atorvastatin improved heart function (increased LV-EF%, LV-fraction shorting (LV-FS%), and E/A velocity ratio; decreased LV-end diastolic diameter (LV-EDD) and LV-end systolic diameter (LV-ESD)) and significantly reduced serum lipid profiles, cTnT, hs-CRP and MDA versus patient controls. In rats, atorvastatin improved signs of CHF, systolic blood pressure, reduced

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serum lipid profiles, cTnT, hs-CRP and tissue MDA; less cardiac necrosis and fibrosis with enhancement of neo-vascularization versus other doxorubicin-treated rats.

Conclusions: Atorvastatin with standard CHF therapy improved cardiac function and remodeling. Cardio-protective "pleiotropic" actions of atorvastatin are anti-inflammatory, anti-fibrotic and anti-oxidative. Thus, atorvastatin has a potential therapeutic value in the management of CHF patients.

1. Introduction

Although 3-hydroxy-3-methylglutaryl (HMG) coenzyme-A reductase inhibitors (statins) prevent important causative factors for chronic heart failure (CHF), myocardial damage and ischemia, the benefits of statins and lowering low-density lipoprotein (LDL-c) in CHF patients have been questioned.[1] Statins, in addition to standard medications for heart failure, are associated with an improvement in morbidity and mortality and significantly reduced subsequent hospitalizations among patients taking them as compared to the placebo group.[2,3] Statin therapy was associated with improved survival in both; patients with ischemic heart failure and patients with non-ischemic heart failure.[4] A previous meta-analysis[2,3,5] of 10 randomized placebo-controlled trials (6 for atorvastatin, 3 for rosuvastatin, and 1 for simvastatin) suggest that statins may be safe and improve left ventricular (LV) ejection fraction (LV-EF), decrease brain natriuretic peptide (BNP) levels and decrease hospitalizations for worsening CHF.[4,6]

Despite recent therapeutic advances, the appropriate role of drug-specific statin therapy in the CHF population, however, remains unclear.[2] There exists an increasing need to find new therapeutic strategies to reduce high mortality and morbidity in this population. Statins, a class of agents for lowering blood lipids, have been shown to reduce adverse cardiovascular events in atherosclerosis related diseases in patients with documented coronary artery disease (CAD).[7] Consequently, the current guidelines for the management of CHF target the causal pathological disease, control of heart rate, and reduction of fluid retention. Thus, combined therapy with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers, β-adrenergic or aldosterone blockers, and diuretics seems to be the current (standard) optimal therapeutic strategy.[4,7] Most of these effects can target important components of the complex physiopathology of heart failure.[8] Thus, concern has been raised about the potential benefits of statins in patients with CHF of multiple etiologies.[4,6]

Several studies in rat models and patients have suggested that statins may directly improve LV relaxation and function by reducing LV hypertrophy and fibrosis, and increasing arterial compliance.[9,10] Some of the effects of statins improve endothelial function.[11,12] However, the best-characterized pleiotropic effects of statins are their anti-inflammatory[13] and antioxidant actions[14,15] and their protective effect against endothelial and LV dysfunction.[12,16] We chose a rat model with doxorubicin induced heart failure because of the commonly known cumulative cardiotoxic effect in cancer patients.[17–20] To date, no such studies have been performed on the cardio-protective potential of atorvastatin in doxorubicin-induced cardiomyopathy.

In this study, we investigated whether the early use of atorvastatin in combination with standard CHF therapy might improve cardiac function and remodeling in CHF patients and also further delay the progression of CHF in doxorubicin-induced heart failure in rat models.

2. Methods

2.1. Clinical study

2.1.1. Subjects

The present study included 40 patients recruited from the Cardiology Department outpatient clinic at Menoufiya University Hospital between January 2010 and March 2012. Eligible patients were men and women of 35–75 years of age with a clinical diagnosis of CHF. Written informed consent was obtained from each participant before inclusion in the study. Ethical approval for this investigation was obtained from the Research Ethics Committee, Faculty of Medicine, Menoufiya University.

CHF diagnosis was determined by echocardiography evaluation. These patients were chosen according to the following inclusion and exclusion criteria: *Inclusion Criteria:* (1) patients who had symptoms according to New York Heart Association (NYHA) functional class II or III, assessed by a detailed history and clinical examination; (2) patients with non-ischemic heart failure with no history of myocardial infarction and cardiac catheterization without significant CAD; (3) patients with findings of a dilated LV end diastolic diameter (LV-EDD) (>60 mm), affected LV fraction shortening (LV-FS) ($<25\%$) and LV-EF ($<45\%$); and (4) patients who used standard anti-heart failure drugs in the form of loop diuretics and spironolactone, β-blockers, ACE inhibitors or angiotensin II receptor blockers with or without digoxin, regularly, for at least 1 month prior to the study. *Exclusion criteria:* (1) patients with CHF caused by ischemic heart diseases; (2) valvular heart diseases; (3) rhythmic dysfunction; (4) renal, hepatic, or pulmonary dysfunction; (5) patients with uncontrolled diabetes; and (6) patients with uncontrolled hypertension (HTN).

All patients with non-ischemic CHF were randomized and divided into two groups (20 patients in each group): (a) Control group: received standard anti-failure treatment in unchanged dose for at least one month before the study and for 6 weeks during the study without any statin therapy. (b) Statin-treated group: received standard anti-failure treatment in unchanged dose for at least one month before the study

and concomitantly with oral atorvastatin therapy (Lipitor® tablets, Pfizer, Egypt), of 40 mg/day for 6 weeks. The dose of 40 mg per day atorvastatin was chosen because this dose was within the dose range observed in the clinical setting, based on previous studies.[21–24]

2.1.2. Echocardiography in patients

Baseline echocardiography was performed on all patients with CHF at the beginning of study and again after 6 weeks of treatment. Two-dimensional and Doppler echocardiography (Vivid 7, Milwaukee, USA) were performed in the left lateral decubitus position with a broadband (1.5–4 MHz) phased array transducer at rest. Echo evaluations included: LV-EDD, end systolic diameter (LV-ESD), LV-FS and LV-EF. Doppler flow velocities (*E* and *A* waves) were taken at the level of the mitral valve in the apical four-chamber view in diastole with the Doppler probe placed at the edge of the mitral leaflets. The peak of early (*E*) and late filling waves (*A*) was measured (*E*/*A* ratio). Echocardiograms were read centrally in a blinded manner.

2.1.3. Laboratory analysis in patients

Baseline venous blood samples were collected from all patients at the beginning of the study and after 6 weeks of treatment. The serum was separated by centrifugation and frozen at −80 °C until analysis. Biochemical parameters in serum were evaluated using commercially available kits such as total cholesterol, triglycerides, high density lipoprotein-cholesterol (HDL-c) (Stanbio Laboratory, USA) and low density lipoprotein-cholesterol (LDL-c) (Quimica Clinica, Aplicada, Spain). Serum cardiac troponin T (cTnT, Boehringer, Germany) and high sensitive C-reactive proteins (hs-CRP, Monobind, USA) were measured by using enzyme-linked immunosorbent assay (ELISA). Serum malondialdehyde (MDA) activity was measured by the NWLSS™ NWDA01 assay (Northwest Life Science Specialties, LLC). This assay is based on the reaction of MDA with thiobarbituric acid (TBA) using spectrophotometry.

2.2. Pre-clinical study

2.2.1. Animals

Forty male albino rats (total body weight, 150–200 g) were acclimated for one week prior to the experiment. Rats were housed in plastic cages, had free access to water and were given a semi-synthetic balanced diet with controlled temperature (21–23 °C) and lighting (12 h light/dark cycles). This study was approved by the Animal Experimentation Ethics Committee of the Egyptian National University.

Rats were divided into 4 groups with 10 rats per group: (a) Control group: Rats received 1 ml of normal saline (El-Nasr Company, Egypt) intra-peritoneally (IP) for 4 weeks. (b) Doxorubicin group: Rats were injected IP with doxorubicin (doxorubicin hydrochloride, Adriblastina® vials 10 mg powder dissolved in normal saline, Farmitalia, Italy) doses of 2.5 mg/kg (body weight) every other day over a period of two weeks (i.e. six equal injections) for a cumulative dose of 15 mg/kg and then they were given 1 ml saline IP for 2 weeks as previously described.[20] (c) Atorvastatin–doxorubicin group: Rats were treated with atorvastatin (Lipitor® tablets 10 mg dissolved in normal saline, Pfizer, Egypt) doses of 10 mg/kg/day given by oral gavage for 4 weeks and on the 15th day were injected with 2.5 mg/kg of doxorubicin IP every other day for 2 weeks. The dose of 10 mg/kg per day atorvastatin was chosen because the plasma concentration achieved in rats with this dose was within the dose range observed in the clinical setting.[25–27] (d) Digoxin–doxorubicin group: Rats were treated with digoxin (Lanoxin® tablets 0.25 mg dissolved in normal saline, GlaxoSmithKline, Egypt) by oral gavage for 4 weeks with doses of 0.02 mg/kg/day[28], and on the 15th day were injected with 2.5 mg/kg doxorubicin IP every other day for 2 weeks.

2.2.2. Systolic blood pressure measurement in rats

Non-invasive systolic blood pressure (SBP) was measured in conscious animals with a tail cuff sphygmomanometer (Harvard, UK). For each animal, the SBP was calculated as the average of three independent measurements at each session, as described previously.[9,27]

2.2.3. Laboratory assays in rats

After 4 weeks of treatment, the blood was collected from the retro-orbital plexus of overnight fasted rats using micro-capillary tubes. The serum was separated using a sterile pipette after centrifugation at 3000 rpm for 15 min. Similarly serum lipid profiles (total cholesterol, triglyceride, LDL-c and HDL-c), hs-CRP, cTnT and MDA activity the patients were analyzed. The rats were then euthanized by an overdose of anesthetic ether vapors. Total body weight was obtained prior to surgical removal of the heart. Heart weights were measured and cut into two parts: one for histopathology and the other for homogenization with MDA assay (see below).

Myocardial lipid peroxidation was assessed by determining TBA reactive substances using a modified TBA method.[20] Briefly, the heart tissue from treated rat groups were washed in ice-cold 0.9% saline and homogenized using a tissue homogenizer to get 10% homogenate and buffered in 0.9% KCl (pH 7.4). The homogenate was centrifuged for 10 min and the supernatant was used for measurement of MDA activity and protein levels using the Lowry protein assay. Cardiac tissue of MDA was measured by spectrophotometry (see above).

2.2.4. Rat heart histopathology

The heart was excised and fixed in neutral buffered formalin. The tissues were embedded in paraffin and sectioned at 5 μm for staining. Heart sections were stained with Hematoxylin and Eosin (H&E) for evaluation of histology. Weigert's Resorcin-Fuchsin stain was used for elastic fiber staining in the blood vessels. Masson's trichrome was used as fibrosis assay for distinguishing muscle from interstitial connective tissue. Images were analyzed under optical microscopy at magnifications of 400–1000×.

2.3. Statistical analysis

Variables are presented as numbers, percentages (%), or mean ± standard error (SE) as indicated. Student's *t*-test, Chi-Square (χ^2) test, or Fisher's exact test and a one way ANOVA test and an un-paired *t*-test were used to compare mean values between the treatment groups and the controls,

as indicated. Bonferroni procedure was applied to the raw two-sided P value. The adjusted P value of <0.05 was considered as being statistically significant. Results were analyzed by the statistical software package SPSS version 11 for window (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Results of clinical study

In non-ischemic CHF patients, there was no significant difference between patient groups regarding age, sex, HTN, smoking, diabetes and standard anti-heart failure therapy ($P > 0.05$) (Table 1).

After 6 weeks of treatment, atorvastatin-treated patients showed a significant decrease in both LV-EDD and LV-ESD, but showed a significant increase in LV-EF%, LV-FS% and E/A velocity ratio compared to those of control patients ($P < 0.05$) (Fig. 1).

Atorvastatin-treated patients showed a significant decrease in serum of total cholesterol, triglyceride and LDL-c ($P < 0.05$), hs-CRP, cTnT and MDA ($P < 0.01$), and no significant increase in serum HDL-c compared to control patients (Fig. 2).

3.2. Results of animal data

After 4 weeks of treatment, doxorubicin-treated or digoxin–doxorubicin-treated rats appeared more fatigue; had a significantly large amount of ascites, had lower SBP compared with atorvastatin–doxorubicin-treated rats (Table 2) ($P < 0.05$). There was an increase in the final body weight of atorvastatin–doxorubicin-treated rats compared to their basal body weight. However, there was a decrease in the final body weight of the doxorubicin-treated and digoxin–doxorubicin-treated rats (Table 2). Moreover, heart weight/body weight ratio (cardiac mass index) was significantly increased in atorvastatin–doxorubicin-treated rats compared to those of doxorubicin-treated and digoxin–doxorubicin-treated rats ($P < 0.05$) (Table 2).

After 4 weeks of treatment, laboratory assay results in the doxorubicin-treated rats produced a significant ($P < 0.05$) increase in the levels of total cholesterol, triglycerides and LDL-c, but no significant increase ($P > 0.05$) in the HDL-c levels compared to those of saline-treated rats. Atorvastatin–doxorubicin-treated rats produced a significant decrease in the level of total cholesterol, triglycerides and LDL-c, and a significant increase in the level of HDL-c compared to doxorubicin-treated rats ($P < 0.05$). Digoxin–doxorubicin-treated rats showed no significant changes in their lipid profile from those of doxorubicin-treated rats ($P > 0.05$) (Fig. 3).

There was a significant increase in serum levels of hs-CRP and cTnT and cardiac tissue MDA in doxorubicin-treated rats compared with those of saline-treated rats ($p < 0.01$). The atorvastatin–doxorubicin treated group produced a significant decrease in the serum levels of hs-CRP and cTnT and cardiac tissue MDA compared to doxorubicin treated rats ($P < 0.01$). Digoxin–doxorubicin treated rats showed no significant changes in their serum levels of hs-CRP and cTnT and cardiac tissue MDA compared to those of doxorubicin-treated rats ($P > 0.05$) (Fig. 3).

3.2.1. Rat histopathology

After 4 weeks of treatment followed by H&E staining, the myocardial fibers of the saline group were arranged regularly with clear striations. No apparent degeneration or necrosis was observed; the cardiac muscle fibers had uniform size and acidophilic cytoplasm. The nuclei appeared rounded or oval and centrally located. Doxorubicin-treated rats showed focal loss in the normal architecture of the cardiac muscle fibers (myocyte vacuolization and degeneration), with deeply stained peripheral pyknotic nuclei. Occasionally, some demonstrated lost nuclei, inflammatory cells, and hyalinosis in the wall of the congested vessels. Atorvastatin–doxorubicin treated rats displayed overall improvement with a nearly normal appearance of most of the cardiac muscle fibers having centrally

Table 1 Baseline clinical data of all chronic heart failure (CHF) patients.

	Control group	Atorvastatin-treated group
Age (years)	59.5 ± 3.5	60.5 ± 3.9
Sex		
Male	14 (70%)	16 (80%)
Female	6 (30%)	4 (20%)
Risk factors		
Hypertension	10 (50%)	12 (60%)
Diabetes mellitus	4 (20%)	6 (30%)
Smoking	12 (60%)	14 (70%)
Family history of CHD	6 (30%)	4 (20%)
Concomitant drugs		
Digoxin	14 (70%)	16 (80%)
Diuretic	18 (90%)	16 (80%)
Nitrates	6 (30%)	4 (20%)
B-blockers	8 (40%)	6 (30%)
ACE inhibitors	16 (80%)	18 (90%)

Data are the mean ± SD ($n = 20$). There was no significance between the control and atorvastatin treated patients ($P > 0.05$); CHD, coronary heart diseases.

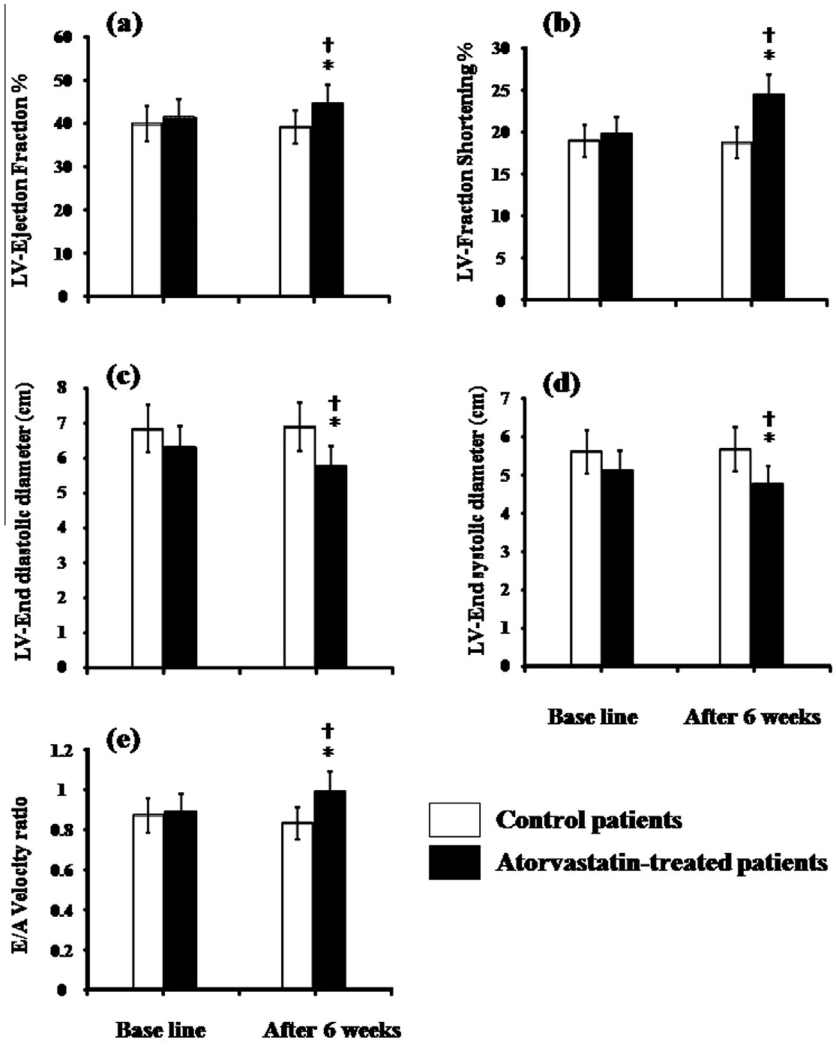

Figure 1 Echocardiographic data in both patients groups. (a) Left ventricle ejection fraction (LV-EF%), (b) fraction shortening (LV-FS%), (c) end-diastolic diameter (LV-EDD), (d) end-systolic diameter (LV-ESD) and (e) E/A velocity ratio in control patients (□) and atorvastatin treated patients (■). Data are given as mean ± SD ($n = 20$). $^{*}P < 0.05$ compared with control-atorvastatin basal. $^{\dagger}P < 0.05$ compared with control-atorvastatin after 6 weeks.

located vesicular nuclei while few fibers were degenerated showing pyknotic peripheral located nuclei. Digoxin–doxorubicin-treated rats showed less or no improvement in the histological pictures as seen by the loss of the normal architecture of the cardiac muscle fibers with degeneration and pyknosis of the nuclei and extravasation of blood between cardiac muscle fibers (Fig. 4A–D).

Resorcin-Fuchsin staining for elastic fibers demonstrated the normal distribution of vessels among cardiac muscle fibers in the control group. Doxorubicin treated rats showed elastic fibers in the wall of the congested vessel among the cardiac muscle fibers. Atorvastatin treated rats showed the appearance of many vessels in between the cardiac muscle fibers (neoangiogenesis) compared to other groups while digoxin treated rats revealed no change in the blood vessel appearance (Fig. 4E–H).

Masson's trichrome staining showed a slight blue color in the connective tissue in the interstitium and around blood vessels in the control group. Doxorubicin treated rats showed marked collagen fibers around the blood vessels and in the interstitium compared with saline treated rats. Atorvastatin treated rats showed few collagen fibers in between the cardiac muscle fibers, and lower fibrotic content than non-treated rats. Digoxin treated rats showed many collagen fibers around blood vessels and in the degenerated myocardium (Fig. 4I–L).

4. Discussion

For the first time, we have demonstrated that the addition of atorvastatin to standard heart failure therapy further prevented LV dilatation (decreased LV-ESD and LV-EDD) and normalized cardiac function (increased LV-EF% and LV-FS%) compared with standard heart failure therapy in CHF patients, indicating an additional benefit. Our explanations of the beneficial effects of atorvastatin were beyond those it has on plasma lipid levels (decreased total cholesterol,

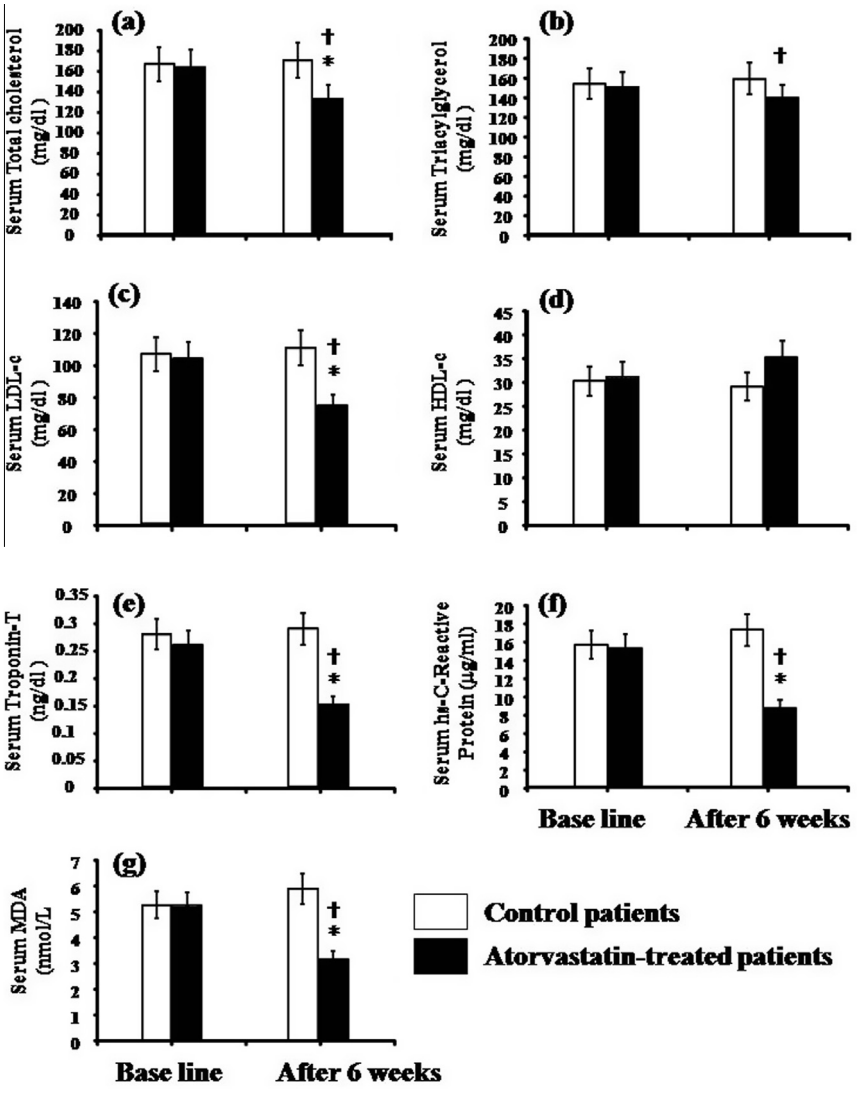

Figure 2 Laboratory parameters in both CHF patient groups. (a) Serum total cholesterol, (b) serum triglycerides, (c) serum LDL-c, (d) serum HDL-c, (e) serum cardiac troponin-T (cTnT), (f) serum hs-CRP, and (g) serum malondialdehyde (MDA) are measured in CHF patient controls (□) and atorvastatin-treated groups (■). Data are given as mean ± SD ($n = 20$). $^{*}P < 0.05$ compared with control-atorvastatin basal; $^{\dagger}P < 0.05$ compared with control-atorvastatin after 6 weeks. $^{**}P < 0.01$ compared with control-atorvastatin after 6 weeks.

Table 2 General animal characteristic parameters and tissue weight ratios in different groups of the experimental study.

	Saline-treated	Doxorubicin-treated	Atorvastatin–doxorubicin-treated	Digoxin–doxorubicin-treated
Baseline SBP (mmHg)	110.9 ± 1.96	110.2 ± 2.14	110.6 ± 1.89	110.3 ± 2.11
Final SBP (mmHg)	111.5 ± 1.42	82.4 ± 1.82***,‡	91.25 ± 1.28*,†,§,‡	80.33 ± 1.96**,‡
Basal body weight (g)	163.2 ± 2.8	162.5 ± 1.26	163.5 ± 1.64	162.6 ± 1.64
Final body weight (g)	210.0 ± 3.26‡	143.8 ± 2.91***,‡	172.0 ± 1.98*,†,§,‡	142.0 ± 2.78**,‡
Heart weight (g)	0.755 ± 0.0193	0.485 ± 0.0078***	0.602 ± 0.0035*,†,§	0.482 ± 0.0083**
Heart weight/final body weight ratio (g/g)	0.00360 ± 0.00073	0.00337 ± 0.00032***	0.00350 ± 0.00055*,†,§	0.00336 ± 0.00033**
Volume of ascites (ml)	0	2.75 ± 0.02***	1.25 ± 0.01*,†,§	2.80 ± 0.03**
Mortality rate %	0	30***	20*,§	40**

Data are given as mean ± SD ($n = 10$). Baseline systolic blood pressure (SBP) and body weight were measured immediately prior to drug administration. The final SBP and body weight were measured 4 weeks after drug administration.

* $P < 0.01$ compared with saline-treated.

** $P < 0.01$ compared with saline-treated.

*** $P < 0.01$ compared with saline-treated.

† $P < 0.05$ compared with doxorubicin-treated.

§ $P < 0.05$ compared with digoxin-treated.

‡ $P < 0.01$ compared with basal parameters in each group.

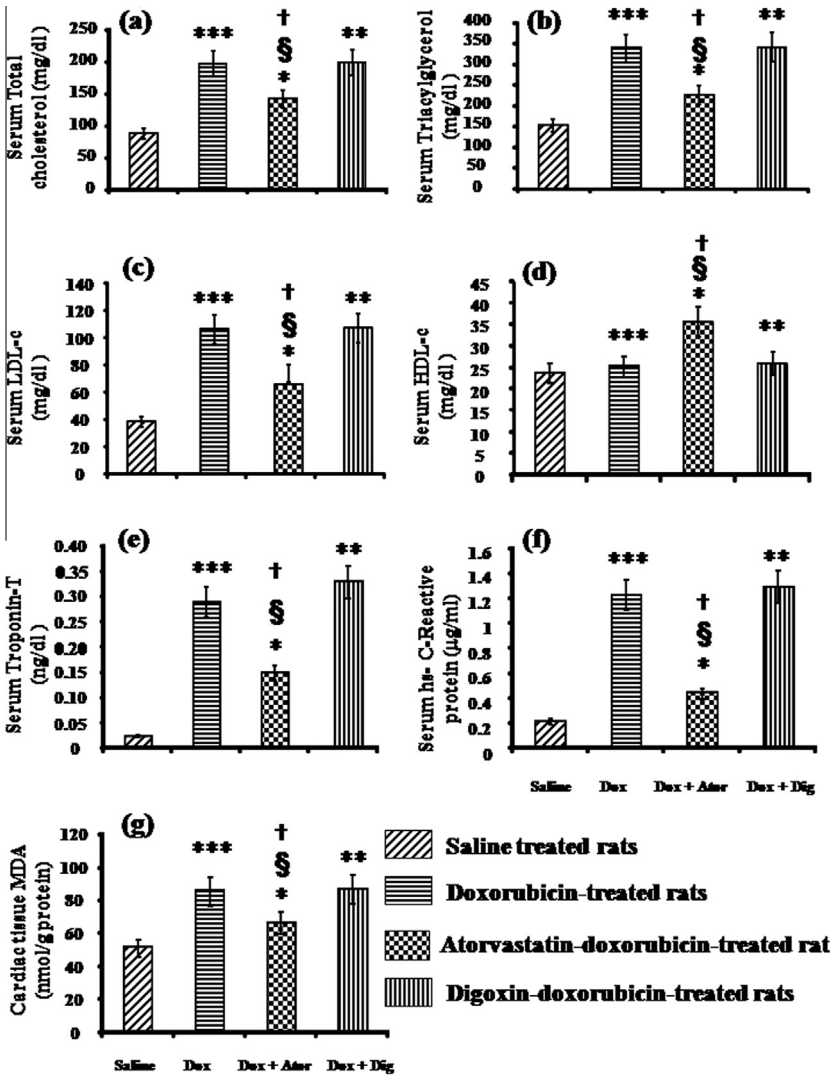

Figure 3 Laboratory parameters in the experimental study. (a) Serum total cholesterol, (b) serum triglycerides, (c) serum LDL-c, (d) serum HDL-c, (e) serum cardiac troponin-T (cTnT), (f) serum hs-CRP, and (g) cardiac tissue malondialdehyde (MDA) were measured in all treated rat groups; saline-treated control rats (saline), doxorubicin-treated rats (Dox), atorvastatin–doxorubicin treated rats (Dox + Ator) and digoxin–doxorubicin treated rats (Dox + Dig). Data are the mean ± SD ($n = 10$). $^{*}P < 0.01$ compared with saline-treated rats; $^{**}P < 0.01$ compared with saline-treated rats; $^{***}P < 0.01$ compared with saline-treated rats; $^{\dagger}P < 0.05$ compared with doxorubicin-treated rats; $^{\S}P < 0.05$ compared with digoxin-treated.

triglyceride, LDL-c), and seems to be linked to improved myocardial remodeling as shown in our doxorubicin cardiomyopathy rat model by the prevention of cardiac inflammation (decreased serum cTnT, hs-CRP, and MDA), the reduction of cardiac fibrosis, and associated with neovascularization. Further, clinically, atorvastatin exerted cardioprotective effect (anti-inflammatory and anti-oxidant) against the development of chronic heart failure, whatever the etiology, or it can be used routinely for improving cardiomyopathy in patients with malignant tumors that are being treated by anthracycline anticancer antibiotics.[17,18]

4.1. Clinical statin therapy effect

Our clinical echocardiographic data were in agreement with other clinical results that reported atorvastatin had beneficial effects for improving heart function (increase in LV-EF%) in CHF patients.[13,29] Moreover, many types of statin therapy improved cardiac function in patients with CHF.[13,16,30]

Clinically, cTnT and hs-CRP were used as prognostic markers and indicators of acute coronary syndromes and heart failure managements.[5,31] Our laboratory results showed that atorvastatin improved cardiac function via exerting potent anti-inflammatory properties. These results were supported by previous clinical results, that also reported many types of statins (atorvastatin, lovastatin, pravastatin, fluvastatin, cerivastatin and simvastatin) reduced the inflammatory biomarkers such as TNF-α, IL1, IL6, IL-10, CRP, cTnT, BNP,[26,30,32–34] and MDA[15] in patients with heart failure.

4.2. Animal statin therapy effect

Atorvastatin treatment protected rats against doxorubicin cardiotoxicity by increasing the body weight, and the heart

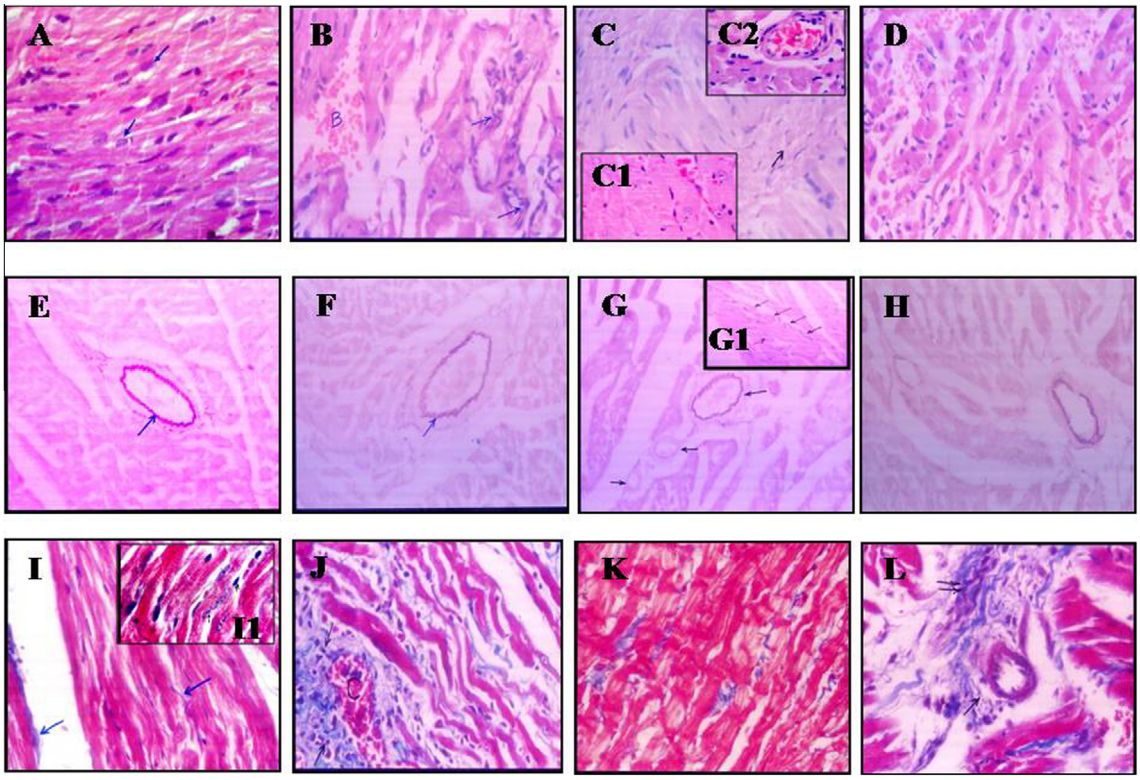

Figure 4 Histopathological characteristics of all groups after 4 weeks of treatment. Hematoxylin and Eosin (H&E) staining (A, B, C and D), Resorcin Fuchsin staining (E, F, G and H), Masson's trichrome staining (I, J, K and L) in sections of cardiac tissue from saline-treated control rats (saline) (A, E and I), doxorubicin-treated rats (Dox) (B, F and J), atorvastatin–doxorubicin treated rats (Dox + Ator) (C, G and K) and digoxin–doxorubicin treated rats (Dox + Dig) (d, h and l). All images are at 400× magnification (scale bar = 20 μm) except in the atorvastatin treated group (G1) which was at 200× to show numerous blood vessels (↑). A higher magnification of 1000× (scale bar = 5 μm) was used in (C1 and C2) to show the congestion of vessels between the cardiac muscle fibers and the centrally located vesicular nuclei that are in between the cardiac muscle fibers (↑) in the atorvastatin treated rats and it was also used in (I1) to show the elongated vesicular nucleus of the cardiac muscle fibers (↑) and the fibroblast in the interstitium (↑↑) in the control group.

weight/body weight ratio in comparison to doxorubicin-treated or digoxin–doxorubicin-treated rats. These results were in agreement with others.[20]

Atorvastatin treatment improved SBP in atorvastatin–doxorubicin treated rats in comparison to doxorubicin-treated or digoxin–doxorubicin-treated rats. These results were similar to other studies on rats with experimental heart failure from simvastatin and rosuvastatin.[9] Statins exert cardiovascular and renal protection, which is accompanied by blood pressure reduction; irrespective of serum cholesterol levels.[9]

Also, in our experimental CHF results were similar to the clinical results obtained in laboratory serum evaluations. Additionally, in rats, cTnT may be a useful marker for the assessment of experimentally doxorubicin-induced cardiotoxicity.

Our results of atorvastatin–doxorubicin treated rats were in agreement with others showing a significant decrease in serum cTnT,[35] serum hs-CRP[9] and cardiac tissue MDA.[14,27] The effect of atorvastatin in reducing MDA could be attributed to its antioxidant and lipid-lowering properties.

Histology of heart tissues in atorvastatin–doxorubicin treated rats, showed a reduction in loss of myofibrils, vacuolization of the cytoplasm and swelling of mitochondria, coagulative necrosis with focal areas of fibrosis, vascular congestion, as well as decreased mononuclear cellular infiltration. These results were in agreement with other experimental results.[9,20,36]

Atorvastatin increased the formation of new blood vessels (neo-angiogenesis) between cardiac muscle fibers, but was absent in both the doxorubicin-treated group and the digoxin–doxorubicin-treated group. This result previously reported that statins mobilize endothelial progenitor cells from the bone marrow that play a role in maintenance of vasculogenesis and new blood vessels' formation.[37,38]

The use of atorvastatin might be a useful cardio-protective measure for limiting adverse LV remodeling because of our demonstration of the decrease of fibrosis and necrosis that has been similarly observed by other studies.[16,19,27] Theses studied have also shown reduction of hydroxyproline content, collagen deposition, fibrosis and the apoptosis of cardiomyocytes.[16,19,27] Moreover, statin treatment in rats with heart failure, showed attenuated LV dilatation, improved LV-EF%, LV-FS% and E/A velocity ratio, and also demonstrated attenuated cardiac hypertrophy, fibrosis and inflammation when compared with control rats with heart failure.[9,27]

Pharmacologically, beyond their lipid-lowering actions, statins have other potentially favorable "pleiotropic" effects[10], including anti-inflammatory,[13,39] anti-fibrotic,[40]

anti-apoptosis,[19] antioxidant effects,[14,15] anti-hypertrophic,[41] inhibition of neurohormonal activation,[29] decrease immune activation,[35] alter metalloproteinase activity,[32] and prevention of cardiac arrhythmias[42]; all of which can contribute to the improvement of LV function and prevention or attenuation of progressive LV remodeling in heart failure. However, for this study, our aim was focused on the anti-inflammatory, anti-fibrotic and antioxidant effects of atorvastatin.

5. Conclusion

The results of the present clinical and pre-clinical studies suggest that atorvastatin has a beneficial cardio-protective effect in heart failure. Statins have also been shown to possess a host of other non-cholesterol lowering properties including the ability to decrease LV fibrosis and necrosis, decrease inflammation (cTnT and hs-CRP), decrease oxidative stress (MDA), and induce neo-angiogenesis, all of which can contribute to the improvement of LV function and prevention or attenuation of progressive LV remodeling in heart failure. Hence, these potential cardio-protective pleiotropic actions of atorvastatin may be of good therapeutic value in management of heart failure, even in patients for whom the medication may not otherwise be indicated. Further clinical studies with large number of patients are now needed in order to clarify whether this beneficial effect can be translated into clinical practice.

Conflict of interest

We have no conflict of interest to declare.

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