JPP Journal of Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 1604–1612

Received May 7, 2011
Accepted August 22, 2011
DOI
10.1111/j.2042-7158.2011.01363.x
ISSN 0022-3573

Research Paper

Curcumin: a potential therapeutic polyphenol, prevents noradrenaline-induced hypertrophy in rat cardiac myocytes

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Abstract

Objectives This study was designed to evaluate the effect of curcumin on H9c2 cardiac cell line and primary rat cardiac myocytes, using purified noradrenaline as a hypertrophy-inducing agent.

Methods The concentration of curcumin at which cells were treated was determined by MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay. The effect of this safe dose in preventing noradrenaline-induced cardiac hypertrophy was assessed by biochemical analysis (estimating total protein content), molecular analysis (using RT-PCR to study the expression of fetal genes like ANF), immunological analysis (by determining the nuclear localization of GATA-4) and electrophoretic mobility shift assay (EMSA; to study DNA binding activity of GATA-4).

Key findings Curcumin at a concentration of 8 μM was found to suppress the increase in cell size, protein content and enhanced marker gene expression (ANF) caused by noradrenaline. Immunocytochemistry and Western blot analysis showed that curcumin suppressed the localization of transcription factor GATA-4 in the nucleus. It also showed a reduced DNA-binding activity in the presence of noradrenaline as confirmed by EMSA.

Conclusions These findings suggest that curcumin reduces the hypertrophic marker gene expression by inhibiting nuclear localization and DNA binding activity of GATA-4. Thus it has a great anti-hypertrophic potential.

Keywords cardiac hypertrophy; curcumin; GATA-4; noradrenaline; therapeutics

Introduction

Cardiomyocytes are terminally differentiated and undergo hypertrophy under stress conditions.[1,2] Hypertrophy may be initially a favourable response, but prolonged hypertrophy leads to heart failure and sudden death.[3,4] During hypertrophy, myocytes increase in size along with enhanced protein synthesis, sarcomereric re-organization and re-expression of fetal genes such as atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and beta-myosin heavy chain (β-MHC).[5–7] There is a great cross-talk between complex signalling pathways implicated in cardiac development and disease and, eventually, they converge on a common cardiac gene activation program regulated by transcription factors such as GATA-4,5,6, myocyte-enhancing factor 2 (MEF2), Csx/Nkx-2.5, eHAND, dHAND, FOG-2 and myocardin.[8–13] These transcription factors can be viewed as potential therapeutic targets for designing anti-hypertrophic drug therapy. Presently, drugs with anti-hypertrophic activity target the outside–in signalling. These therapies, however, vary greatly in effectiveness depending on the signalling pathway targeted and despite such conventional therapy, cardiomyopathies are still an increasing cause of mortality worldwide.[14]

Developing drug molecules that will selectively suppress transcription factor activity can be of great therapeutic interest. Inhibition of transcription factor activity down-regulates gene-expression, thus preventing the occurrence of disease. In this regard, nuclear factor-κB (NF-κB) has been shown to be a potential drug target for developing anti-cancer therapy.[15]

Curcumin, a polyphenol and bioactive compound of *Curcuma longa*, is known to have beneficial properties that include anti-cancer, antimicrobial, anti-inflammatory and, very recently discovered, cardioprotective activity.[16–18] Recent studies show that dietary

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curcumin inhibits p300 histone acetyl transferase (HAT) activity and prevent heart failure in rats.[19,20] Treatment of cardiomyocytes with curcumin also prevents the diabetes-induced expression of p300 and MEF2 transcription factors.[21] However, to our knowledge, no study has focused on identifying the potential of curcumin in prevention of noradrenaline (norepinephrine)-induced cardiac hypertrophy by targeting the nuclear localization and binding activity of GATA-4 to its cognate sequences.

Materials and Methods

Chemicals

All the chemicals were purchased from Sigma-Aldrich (Saint Louis, MO, USA) unless or otherwise stated.

Cell culture

Heart-derived H9C2 cardiomyoblast cells were obtained from the National Centre for Cell Sciences (NCCS), Pune. H9C2 cells were cultured with Dulbecco's modified Eagle's medium (DMEM), supplemented with penicillin (60 U/ml), streptomycin (60 µg/ml), gentamicin (10 µg/ml), amphotericin B (2 µg/ml), glucose (3.5 g/l), L-glutamine (2.5 mM), sodium bicarbonate (2 mg/ml) and 10% fetal calf serum (FCS), in a humidified CO_2 incubator with 5% CO_2 at 37°C. Cells were routinely subcultured at a split ratio of 1:3. Noradrenaline was used for the induction of hypertrophy.[22] Cells were treated with noradrenaline (2 µM) under serum-free conditions in DMEM containing ITS supplement (insulin 50 µg/ml, transferrin 27.5 µg/ml and selenium 0.025 µg/ml) for 48 h. The cells were harvested using trypsin-EDTA and counted. The cell pellet was then used for further experiments.

Primary cultures of cardiac myocytes were prepared as described previously, with slight modification.[23] Cells from the hearts of two-day-old Sprague–Dawley rats (Department of Zoology, Govt. PG College, Noida, India) were seeded at a density of 1×10^6 cells/well in plating medium consisting of DMEM/F12 (1 : 1) medium supplemented with 10% FCS and penicillin/streptomycin/amphotericin B/gentamicin. After 48 h, the culture medium was replaced with medium containing ITS supplement and induction was carried out as previously described.

Cell viability by trypan blue exclusion

The trypsinized cell suspensions were taken and mixed with an equal volume of viability stain (0.4% trypan blue in phosphate-buffered saline (PBS)). A 20-µl volume of the mixture was loaded onto the counting chamber of a haemocytometer and viewed to count the viable cells (unstained) and non-viable cells (stained blue). The percentage viability was calculated as the ratio of viable cells and total cells.

In-vitro cytotoxicity for curcumin

Cell viability and proliferation was measured by 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) as previously described, with slight modifications.[24] Curcumin was dissolved in pure dimethyl sulfoxide (DMSO). For the assay, 7500 cells were plated in 96-well plates. After treatment with curcumin for 48 h at different concentrations ranging from 2 µM to 20 µM, 10 µl MTT solution (5 mg/ml) was added and incubated at 37°C for 3 h. Supernatant was then aspirated and formazan salt crystals were dissolved in 200 µl DMSO. Plates were analysed in an ELISA plate reader (Bio-Rad) at 570 nm. Cell viability was defined relative to untreated control cells as follows: cell viability = absorbance of treated sample/absorbance of control.

Treatment of cells with curcumin

To see the effect of the optimum concentration of curcumin on hypertrophy, H9C2 cells were cultured in serum-free DMEM supplemented with ITS in the presence of an optimized concentration of noradrenaline and curcumin for 48 h. Similar treatments were carried out on primary cardiac myocytes. Treatment with curcumin was followed by morphological and biochemical analysis.

Morphological analysis

Cells were visualized with an inverted microscope and the images were captured at 40 × magnification. Cells were observed for increase in cell size to assess cellular hypertrophy. Cell size was quantified for cells in different fields using Image J software (National Institutes of Health).

Protein analysis

Cell pellets were washed with ice-cold PBS and total protein was obtained using protein extraction buffer (20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 20% glycerol, 500 mM SODIUM Chloride (NaCl), 0.2 mM ethylenediaminetetraacetic acid (EDTA), 0.1% Triton-X, 100 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM dithiothreitol (DTT)). The total protein was estimated using Lowry's method.[25]

RNA analysis

Total RNA was extracted from the cell pellet using TRIzol reagent (Ambion, Foster City, CA, USA). The integrity of RNA was assessed by electrophoresis on a 1.2% agarose gel containing formaldehyde. The RNA was reverse transcribed using gene-specific ANF primers. The cDNA was then amplified by polymerase chain reaction (PCR) using Taq Polymerase on performing 40 cycles with parameters: 95°C for denaturation (30 s), annealing temperature of 60°C (1 min) and extension at 72°C (1 min). RT-PCR was also done for the constitutively expressed gene, β-actin, for 30 cycles at annealing temperature of 57.5°C (1 min) with the same parameters for denaturation and extension. The primer sequences used were as follows:

ANF: 5′-CTGCTAGACCACCTGGAGGA-3′(F), 5′-AAGCT GTTGCAGCCTAGTCC-3′ (R);

β-Actin: 5′-CATCGTACTCCTGCTTGCTG-3′ (F), 5′-CCT CTATGCCAACACAGTGC-3′(R)

Immunocytochemistry

Immunocytochemistry was performed for cells treated with noradrenaline alone and those treated with curcumin in the presence of noradrenaline. A control set was also used where the cells were cultured without noradrenaline or curcumin.

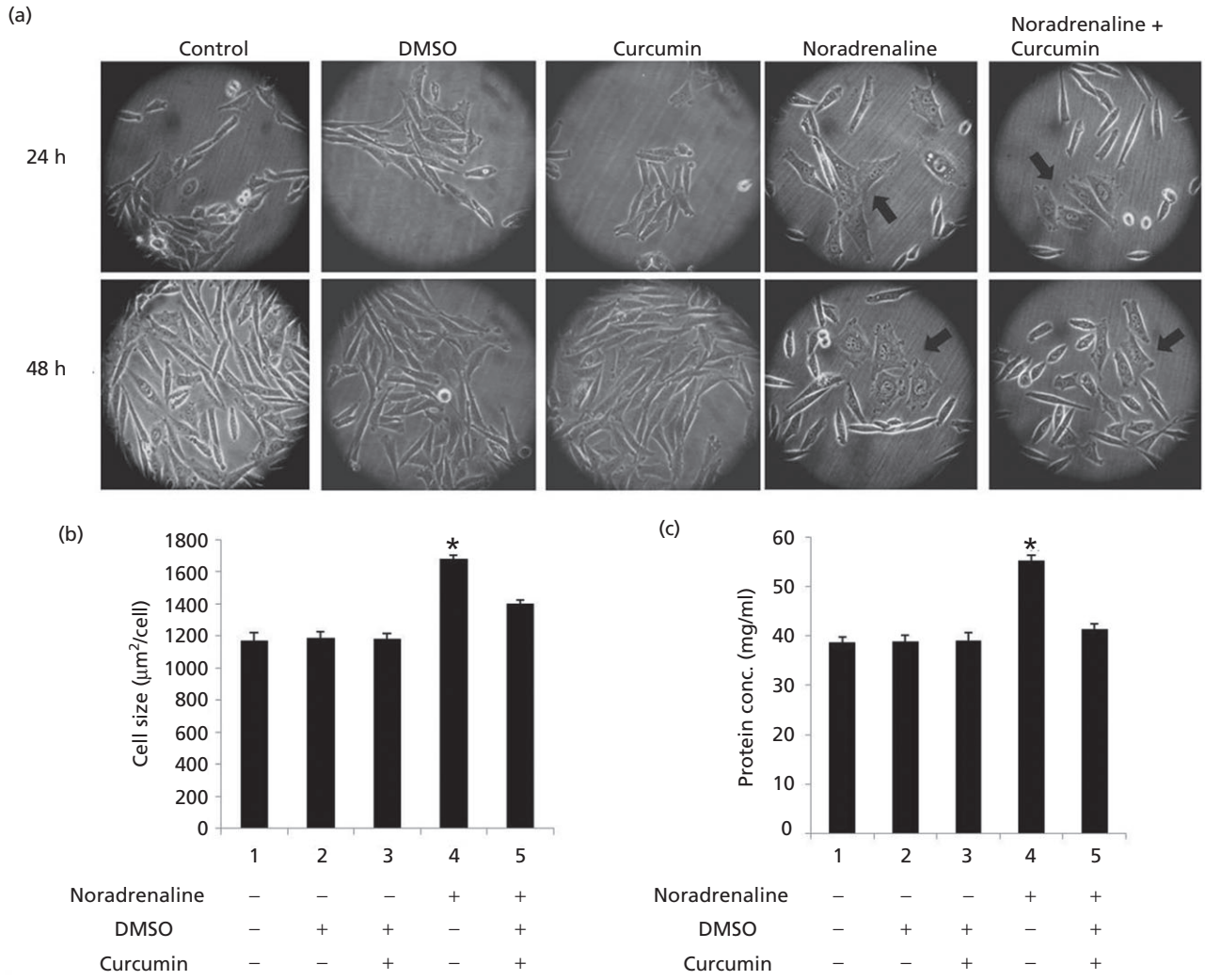

Figure 1 (a) Morphological analysis of rat cardiac myocytes. Comparison of cell size was made between control, noradrenaline-treated and curcumin-treated hypertrophied cells. Arrows indicate cells undergoing hypertrophy on treatment with noradrenaline and its prevention on treatment with curcumin in the presence of noradrenaline. (b) Quantitation of cell size: cell size from different fields was quantitated using Image J software for different experiments from 1 to 5. '+' Indicates presence of a particular factor (noradrenaline, curcumin or 0.1% DMSO) and '–' indicates absence of that factor in the experiment. The results were plotted as a histogram to validate the results obtained from microscopic analysis. (c) Estimation of protein content: similar experiments (1–5) as above (b) were carried out for protein estimation and plotted as a histogram (*$P < 0.05$).

Cells were first methanol-fixed and blocked using 1% bovine serum albumin (BSA) prepared in 0.2% goat serum. A dilution of 1 : 300 was prepared for GATA-4 and β-MHC primary antibodies. Anti-goat secondary antibody conjugated with fluorescein isothiocyanate (FITC) was used at 1 : 250 dilution. Nuclear staining was performed using 4',6-diamidino-2-phenylindole (DAPI) staining dye. All antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA).

Nuclear and cytosolic extract preparation

Nuclear and cytosolic extracts of the cells were prepared by incubating the cells firstly with buffer A (20 mM HEPES, 20% glycerol, 10 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.1% Triton X-100, 1 mM DTT, 100 mM PMSF, protease inhibitor cocktail) in ice for 15 min. This was followed by centrifugation at 1500*g* for 10 min. Cytosolic extract was obtained in the supernatant and the pellet obtained was resuspended in ice-chilled buffer B (20 mM HEPES, 20% glycerol, 500 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.1% Triton X-100, 1 mM DTT, 100 mM PMSF, protease inhibitor cocktail). The nuclei were lysed by intermittent tapping during incubation for 60 min at 4°C. This was centrifuged at 15 000*g* for 10 min and nuclear proteins were obtained in supernatant and frozen at −80°C in small volumes.[26] The nuclear protein extract thus obtained was utilized in electrophoretic mobility shift assay and Western blotting.

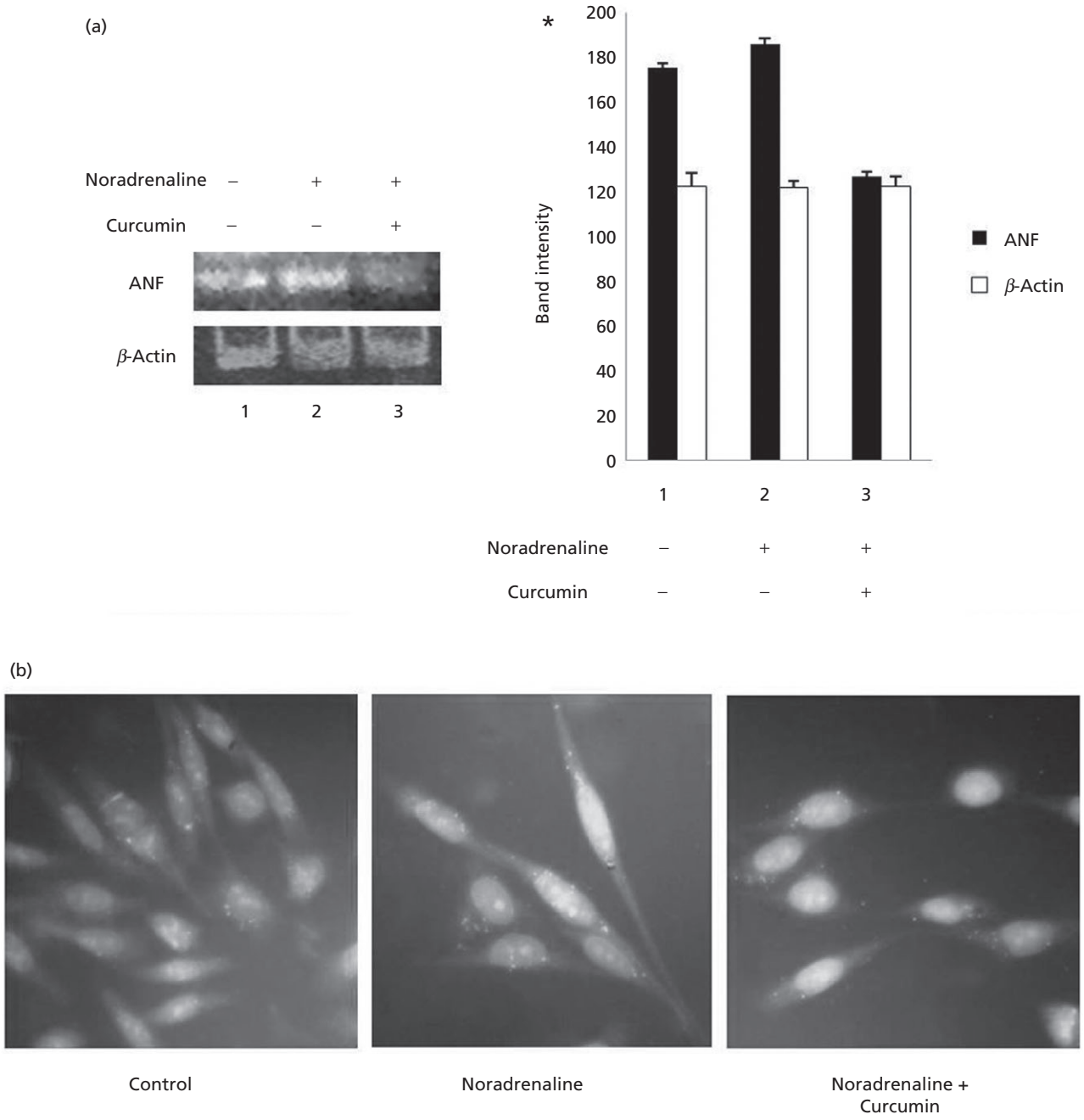

Figure 2 Expression of marker genes in rat cardiac myocytes. (a) RT-PCR analysis: RT-PCR with equal amount of RNA. Bands are representative of three experiment sets: control; noradrenaline-treated; and noradrenaline + curcumin-treated cells. ANF gene showed enhanced expression under hypertrophic condition. The expression was lowered when the cells were treated with curcumin. Change in the expression of constitutively expressed β-Actin was negligible. Results obtained by RT-PCR were quantitated by Image J software and analysed. (b) Immunocytochemistry: β-MHC gene showed enhanced protein expression under hypertrophic condition and the expression was decreased when cells were treated with curcumin. This implies that curcumin lowers the expression of hypertrophic marker gene ($^*P < 0.05$).

Western blotting

Nuclear and cytosolic protein extracts were isolated separately from all three experimental sets. Samples containing equal amount of proteins were separated on a 10% SDS–polyacrylamide gel, transferred to a polyvinylidene difluoride membrane (PVDF) (Amersham Pharmacia Biotech, Buckinghamshire, UK), and blocked in blocking solution (5% BSA in Tris-buffered saline-Tween) overnight at 4°C. Next day, the membranes were incubated for 1 h at 37°C with 1 : 1000 diluted primary antibodies against GATA-4, in blocking solution. Horse radish peroxidase-conjugated secondary antibody (1 : 8000) was incubated for 1 h at room temperature in blocking solution and processed for enhanced chemiluminescent

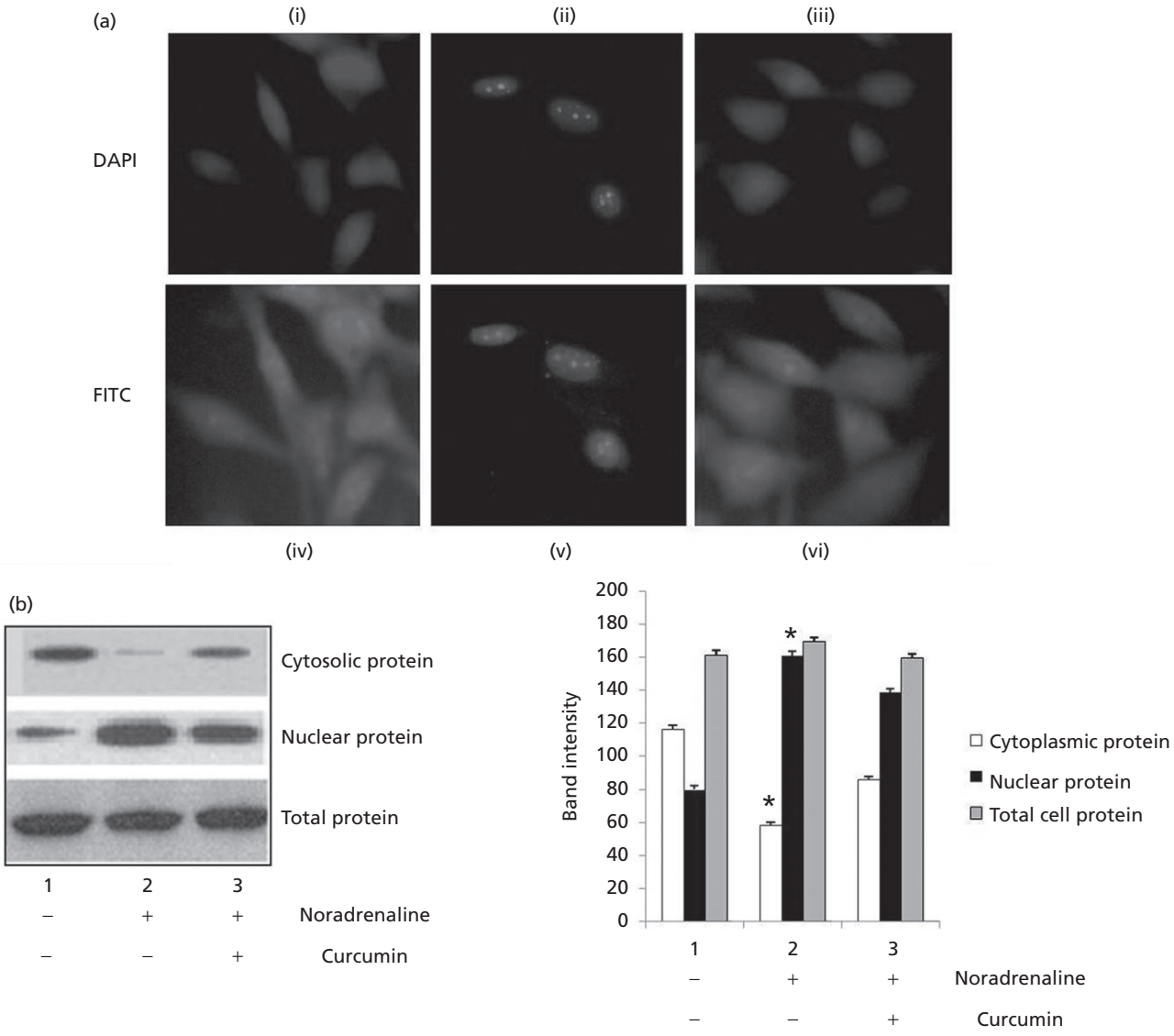

Figure 3 Nuclear localization of GATA-4 in H9C2 rat ventricular myocytes. (a) Single immunofluorescence for GATA-4: nuclear localization of GATA-4 was compared between H9C2 control cells (uninduced), noradrenaline-treated cells (induced) and curcumin-treated hypertrophied cells. FITC tagged secondary antibody was used to observe nuclear localization of GATA 4. DAPI staining of nucleus is shown in (i) control (uninduced cells), (ii) noradrenaline-treated, (iii) curcumin-treated hypertrophied cells. FITC stained GATA-4 is shown in (iv) control (uninduced cells), (v) noradrenaline-treated, (vi) curcumin-treated hypertrophied cells. (b) Western blotting was performed in three experimental sets: control (uninduced cells); noradrenaline-treated; and curcumin-treated cells, from their respective cytosolic and nuclear protein extracts. '+' indicates presence of a particular factor (noradrenaline or curcumin); '–' indicates absence of that factor in the experiment. The expression level was quantitated and plotted as histogram using Image J software (*$P < 0.05$).

(ECL) detection as described by the manufacturer (Amersham Pharmacia Biotech). After immunoblotting, the film was scanned and the intensity of immunoblot bands was detected with NIH Image J Software.

Electrophoretic mobility shift assay

The oligonucleotide sequences used were GATA (5′ CAC TTG ATA ACA GAA AGT GAT AAC TCT 3′ and 5′ AGA GTT ATC ACT TTC TGT TAT CAA GTG 3′) and Oct-1 (5′ TGT CGA ATG CAA ATC ACT AGA A 3′ and 5′ T TCT AGT GAT TTG CAT TCG ACA 3′). Protein–DNA binding reactions were carried out in 40 µl volume containing 40 µg nuclear extract, 40 ng oligonucleotide and gel-shift assay buffer (20 mM HEPES pH 7.9, 5% glycerol, 60 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT) for 45 min in ice. DNA–protein complexes were fractionated on a 6% polyacrylamide gel in 0.5 × Tris-Borate-EDTA for 3 h at 200 V, 4°C. After this the gel was stained using chemilabelling kit as per manufacturer's instructions (Invitrogen, Foster City, CA, USA). Stained bands were detected using a standard 300 nm UV transilluminator.

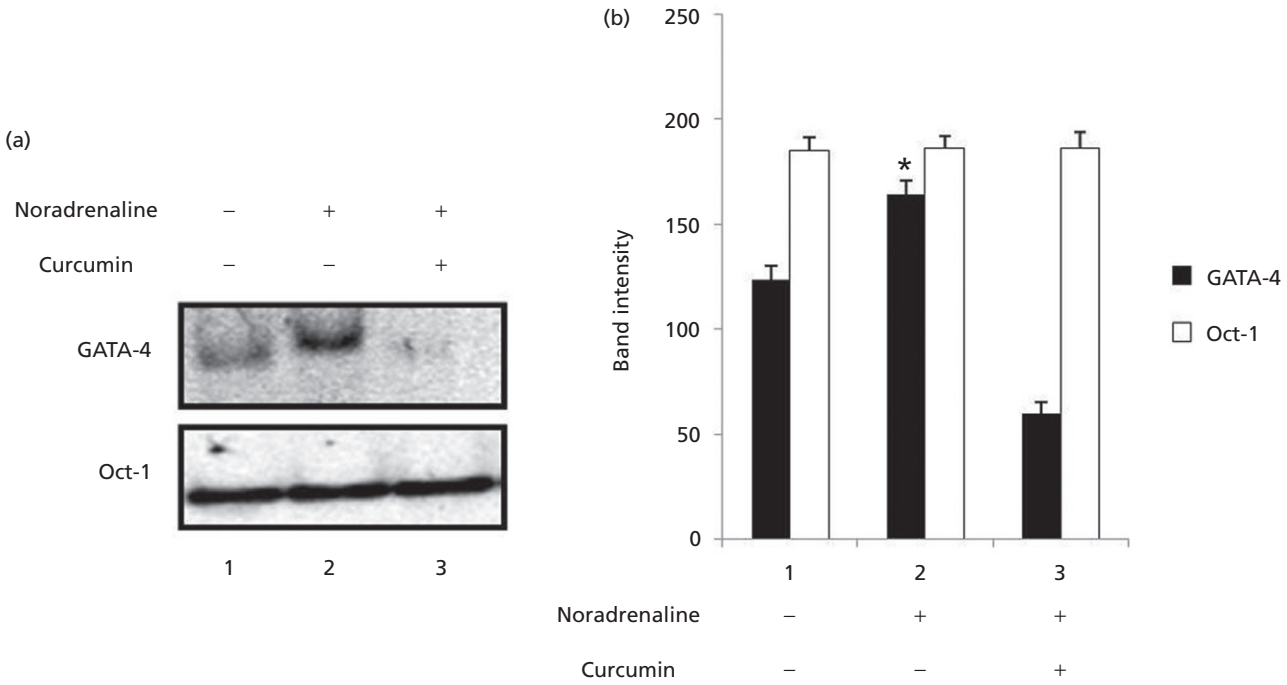

Figure 4 (a) Electrophoretic mobility shift assay (EMSA) in H9C2 rat ventricular myocytes. EMSA was performed by using nuclear protein extracts from control (uninduced cells), noradrenaline-treated and curcumin-treated hypertrophied cells with GATA-4 and Oct-1 (internal control) oligonucleotides. (b) Quantitation of band intensity. Bands obtained in EMSA were analysed by Image J software and plotted as histogram. Band intensity for GATA-4 was found to be higher in the noradrenaline-treated cells and significantly lower when cells were treated with curcumin. Negligible difference in band intensity was observed for Oct-1. This showed that curcumin prevents the DNA-binding activity of GATA-4 (data shown are ± SD, $n = 3$, $^*P < 0.05$).

Statistical analysis

For quantitative analysis, all data were expressed as means ± SD, The significance of differences in the data was evaluated by one-sided analysis of variance. $P < 0.05$ was considered statistically significant and the null hypothesis was rejected in that case. Standard error as calculated is shown in the graphs.

Results

Treatment with curcumin prevents noradrenaline-induced cardiac hypertrophy in H9C2 rat ventricular myocytes

The effects of curcumin on hypertrophic responses in cardiomyocytes were first examined. H9C2 rat ventricular myocytes were cultured to more than 95% viability as confirmed using trypan blue exclusion analysis. To identify the optimal concentration of curcumin, MTT assay was performed. In our study, DMSO was used as a solvent for curcumin, which did not show any cytotoxic effects. When curcumin was applied to cultured cardiomyocytes at a concentration of 2–20 μM for 48 h, cells were almost 100% viable in the presence of curcumin up to a concentration of 8 μM. Above this concentration, the viability significantly decreased. Moreover, at 20 μM, the viability was less than 80%, leading us to choose 8 μM as the optimal concentration for the experiments.

Increase in cardiomyocyte cell size, protein content and induction of fetal gene program are indicators of hypertrophy. H9C2 cells were treated with 8 μM curcumin in the presence of 2 μM noradrenaline and incubated for 48 h. A reduction in the size of cardiac myocytes was observed after treatment with curcumin under hypertrophic conditions (Figure 1a and 1b). It was also noted that curcumin reduced the increase in protein content of the myocytes that had been caused by noradrenaline (Figure 1c). Based on these results, the study was limited to three experimental sets (i.e. control, noradrenaline and noradrenaline + curcumin).

The level of expression of ANF, which is a well-established marker gene for myocardial cell hypertrophy, was also assessed. Curcumin treatment resulted in reduced ANF expression (Figure 2a). Immunocytochemistry showed that curcumin also reduced the expression of β-MHC, a marker gene up-regulated in cardiac hypertrophy (Figure 2b). These data suggest that curcumin prevents noradrenaline-induced cardiac hypertrophy in H9C2 cells.

Curcumin prevents nuclear localization of GATA-4 in H9C2 rat ventricular myocytes

To find out the players involved in preventing hypertrophy, immunofluorescence studies were carried out with GATA-4 antibody to determine the localization of GATA-4, a major transcription factor involved in cardiac hypertrophy. Cultured H9C2 cells were exposed to 2 μM noradrenaline with and without curcumin. Noradrenaline resulted in increased localization of GATA-4 inside the nucleus. This triggers the transcriptional machinery and further expression of genes involved in cardiac hypertrophy. Curcumin-treated cells

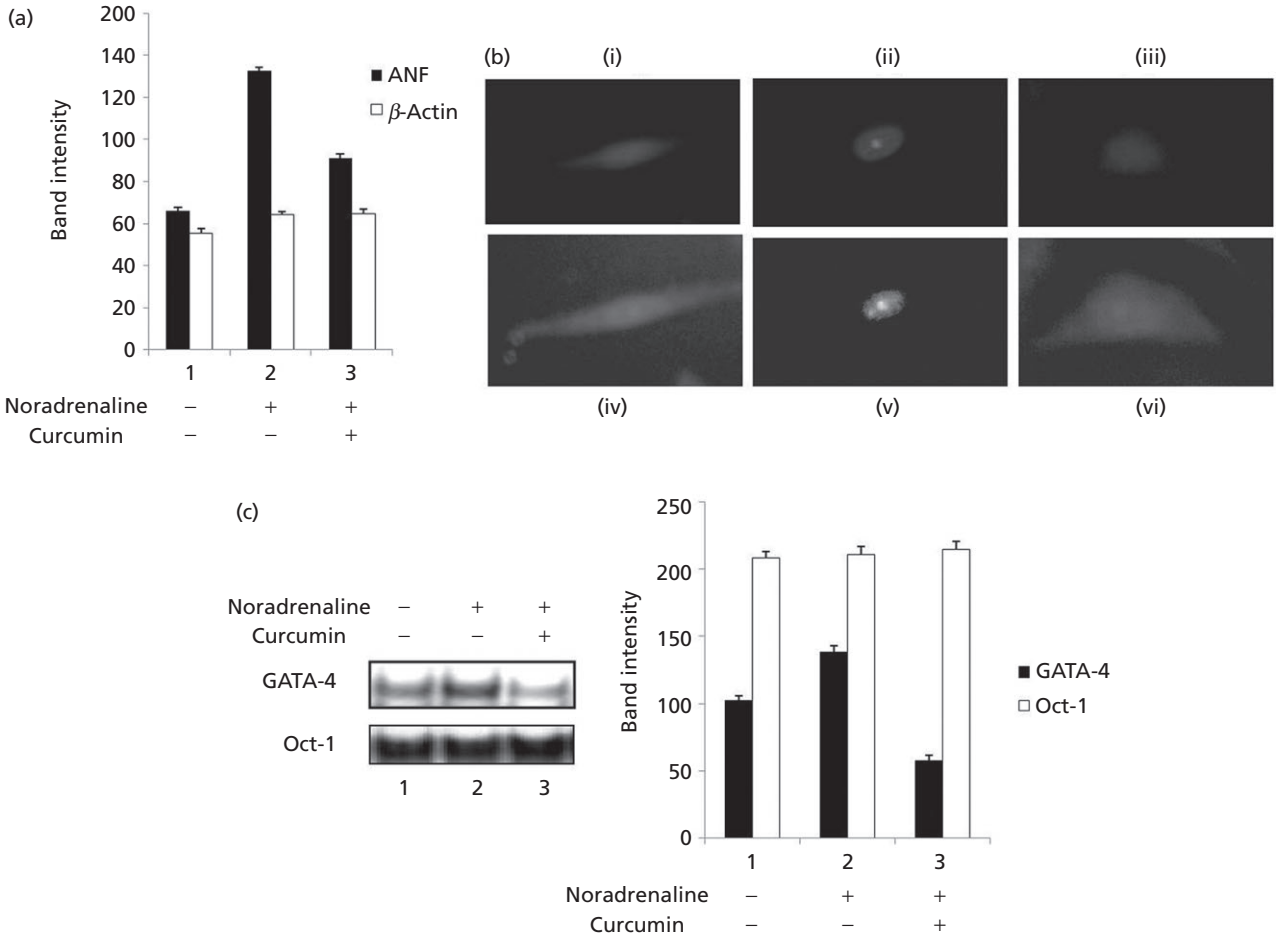

Figure 5 Effect of curcumin on primary neonatal rat cardiac myocytes. (a) RT-PCR analysis: the results obtained were similar to those obtained with H9C2 cells. The marker gene ANF showed enhanced expression on treatment with noradrenaline and the expression level was found to be decreased on treatment with curcumin. (b) Immunocytochemistry: localization of GATA-4 was studied in primary myocytes. Image shows DAPI staining of nucleus in (i) control, (ii) noradrenaline-treated, (iii) curcumin-treated hypertrophied cells and FITC stained GATA-4 in (iv) control, (v) noradrenaline-treated, (vi) curcumin-treated hypertrophied cells. (c) Electrophoretic mobility shift assay (EMSA): curcumin resulted in a decrease in the DNA-binding activity of GATA-4 in the primary myocytes as compared with enhanced DNA-binding activity in noradrenaline-treated myocytes ($*P < 0.05$).

showed a reduced localization of GATA-4 inside the nucleus. GATA-4 was found to be localized majorly in the cytoplasm (Figure 3a). This result was also confirmed by performing Western blot analysis of nuclear and cytoplasmic protein extracts separately. Expression of GATA-4 was greater in the nuclear extract of noradrenaline-induced cells and the cytosolic extracts of control and curcumin-treated cells (Figure 3b). These findings suggest that curcumin, under hypertrophic conditions, supresses the entry of GATA-4 into the nucleus.

Curcumin reduces the DNA-binding activity of GATA-4 in H9C2 rat ventricular myocytes

To determine the inhibitory effect of curcumin on the DNA–protein interactions involved in noradrenaline-induced cardiac hypertrophy, EMSA was carried out. Nuclear proteins were isolated from the cells treated with and without curcumin in the presence of noradrenaline and the binding activity was compared using the GATA oligonucleotide sequence. The results show that curcumin was able to suppress the DNA-binding activity of GATA-4. The intensity of shifted bands obtained was much higher in nuclear proteins isolated from cells treated with noradrenaline alone. A significant reduction in the band intensity was found in nuclear proteins isolated from cells treated with noradrenaline in the presence of curcumin. Interestingly, this band intensity was found to be less than in controls (Figure 4). The specificity of GATA-4 binding activity was further confirmed by super shift assay (data not shown). These data imply that enhanced GATA-4 DNA binding activity during noradrenaline-induced cardiac hypertrophy is suppressed by curcumin.

Effect of curcumin on primary neonatal rat cardiac myocytes

The study was further validated by performing similar experiments in a primary culture. A primary culture of two-day-old neonatal rat cardiac ventricular myocytes was established and

induced with 2 μM noradrenaline and the effect of curcumin in the presence of noradrenaline was analysed. Results obtained were consistent with those obtained in the H9C2 rat cardiomyocyte cell line, when treated with noradrenaline and curcumin. Localization of GATA-4 was observed in the nucleus when cells were treated with noradrenaline and in the cytoplasm on treatment with curcumin in the presence of noradrenaline. A significant enhancement of DNA binding activity of GATA-4 was observed on treatment with noradrenaline, which was substantially reduced by curcumin treatment. These results thus further validate the results obtained in the H9C2 cell line (Figure 5).

Discussion

Cardiovascular disorders have proven to be one of the major causes of mortality. Myocardial hypertrophy is an independent risk factor for the development of congestive heart failure. An increasing concern, therefore, is to develop affordable cardioprotective drugs that combine the characteristics of higher efficacy along with less or, possibly, no side effects in comparison with allopathic drugs.[27] In this context, several plant products have been reported to have cardioprotective properties. It is however necessary to elucidate the pharmacological actions of cardiopotent herbal medicines.

Curcumin, the principal component of dietary turmeric, a naturally occurring low-molecular-weight polyphenol, is one such compound, which has been tested to have beneficial actions of clinical relevance in treating a wide spectrum of diseases, such as cancer, rheumatoid arthritis, cystic fibrosis, psoriasis, pancreatitis and other disorders.[18] Several clinical studies have used pure curcumin in humans with no reports of toxicity or adverse outcomes.[16,28] Meager information is available regarding the effects of curcumin in a cardiac context. There is recent evidence showing that dietary curcumin possesses p300 HAT inhibitory activity, which may provide a novel therapeutic strategy for heart failure in humans.[19,20]

GATA-4 transcription factor plays an essential role in transcriptional regulation during the generation of cardiac hypertrophy. Cumulative evidence suggests that GATA-4 DNA-binding activity increases during initiation of the hypertrophic response of cardiac myocytes *in vitro* and *in vivo*.[12] Therefore, since GATA-4 is the main trancription factor whose activity is upregulated in hypertrophic signalling pathways, inhibition of its nuclear localization and DNA-binding activity could be a very promising strategy for the development of an anti-hypertrophic therapeutic agent.

The rationale of this study was to discover the molecular mechanism by which curcumin is able to prevent noradrenaline-induced cardiac hypertrophy. The findings of our study revealed that curcumin is able to prevent cardiac hypertrophy in an in-vitro model, H9c2 cardiac cells. Curcumin treatment of H9c2 cells in the presence of noradrenaline clearly circumvented the occurrence of cardiac hypertrophy by preventing the increase in cell size and cell protein content and, most importantly, reduced nuclear localization and DNA-binding activity of GATA-4, thus reducing the expression of fetal genes. A model can therefore be proposed showing that curcumin targets the nuclear localization and DNA-binding activity of GATA-4 to its hypertrophic marker genes thus preventing noradrenaline-induced cardiac hypertrophy (Figure 6). Its effect on other transcription factors involved in hypertrophy is still unknown and yet to be explored.

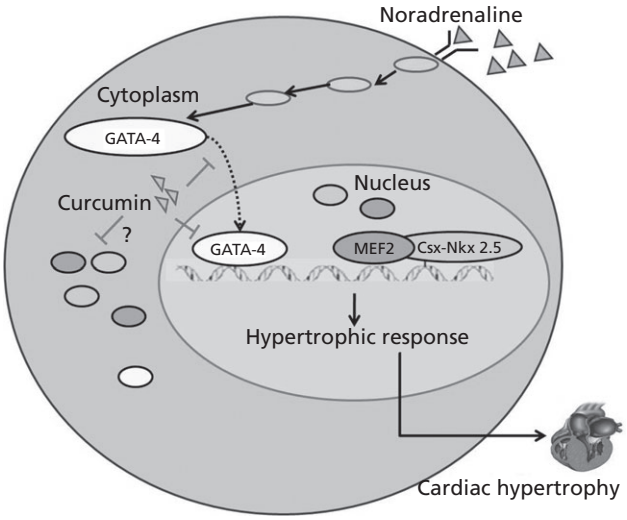

Figure 6 Proposed model for the molecular mechanism by which curcumin is able to prevent noradrenaline-induced cardiac hypertrophy. Curcumin can reverse cardiac hypertrophy but the mechanism of action is unclear. This model depicts direct inhibition of GATA-4 nuclear localization and DNA-binding activity by curcumin, including its possible actions on other transcription factors (indicated by question marks).

Inhibition of the DNA-binding activity of GATA-4 can hence be viewed as an effective therapeutic target for cardiac hypertrophy. Continuing research is necessary to elucidate the pharmacological activity and mechanism of action of curcumin, which will further stimulate future pharmaceutical development of this therapeutically beneficial herbal drug. The data of this study should be further validated on in-vivo models, which can be followed by well-designed clinical trials, and pharmacokinetics and pharmacodynamics data, to develop a potent cardioprotective drug for the future.

Conclusions

The study demonstrates that curcumin, a polyphenol, inhibits noradrenaline-induced hypertrophy in cultured cardiomyocytes. Our data show that the inhibitory action of curcumin targets the principal cardiac transcription factor GATA-4 nuclear localization and its DNA-binding activity, which further alters the hypertrophic gene program in cardiomyocytes. The study is thus relevant to understanding the molecular mechanism regulating the inhibitory effect of curcumin on noradrenaline-induced cardiomyocyte hypertrophy.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This work was supported by a research grant awarded to Dr Vibha Rani by the Department of Science and Technology, Government of India (SR/FT/LS-006/2009: Sept 4, 2009).

Acknowledgements

We acknowledge Jaypee Institute of Information Technology, Deemed University for providing the infrastructural support. We are grateful to the Department of Zoology, Government Post Graduate College for providing the neonatal rats. We would also like to thank Aastha Chhabra, Astha Jaiswal and Umang Malhotra for linguistic improvement and completion of the manuscript.

References

1. Ho CY. Hypertrophic cardiomyopathy. *Heart Fail Clin* 2010; 6: 141–159.
2. Lorell BH, Carabello BA. Left ventricular hypertrophy: pathogenesis, detection, and prognosis. *Circulation* 2000; 102: 470–479.
3. Beisvag V *et al*. Pathological and physiological hypertrophies are regulated by distinct gene programs. *Eur J Cardiovasc Prev Rehabil* 2009; 16: 690–697.
4. Molkentin JD, Dorn GW 2nd. Cytoplasmic signaling pathways that regulate cardiac hypertrophy. *Annu Rev Physiol* 2001; 63: 391–426.
5. Frey N, Olson EN. Cardiac hypertrophy: the good, the bad, and the ugly. *Annu Rev Physiol* 2003; 65: 45–79.
6. Akazawa H, Komuro I. Roles of cardiac transcription factors in cardiac hypertrophy. *Circ Res* 2003; 92: 1079–1088.
7. Sundaresan NR *et al*. Sirt3 blocks the cardiac hypertrophic response by augmenting Foxo3a-dependent antioxidant defense mechanisms in mice. *J Clin Invest* 2009; 119: 2758–2771.
8. Sugden PH. Signalling pathways in cardiac myocyte hypertrophy. *Ann Med* 2001; 33: 611–622.
9. Palaniyandi SS *et al*. Protein kinase C in heart failure: a therapeutic target? *Cardiovasc Res* 2009; 82: 229–239.
10. Oka T, Komuro I. Molecular mechanisms underlying the transition of cardiac hypertrophy to heart failure. *Circ J* 2008; 72(Suppl A): A13–A16.
11. Sugden PH. Signaling in myocardial hypertrophy: life after calcineurin? *Circ Res* 1999; 84: 633–646.
12. Pikkarainen S *et al*. GATA transcription factors in the developing and adult heart. *Cardiovasc Res* 2004; 63: 196–207.
13. Xing W *et al*. Myocardin induces cardiomyocyte hypertrophy. *Circ Res* 2006; 98: 1089–1097.
14. Fiedler B, Wollert KC. Interference of antihypertrophic molecules and signaling pathways with the Ca2+-calcineurin-NFAT cascade in cardiac myocytes. *Cardiovasc Res* 2004; 63: 450–457.
15. Paul AG. NF-κB: a novel therapeutic target for cancer. *Eukaryon* 2005; 1: 4–5.
16. Hsu CH, Cheng AL. Clinical studies with curcumin. *Adv Exp Med Biol* 2007; 595: 471–480.
17. Ammon HP, Wahl MA. Pharmacology of Curcuma longa. *Planta Med* 1991; 57: 1–7.
18. Epstein JA. Currying favor for the heart. *J Clin Invest* 2008; 118: 850–852.
19. Morimoto T *et al*. The dietary compound curcumin inhibits p300 histone acetyltransferase activity and prevents heart failure in rats. *J Clin Invest* 2008; 118: 868–878.
20. Morimoto T *et al*. Novel heart failure therapy targeting transcriptional pathway in cardiomyocytes by a natural compound, curcumin. *Circ J* 2010; 74: 1059–1066.
21. Feng B *et al*. Regulation of cardiomyocyte hypertrophy in diabetes at the transcriptional level. *Am J Physiol Endocrinol Metab* 2008; 294: E1119–E1126.
22. Simpson P. Norepinephrine-stimulated hypertrophy of cultured rat myocardial cells is an alpha 1 adrenergic response. *J Clin Invest* 1983; 72: 732–738.
23. Sreejit P *et al*. An improved protocol for primary culture of cardiomyocyte from neonatal mice. *In Vitro Cell Dev Biol Anim* 2008; 44: 45–50.
24. Plumb JA *et al*. Effects of the pH dependence of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide-formazan absorption on chemosensitivity determined by a novel tetrazolium-based assay. *Cancer Res* 1989; 49: 4435–4440.
25. Lowry OH *et al*. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265–275.
26. Sindhu KV *et al*. Isolation of a library of target-sites for sequence specific DNA binding proteins from chick embryonic heart: a potential tool for identifying novel transcriptional regulators involved in embryonic development. *Biochem Biophys Res Commun* 2004; 323: 912–919.
27. Lampronti I *et al*. Inhibitory effects of Bangladeshi medicinal plant extracts on interactions between transcription factors and target DNA sequences. *Evid Based Complement Alternat Med* 2008; 5: 303–312.
28. Hatcher H *et al*. Curcumin: from ancient medicine to current clinical trials. *Cell Mol Life Sci* 2008; 65: 1631–1652.