

Ginsenoside Rb1 Attenuates Post Doxorubicin Induced Myocardial Hypertrophy through the CaN NFATc4 GATA4 Pathway

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ABSTRACT

Heart muscle cell enlargement, referred to as myocardial hypertrophy, serves as a critical disease process observed in the cardiomyopathy phase that appears after patients undergo anthracycline medication. The capacity of ginsenoside Rb1 (Rb1) to influence hypertrophy caused by these agents has not been determined clearly until now. The goal of the present research was to test the ability of Rb1 to counteract heart muscle cell growth in the period following doxorubicin (DOX) exposure while also clarifying the biological route through which it might act. The model of myocardial hypertrophy in the post-DOX phase was produced by giving C57BL/6 mice a 15 mg/kg injection of DOX and then studying the hearts after 12 days or after 22 hours. Parallel cell experiments used H9c2 cardiomyoblasts incubated with 2 μ M DOX for a 2-hour period. Rb1 was supplied to the mice beginning two days ahead of DOX and continued once daily across a 14-day span. In the cell work, Rb1 exposure started 6 hours in advance of DOX and lasted through a total 30-hour incubation. Hypertrophy indicators tracked included heart weight relative to body weight (HW/BW), heart weight relative to tibia length (HW/TL), ultrasound heart imaging, WGA-based cell size staining, plus measurement of α -SMA, BNP, and β -MHC protein amounts. Heart tissue shape and structure were inspected using HE staining, Masson staining, and electron microscope imaging. Free calcium inside cells was quantified through Fluo-3/AM dye fluorescence. Levels and location of proteins in the CaN β /NFATc4/GATA4 route were studied with Western blot analysis, tissue staining by immunohistochemistry, and cell staining by immunofluorescence. Rb1 application caused notable lowering of the HW/BW ratio, HW/TL ratio, and LVd mass/BW ratio. It also shrank the average size of heart muscle cells and brought down the production of BNP, β -MHC, and α -SMA. Rb1 further decreased the amount of scar-like fibrous tissue in the heart, helped restore normal tiny structures inside cells, and supported improved blood flow dynamics in the hearts of DOX-exposed mice. Calcium excess that normally follows DOX was also reduced by Rb1. Matching these protective changes, the unusually high amounts of CaN, NFATc4, and GATA4 proteins detected in both animal and cell experiments returned toward normal. Rb1 lessened the myocardial hypertrophy that occurs after doxorubicin is given. This improvement is likely related to reduced functioning of the CaN/NFAT/GATA4 molecular pathway.

Keywords: Ginsenoside Rb1, Post-doxorubicin treatment, Myocardial hypertrophy, Fibrosis, CaN/NFATc4/GATA4

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Introduction

Antibiotic compounds belonging to the anthracycline family, which encompass doxorubicin (DOX), daunorubicin, and epirubicin, represent essential items listed in the World Health Organization chemotherapy standards and see frequent use against various cancer forms [1]. A key difficulty with these medicines is their tendency to generate ongoing, gradually worsening harm to heart tissue that can persist or first become evident

long after drug administration stops. Following the immediate phase of heart muscle harm and cell loss, anthracyclines drive permanent reorganization of heart architecture, notably through myocardial hypertrophy and the risk of fatal ongoing heart failure in later disease stages. Such damaging heart modifications, which correlate tightly with severe cardiovascular incidents, can develop even when total anthracycline exposure stays at 240 mg/m² [2]. As the number of individuals who recover from cancer rises, anthracycline-induced cardiotoxicity (AIC) has turned into a serious hazard for sustained health and life expectancy. Owing to its slow, hidden progression, available medical approaches like angiotensin-converting enzyme inhibitors, beta-blocking drugs, and agents that bind iron show restricted usefulness for controlling heart structure changes and late cardiotoxicity that emerge after anthracycline courses [3].

Myocardial hypertrophy features unusually large heart muscle cells together with higher total heart weight and forms a pivotal element in AIC development [4]. Should the heart fail to handle continued growth demands, hypertrophy advances into cell death, inadequate blood circulation, and potentially deadly persistent heart failure. Among calcium-triggered routes that encourage hypertrophy, the CaN/NFAT/GATA4 cascade is prominent. Within it, the phosphatase enzyme calcineurin (CaN), which depends on calcium and calmodulin, removes phosphate from nuclear factor of activated T cells (NFAT). This allows NFAT entry into the nucleus where it joins with GATA binding protein 4 (GATA4) to start transcription of genes typical of fetal heart development, thereby driving hypertrophy [5]. A range of earlier reports and our early laboratory tests have established that the CaN/NFAT/GATA4 system activates during AIC and participates in heart cell death as well as cell size increase [6, 7].

The plant *Panax ginseng* Mey. functions as a longstanding herbal treatment and nutritional product widely applied in Asian regions for thousands of years to address disorders of the heart and vessels, inflammatory states, nervousness, and sugar metabolism issues. In more recent times, ginseng has been examined for its role as an add-on therapy capable of strengthening cancer chemotherapy results and decreasing unwanted reactions [8, 9]. Ginsenosides make up the chief bioactive molecules within ginseng. Of the over 40 ginsenosides isolated from ginseng roots, ginsenoside Rb1 (Rb1) occurs in the greatest quantity and accounts for much of the heart-protective activity linked to the herb [10]. Rb1 in particular displays the ability to oppose hypertrophy by interfering with several pathways that promote cell growth, one example being CaN/NFAT/GATA4 [10, 11]. While published work has demonstrated Rb1 blocking DOX-caused programmed cell death in H9c2 cells from rat heart, whether Rb1 can reduce the hypertrophy produced by anthracyclines in heart muscle has remained unknown [12].

The purpose of this investigation was to assess pharmacological benefits of Rb1 against myocardial hypertrophy arising after DOX in living mice and in cultured cells, together with initial exploration of CaN/NFAT/GATA4 involvement.

Materials and Methods

Animal models and Rb1 treatment

Procedures with live animals gained approval from Fujian Medical University's Animal Ethics Committee (IACUC FJMU 2022-0730) and were carried out in line with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (8th Edition, 2011). Six-week-old male C57BL/6J mice of 20 ± 2 g body weight came from Shanghai SLAC Laboratory Animal Company and lived in specific-pathogen-free rooms on a 12-hour light-dark cycle with unlimited food and water. Random assignment created six groups: untreated normal control (CON), Rb1 given alone, DOX given alone, and three Rb1-plus-DOX groups using daily doses of 25 mg/kg, 50 mg/kg, or 100 mg/kg Rb1. Rb1 (HPLC ≥98%, Durst Bio, Chengdu, China, CAS: 41753-43-9) was injected intraperitoneally each day for 14 days in the combined groups, while a single 15 mg/kg DOX injection (Hanhui Pharmaceutical, Shanghai, China, BP: 2200811) occurred on trial day 3, in keeping with methods from earlier publications [10, 13-15]. Rb1 control animals received 100 mg/kg/day Rb1, DOX control animals received only DOX, and normal controls received saline of equal volume. Body weight checks took place every three days.

Cell culture and Rb1 treatment

H9c2 cardiomyoblasts, sourced from the Chinese Academy of Sciences Cell Bank in Shanghai, grew in Dulbecco's modified Eagle's medium plus 10% fetal bovine serum under 37 °C and 5% CO₂ conditions. Following approaches described in prior literature, DOX-treated cells underwent 2 μM DOX for 2 hours then normal medium for 22 hours [16]. Rb1-plus-DOX cells received Rb1 at 1.56 μM, 6.25 μM, 25 μM, 100 μM, or

400 μM concentrations for 30 hours total, with the start 6 hours before DOX addition [12, 17]. Vehicle-only cells acted as controls. All cell work was repeated independently three times.

Western blotting

Samples underwent lysis in cold RIPA solution. Separation of proteins happened on 8% SDS-polyacrylamide gels via wet transfer. Membranes received 5% skim milk blocking for 2 hours, then overnight exposure to antibodies for BNP (1:1000, Absin), GATA4 (1:500, Affinity), CaN (1:500, Affinity), β -MHC (1:10000, Affinity), NFATc4 (1:4000, CST), and β -actin (1:1000, CST). After secondary antibody steps lasting 2 hours, ECL reagent from Thermo Fisher Scientific revealed the bands. ImageJ software quantified optical density values normalized against β -actin.

Histological analysis

Formalin-fixed hearts went through ethanol dehydration steps and were placed in 4% paraffin blocks. Five-micrometer sections received hematoxylin-eosin (HE), Masson's trichrome, and wheat germ agglutinin (WGA) staining. Photographs came from an Olympus IMT-2 microscope, and ImageJ software calculated areas showing hypertrophy or fibrosis.

Immunohistochemical and immunofluorescence staining

Sections freed of paraffin were treated with 10% H_2O_2 before primary antibody incubation targeting NFATc4 (1:1000, Thermo), CaN (1:1000, Proteintech), and α -SMA (1:1000, Boster). Secondary steps used Alexa Fluor 488 anti-rabbit antibody (1:5000, Abcam) or HRP-linked reagents (Biohighgrade), followed by DAPI or hematoxylin nuclear labeling. NIS-Elements software analyzed average optical density and fluorescence from four to six randomly picked microscope fields.

Transmission electron microscopy

Three 1 mm^3 pieces from the left ventricle middle wall were fixed with 2.5% glutaraldehyde, then 1% osmium tetroxide, dehydrated in ethanol steps, and embedded in araldite. Ultrathin 40–60 nm slices underwent double staining with uranyl acetate and lead citrate and were viewed on a JEOL JEM 1010 electron microscope.

Measurement of $[\text{Ca}^{2+}]_i$

H9c2 cells loaded with 5 μM Fluo-3/AM for 30 minutes in darkness at 37 $^\circ\text{C}$ were washed and kept in PBS for 30 minutes to complete dye processing. Confocal microscope captured images at 488 nm excitation and 525 nm emission. Six fields with over 10 cells each were processed via Nikon NIS Elements software. Calcium concentration followed the formula $[\text{Ca}^{2+}]_i = \text{Kd} \times \text{F}$.

Echocardiography

Mice under 1%–2% isoflurane anesthesia were imaged supine with a VINNO 6 Lab system using a 40-MHz probe while heart rates stayed at 400–500 bpm. Parasternal long-axis views allowed assessment of left ventricle size, ejection fraction, fractional shortening, diastolic radial strain, and cardiac index (cardiac output divided by body weight).

Statistical analysis

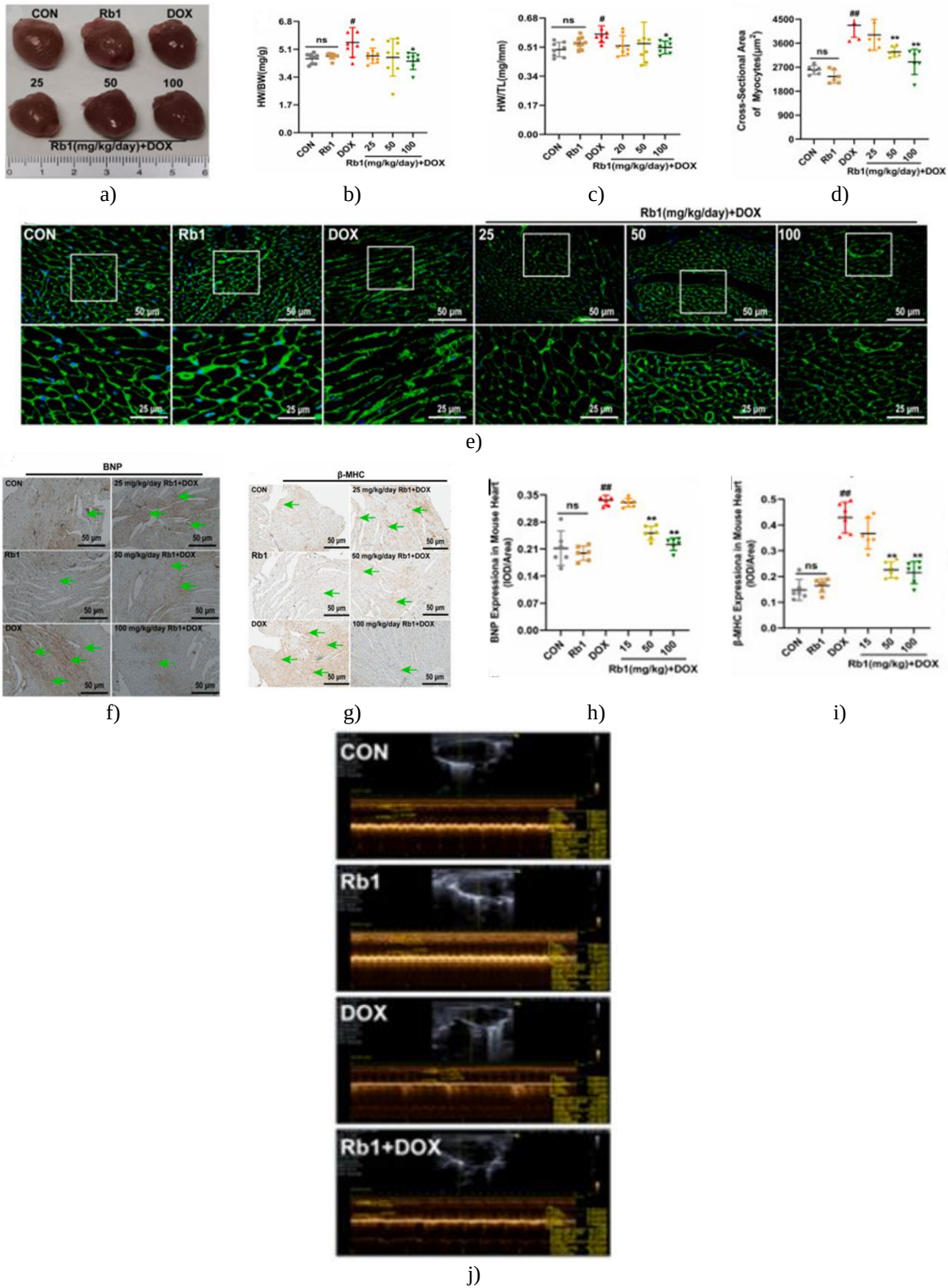
SPSS 23.0 software handled data evaluation. Results appear as mean $\pm$ SEM. Student's t-test compared pairs of groups; one-way ANOVA handled several groups. Findings reached significance at $P < 0.05$.

Results and Discussion

Rb1 protected against post-DOX treatment myocardial hypertrophy in mice

When compared with the normal control situation, DOX administration raised HW/BW by 21.85%, HW/TL by 18.00%, and LVd mass/BW by 16.70%, while lowering LVEDV by 32.92% and LVIDd by 21.08% ($P < 0.05$ versus CON) (**Figures 1a, 1c, 1j, 1m**). Cardiomyocyte cross-section area increased from $2602.58 \pm 167.60 \mu\text{m}^2$ to $4272.95 \pm 445.72 \mu\text{m}^2$ ($P < 0.01$ versus CON) (**Figures 1d, 1e**). Hypertrophy-associated proteins BNP and β -MHC showed elevations of 58.49% and 199.17%, respectively. These shifts established the presence of post-

DOX myocardial hypertrophy together with lowered heart compliance in the mice ($P < 0.05$ versus CON) (Figures 1f, 1i).



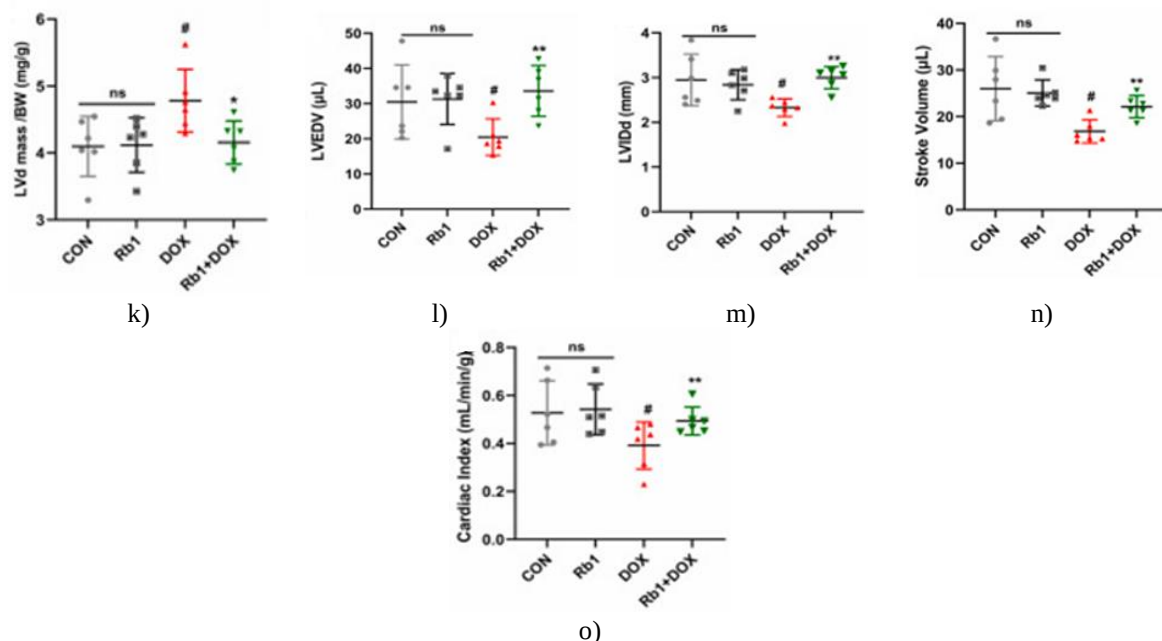


Figure 1. Rb1 reduced myocardial hypertrophy that develops after DOX administration in mice. (a) Typical photographs of whole hearts from the mice. (b) HW/BW and (c) HW/TL values across the study groups, $n = 10$. (d) Quantification of cardiomyocyte cross-sectional areas in the different groups, $n = 6$. (e) Typical images showing WGA-stained heart muscle cross-sections along with immunohistochemical detection for (f) BNP and (g) β -MHC in mouse cardiac tissue. Green arrows highlight the sites and intensity of positive staining for BNP and β -MHC. (h) Quantitative measurement of BNP and (i) β -MHC protein levels in mouse hearts, $n = 6$. (j) Example echocardiographic images and (k–o) corresponding statistical results from mouse heart ultrasound examinations, $n = 6$. Results are shown as Mean $\pm$ SEM. ns: no significant difference; # $P < 0.05$, ## $P < 0.01$ compared with CON; * $P < 0.05$, ** $P < 0.01$ compared with DOX.

Rb1 treatment countered the rises in HW/BW ratio, HW/TL ratio, LVd mass/BW ratio, and cardiomyocyte cross-sectional area while also correcting the reductions seen in LVEDV and LVIDd. Notably, when given at 100 mg/kg/day, Rb1 brought the HW/BW ratio, HW/TL ratio, and cardiomyocyte cross-sectional area nearly back to control levels ($P < 0.05$ versus DOX, $P > 0.05$ versus CON) (**Figures 1a and 1e**). In agreement with these findings, the hypertrophy marker BNP dropped by 33.88% ($P < 0.01$ versus DOX, $P > 0.05$ versus CON) and β -MHC decreased by 49.94% ($P < 0.01$ versus DOX) relative to the DOX-only group.

Rb1 enhanced heart blood flow performance, lessened cardiac fibrosis, and restored subcellular structural alterations in mice after DOX exposure

Body weight showed consistent growth over the experimental period in both the untreated control and the Rb1-only groups. Exposure to DOX interfered with this normal weight increase (**Figure 2a**). Following the DOX injection, animals displayed ongoing body weight reduction ($P < 0.01$ versus CON), lower food consumption, reduced physical activity, dulling of fur, and sporadic diarrhea. Overall survival fell from 100% to 55% (**Figure 2b**). Stroke volume (SV) declined from $26.00 \pm 2.81 \mu\text{L}$ to $16.80 \pm 1.02 \mu\text{L}$, accompanied by a 25.79% reduction in cardiac index (CI) ($P < 0.05$ versus CON) (**Figures 1n and 1o**). Rb1 treatment markedly improved the animals' overall health status and heart function parameters. Survival rates reached 65%, 85%, and 90% in the respective Rb1 + DOX groups. In the group receiving 100 mg/kg/day Rb1 plus DOX, body weight increased by 25.53%, SV by 31.71%, and CI by 26.21% compared with the DOX-only animals ($P < 0.05$ versus DOX).

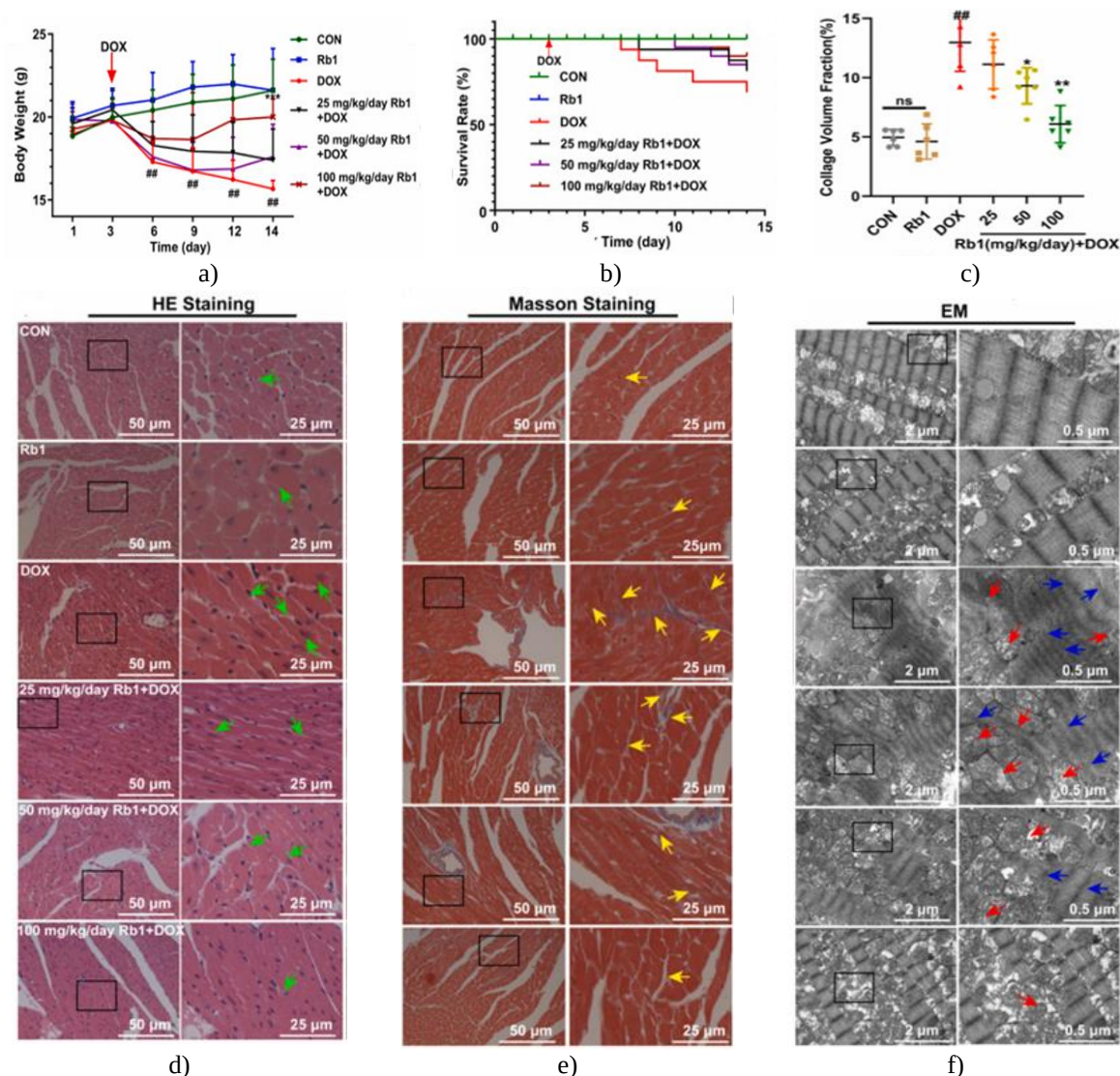


Figure 2. Administration of Rb1 led to better physical status of the animals and more normal heart tissue architecture in mice that had undergone DOX treatment. (a) Time-dependent changes in body weight and (b) curves showing percentage of surviving mice across the various groups, with $n = 20$ per group. (c) Measured collagen volume fraction values, $n = 6$. Example photographs obtained from (d) hematoxylin-eosin staining, (e) Masson trichrome staining, and (f) ultrastructural analysis by electron microscopy of cardiac samples from mice. Green arrows point out regions showing vacuoles in the cytoplasm, loss of normal cardiomyocyte alignment, and breakdown of myofibrils. Yellow arrows designate sites of excess collagen buildup. Blue and red arrows identify disrupted cross-striations, abnormally shaped mitochondria, and mitochondria that are enlarged and contain internal vacuoles. All quantitative data are expressed as Mean $\pm$ SEM. ns indicates no significant difference; # $P < 0.05$, ## $P < 0.01$ in comparison to the CON group; * $P < 0.05$, ** $P < 0.01$ in comparison to the DOX group.

The hearts removed from animals treated solely with DOX presented a darker red shade and exhibited increased firmness upon handling. In contrast, adding Rb1 treatment produced hearts with an external look much nearer to that of healthy specimens, featuring a lighter and more vivid red tone along with reduced firmness (**Figure 1a**). Exposure to DOX resulted in clear damage to heart muscle cells and promoted the formation of fibrotic tissue, which was observable via hematoxylin-eosin staining, Masson trichrome staining, and detailed electron microscopy evaluation (**Figures 2c and 2f**). According to the images in **Figure 2f**, inclusion of Rb1 markedly diminished the structural defects induced by DOX, such as irregular banding patterns, chaotic arrangement of myocardial filaments, deformed mitochondria, and mitochondrial enlargement. When Rb1 was dosed at 50 mg/kg/day, the collagen volume fraction fell by 28.14%, and at 100 mg/kg/day the reduction reached 53.00% ($P < 0.05$ versus DOX in both cases).

Rb1 suppressed the hypertrophic response induced by DOX in cultured H9c2 heart cells

To outline the full shape of H9c2 cardiomyocytes, researchers stained the cytoplasmic region with α -SMA and the nuclear region with DAPI. Following a 22-hour period after DOX was removed from the culture medium, the surface area of these cells had expanded from $4546.25 \pm 353.39 \mu\text{m}^2$ in untreated cells to $13804.5 \pm 909.36 \mu\text{m}^2$, amounting to 3.04 times the size observed in the normal control condition ($P < 0.01$ versus CON) (**Figures 3a and 3c**). Furthermore, cellular protein amounts of α -SMA, BNP, and β -MHC showed increases of 164.18%, 81.43%, and 79.20%, respectively ($P < 0.05$ versus CON) (**Figures 3d and 3f**).

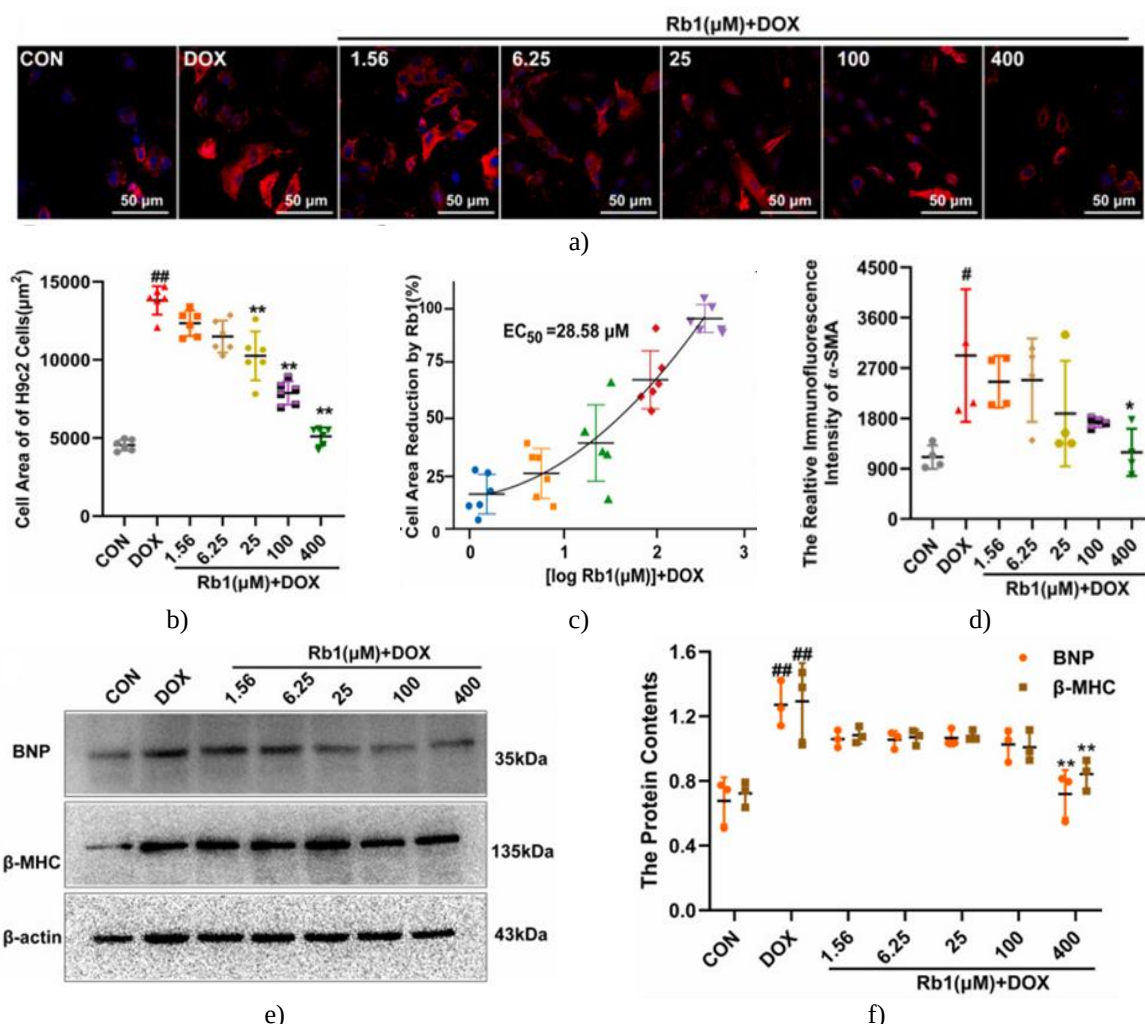
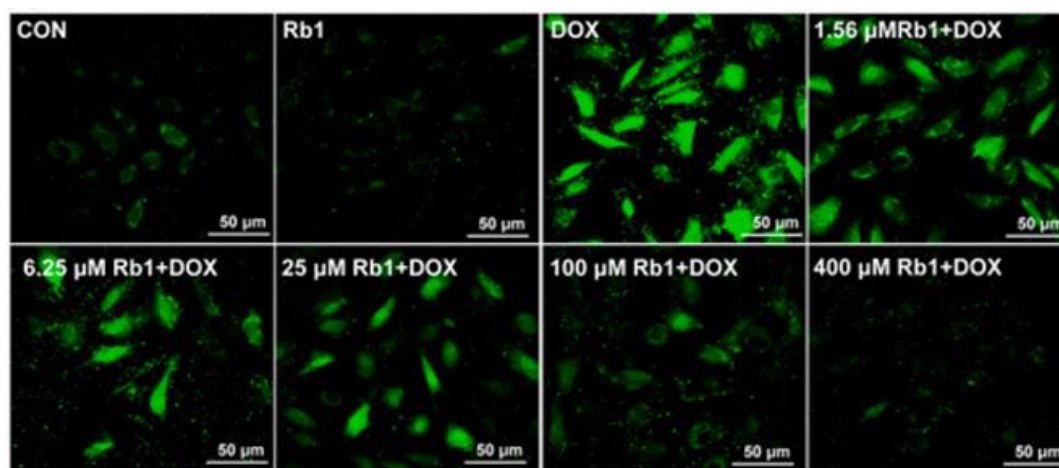


Figure 3. Rb1 blocked the hypertrophic enlargement of H9c2 cardiomyocytes that follows DOX exposure. (a) Example photographs displaying the outlines of H9c2 cells outlined by α -SMA staining. (b) Measurement of H9c2 cell surface area and (c) percentage reduction in cell area achieved by different Rb1 concentrations shown as a dose-response curve, $n = 6$. (d) Average relative immunofluorescence intensity of α -SMA across the experimental groups, $n = 4$. (e) Representative Western blot bands detecting BNP and β -MHC proteins and (f) quantitative analysis of their relative expression levels, $n = 3$. All data are presented as Mean $\pm$ SEM. # $P < 0.05$, ## $P < 0.01$ versus CON; * $P < 0.05$, ** $P < 0.01$ versus DOX.

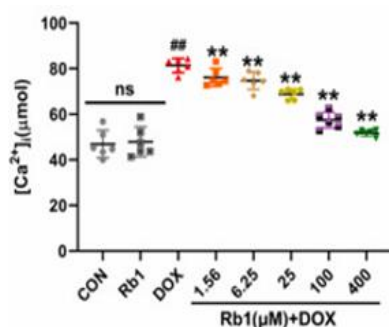
The expanded size of H9c2 cells became progressively smaller as Rb1 concentrations rose. Treatment with 6.25 μM , 25 μM , 100 μM , and 400 μM Rb1 produced cell area reductions of 16.73%, 25.72%, 42.95%, and 63.01%, respectively ($\text{EC}_{50} = 28.58 \mu\text{M}$), all with $P < 0.01$ versus DOX (**Figures 3b and 3c**). Notably, the 400 μM dose of Rb1 fully eliminated the DOX-induced increase in cell area ($P > 0.05$ versus CON) and brought down the elevated levels of α -SMA, BNP, and β -MHC by 59.30%, 71.20%, and 35.0%, respectively ($P < 0.01$ versus DOX).

Rb1 alleviated calcium overload and reduced excessive CaNB β expression in the setting of myocardial hypertrophy that occurs after DOX treatment

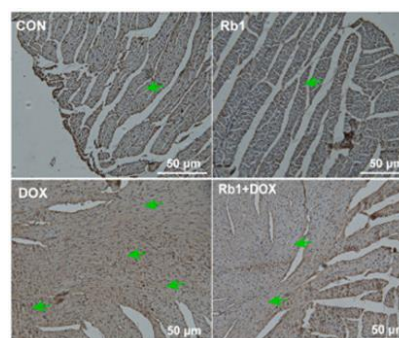
Intracellular calcium levels ($[Ca^{2+}]_i$) were assessed with the fluorescent calcium probe Fluo-3 AM. As depicted in **Figures 4a and 4b**, 22 hours following the removal of DOX, H9c2 cells in the post-DOX condition displayed an 82.23% elevation in $[Ca^{2+}]_i$ ($P < 0.01$ versus CON). Application of Rb1 at concentrations of 1.56 μ M, 6.25 μ M, 25 μ M, 50 μ M, and 100 μ M decreased this elevated $[Ca^{2+}]_i$ by 6.42%, 8.15%, 15.17%, 29.40%, and 36.42%, respectively ($P < 0.01$ versus DOX). When the Rb1 concentration reached 400 μ M, the calcium overload caused by prior DOX exposure was fully reversed, returning $[Ca^{2+}]_i$ to levels indistinguishable from the control group ($P > 0.05$ versus CON).



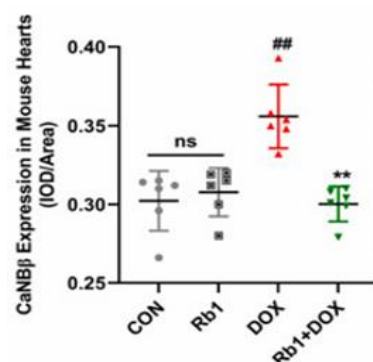
a)



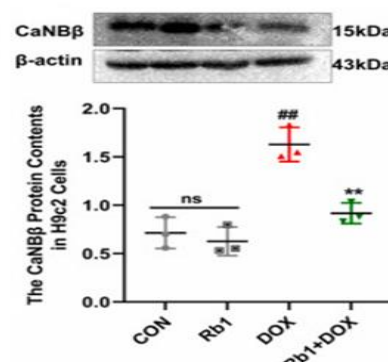
b)



c)



d)



e)

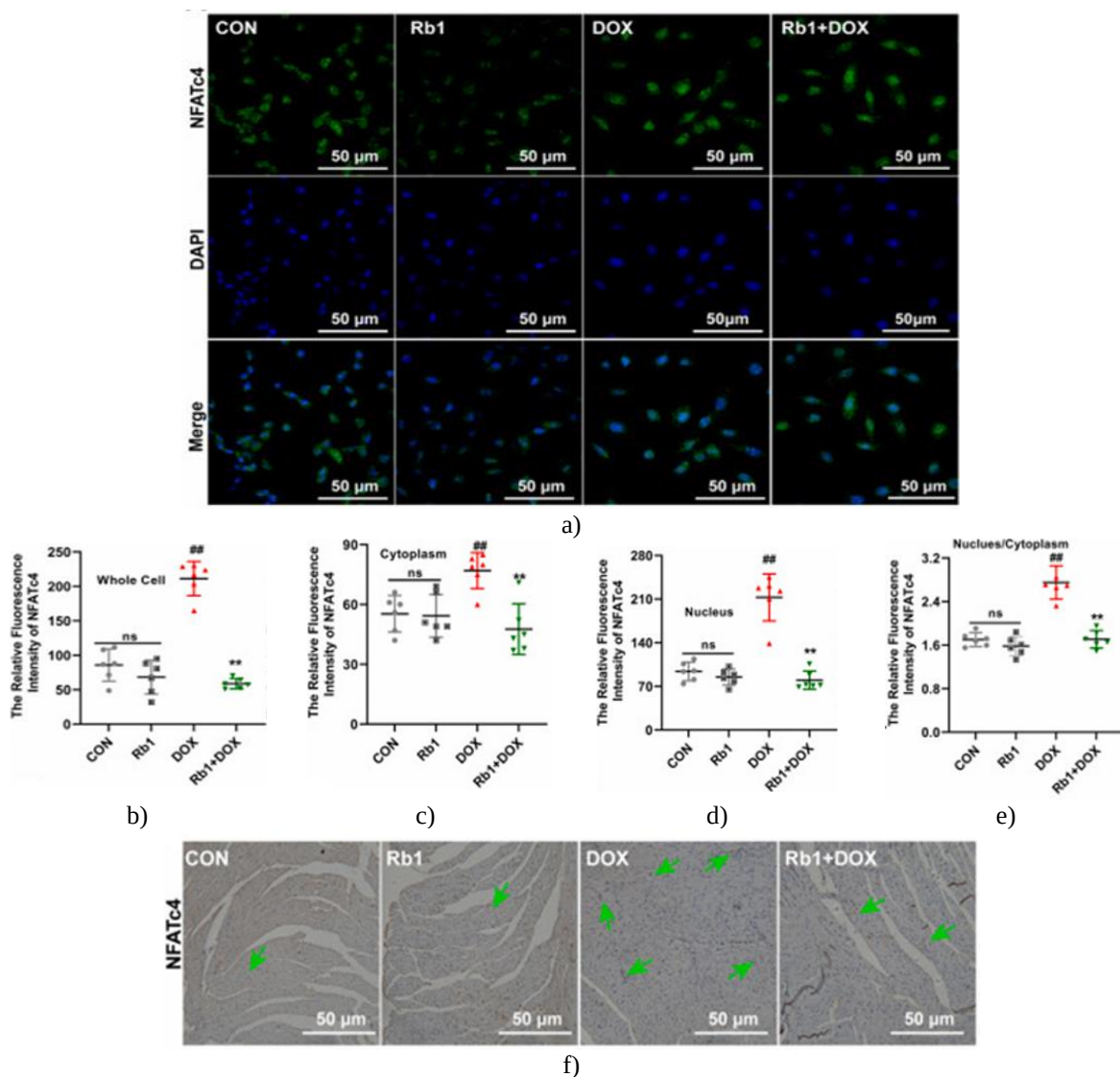
Figure 4. Rb1 decreased intracellular calcium concentration and lowered CaNB β protein levels in cardiomyocytes exposed to DOX. (a) Example fluorescence images captured using Fluo-3/AM in H9c2 cells. (b) Quantitative evaluation of $[Ca^{2+}]_i$ levels, $n = 6$. (c) Typical immunohistochemical staining images for CaNB β in mouse heart sections. The green arrow indicates the location and intensity of positive CaNB β staining. (d) Quantitative assessment of CaNB β expression in mouse hearts, $n = 6$. (e) Representative Western blot image showing CaNB β bands in H9c2 cells together with analysis of relative protein

abundance, $n = 3$. Results are shown as Mean $\pm$ SEM. ns: no significant difference; ## $P < 0.01$ versus CON; ** $P < 0.01$ versus DOX.

The ongoing calcium overload that persists after DOX treatment was accompanied by increased expression of CaNB β . Data presented in **Figures 4c and 4e** demonstrate that CaNB β levels rose by 17.76% in the animal model and by 129.60% in the cell model ($P < 0.01$ versus CON). This elevation was completely prevented when animals received 100 mg/kg/day Rb1 or when cells were treated with 400 μ M Rb1 ($P < 0.05$ versus DOX; $P > 0.05$ versus CON).

Rb1 suppressed the activation of NFATc4 and GATA4 during myocardial hypertrophy that develops after DOX administration

In heart tissue from mice and in H9c2 cells that had been exposed to DOX, total cellular NFATc4 protein increased by 146.53% and by 2.50-fold, while GATA4 levels rose by 53.23% and by 1.53-fold, respectively ($P < 0.01$ versus CON) (**Figure 5**). Treatment with Rb1 produced a clear reduction in these elevated NFATc4 and GATA4 amounts ($P < 0.01$ versus DOX, $P > 0.05$ versus CON). In parallel with the decrease in NFATc4, the ratio of nuclear to cytoplasmic immunofluorescence intensity fell from 2.75 to 1.71, indicating that Rb1 limited the movement of NFATc4 into the nucleus and thereby restricted its activation ($P < 0.01$ versus DOX, $P > 0.05$ versus CON) (**figure 5e**).



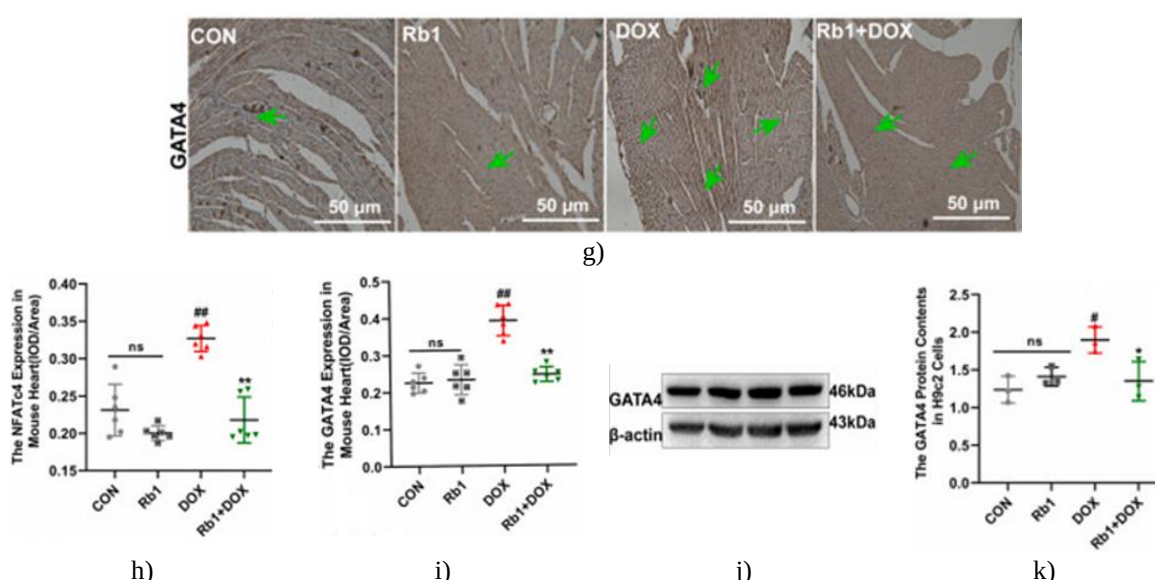


Figure 5. Rb1 reduced the activation of NFATc4 and GATA4 in myocardial hypertrophy occurring after DOX treatment. (a) Typical fluorescence images of NFATc4 in H9c2 cells. Quantitative assessment of NFATc4 relative immunofluorescence intensity at the (b) whole-cell level, (c) cytoplasmic level, (d) nuclear level, and (e) nucleus-to-cytoplasm ratio in H9c2 cells, $n = 6$. Representative immunohistochemical staining images for (f) NFATc4 and (g) GATA4 in mouse heart tissue. Quantitative analysis of (h) NFATc4 and (i) GATA4 expression levels in mouse hearts, $n = 6$. (j) Example Western blot bands for GATA4 in H9c2 cells and (k) corresponding relative protein expression analysis, $n = 3$. Data are presented as Mean $\pm$ SEM. ns: no significant difference; # $P < 0.05$, ## $P < 0.01$ versus CON; * $P < 0.05$, ** $P < 0.01$ versus DOX.

This investigation employed both living animal and cultured cell models of myocardial hypertrophy that develops after DOX exposure to evaluate the potential of Rb1 to oppose hypertrophic growth. Rb1 successfully reduced cardiomyocyte enlargement and overall heart mass while also lowering the amounts of α -SMA, β -MHC, and BNP. By decreasing excessive calcium accumulation and dampening the CaN/NFATc4/GATA4 pathway, Rb1 appeared capable of limiting cardiac hypertrophy in the post-DOX period.

It is well established that harmful cardiac hypertrophy can emerge many months or even years following anthracycline chemotherapy. For this reason, sustained treatment over an extended period that helps preserve normal tissue architecture is essential for controlling long-term anthracycline-induced cardiotoxicity. To replicate this clinical situation, Rb1 was administered before DOX and continued during the entire period when hypertrophy develops. The observed dose-related suppression of hypertrophy, along with substantial decreases in fibrotic tissue and restoration of fine cellular structures, demonstrated that Rb1 strongly improved cardiac remodeling after DOX. These structural benefits likely supported better heart wall flexibility and overall pumping ability.

Further experiments exploring the underlying processes reinforced Rb1's protective role against heart muscle enlargement after anthracycline exposure. Calcium acts as a common intracellular signal involved in normal heart muscle contraction as well as in disease processes such as hypertrophy and scarring. Anthracyclines interfere with calcium regulation by affecting L-type calcium channels, receptor-operated channels, ryanodine receptors, and the sarcoplasmic reticulum calcium pump. These disturbances lead to pronounced calcium buildup, abnormal signaling, increased production of reactive oxygen species, and eventual cardiomyopathy [18-21]. The present findings extend these observations by showing that calcium dysregulation and overload persist into the post-anthracycline phase. Although the exact processes are not yet fully understood, giving Rb1 before and during DOX exposure substantially lowered cytoplasmic free calcium levels. This normalization of calcium signaling is expected to produce beneficial downstream effects.

Calcium-dependent signaling involves complex networks with extensive interactions among various molecules. Previous work has linked calcium-triggered NFAT activation and anthracycline-related reduction in GATA4 to stimulation of cell death pathways during the early stage of anthracycline cardiotoxicity [7, 22]. In the current study, DOX treatment led to elevated CaN levels, increased nuclear presence of NFATc4, and sustained high calcium. As anticipated, Rb1's regulation of cytoplasmic calcium also limited CaN and NFAT activity.

Anthracyclines are known to suppress GATA4 function by reducing its ability to bind DNA [23]. Interestingly, in the post-DOX models used here, GATA4 levels rose substantially in the absence of ongoing DOX. Research by Suzuki has indicated that calcium signaling can positively influence GATA activity [24]. It is therefore reasonable to suggest that the observed increase in GATA4, which Rb1 could suppress, may partly stem from interactions with NFATc4. Although quantitative precision and standardization of immunohistochemistry and immunofluorescence require further careful validation, the combined use of Western blotting together with consistent findings from both cell and animal systems provided robust support for Rb1's inhibitory action on the CaN/NFAT/GATA4 cascade. Since NFAT/GATA4 strongly drives the production of hypertrophy-related genes such as β -MHC and BNP, the suppression of this pathway by Rb1 likely accounts for much of its protective effect against hypertrophy after anthracycline treatment.

Beyond its ability to counteract hypertrophy, practical aspects including safety, availability, and cost of long-term Rb1 use deserve thorough consideration. Earlier research has shown that Rb1 is generally well tolerated with minimal toxicity even when given at high doses in various animal species and in human participants [25-28]. In the present work, a total accumulated dose of 1400 mg/kg Rb1 delivered intraperitoneally produced no obvious adverse effects and markedly enhanced the overall condition of mice that received DOX. Being the most plentiful ginsenoside, Rb1 is relatively easy to source and offers better economic feasibility compared with less abundant analogs. Nevertheless, some reports note that the area under the plasma concentration-time curve for Rb1 is relatively modest, which could limit its therapeutic usefulness and raise concerns about side effects or treatment expenses [15, 29-31]. Additional studies on pharmacology, pharmacokinetics, and safety using different administration routes, along with development of advanced delivery methods or structural modifications of Rb1, will be important for supporting prolonged clinical application.

Conclusion

In summary, the results from animal and cell-based experiments provide clear support for the ability of Rb1 to oppose myocardial hypertrophy that arises after anthracycline treatment. The likely mechanism centers on suppression of the CaN/NFATc4/GATA4 signaling pathway. Rb1 represents a promising natural compound for both preventing and managing cardiomyopathy that occurs following anthracycline therapy.

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Ethics Statement: None

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