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By Inhibiting the NO/cGMP/PKG and ERK1/2/Calcineurin a pathways, KMUP-1 Reduces the Cardiac Hypertrophy that Isoprenaline Causes in Rats

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Abstract:

Background and goal: To find out if the new xanthine-based derivative KMUP-1 reduces isoprenaline (ISO)-induced cardiac hypertrophy in rats and, if so, if the nitric oxide (NO) pathway mediates the anti-hypertrophic action.

Methodology used in the experiment: Wistar rats were given an injection of ISO (5 mg kg d-1 s-c) for ten days to induce cardiac hypertrophy in vivo. KMUP-1 was given to the treatment group one hour prior to ISO. The effects of KMUP-1 on survival, cardiac hypertrophy and fibrosis, as well as the pathways involved in hypertrophy [calcineurin A and extracellular signal-regulated kinase (ERK)1/2] and NO/guanosine 3′5′-cyclic monophosphate (cGMP)/protein kinase G (PKG), were investigated after 10 days. Nω-nitro-L-arginine (L-NNA), a NOS inhibitor, was given in conjunction with KMUP-1 in order to study the part that NOS plays in the effects of KMUP-1. anti-hypertrophic actions in vitro of KMUP-1 in hypertrophy neonatal rat cardiomyocytes caused by ISO.

Important outcomes: Rats treated with ISO had increased survival rates and less heart hypertrophy and fibrosis when KMUP-1 pretreatment was administered in vivo. KMUP-1 enhanced cardiac endothelial NOS, cGMP, PKG, and plasma NOx (nitrite and nitrate). KMUP-1 also reduced the activation of hypertrophic signaling in rats treated with ISO by calcineurin A and ERK1/2. Concurrent dosing of L-NNA reduced all of these effects of KMUP-1. Similarly, in vitro, in newborn rat cardiomyocytes, KMUP-1 reduced the hypertrophic responses and signaling brought on by ISO.

Inferences and conclusions: Rats' ventricular hypertrophy brought on by ISO injection is lessened by KMUP-1. Activation of NOS mediates these effects, at least partially. This innovative substance affects the NO/cGMP pathway, which may have a part in preventing heart hypertrophy.

Keywords: kmup-1; cardiac hypertrophy; isoprenaline; nitric oxide; calcineurin

Abbreviations:

cGMP, guanosine 3′5′-cyclic monophosphate**;**

ERK, extracellular signal-regulated kinase**;**

ISO, isoprenaline; L-NNA**,**

Nw-nitro-L-arginine**;**

NFAT, nuclear factor of activated T cell**;**

NO, nitric oxide**;**

NOS, nitric oxide synthase**;**

PKG, protein kinase G.

Overview:

Heart hypertrophy is frequently brought on by pathological circumstances like pressure or volume overload, and it is thought to represent the heart muscle's adaptive reaction (Sadoshima and Izumo, 1997). On the other hand, according to Levy et al. (1990), chronic cardiac hypertrophy is linked to a markedly elevated risk of heart failure and consequently elevated cardiovascular mortality. Hypertrophic remodeling is facilitated by a variety of signaling and transcription mechanisms (Frey et al., 2004). Furthermore, there is increasing evidence to show that the modulation of cardiac hypertrophy is influenced by the nitric oxide (NO)/guanosine 3′5′-cyclic monophosphate (cGMP)

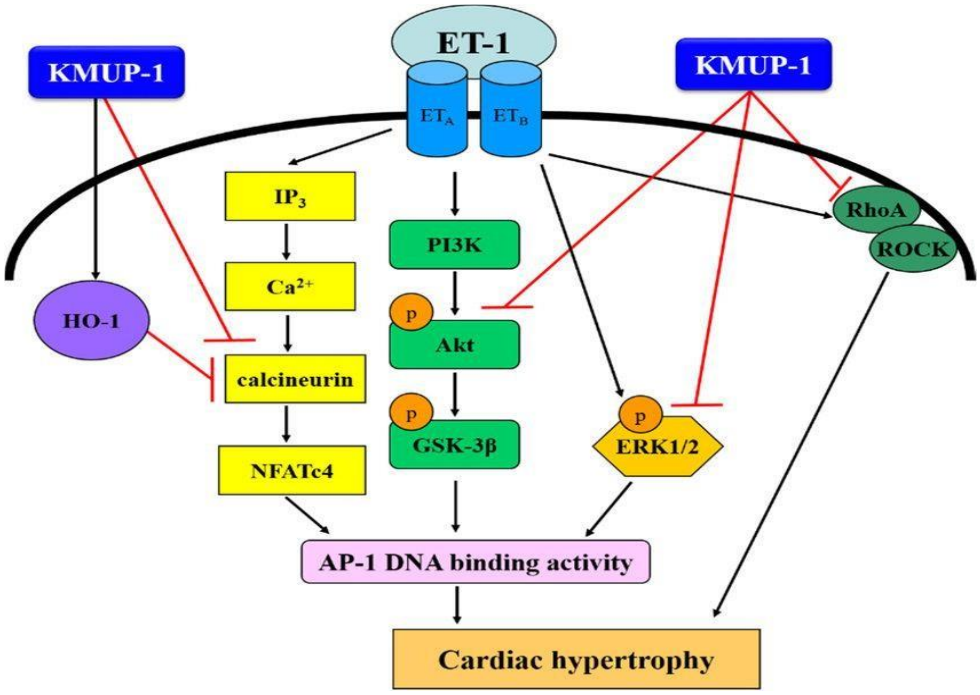

Figure 1: Proposed signaling pathways involved in the protective effects of KMUP-1 on ET-1-induced cardiomyocyte hypertrophy. The underlying mechanisms are associated with inhibition of p-ERK1/2, p-Akt, p-GSK-3β, calcineurin A, nuclear NFATc4 expression and Rho A translocation, subsequent AP-1 expression and up-regulation of HO-1.

pathway. For instance, it has been demonstrated that overexpressing endothelial nitric oxide synthase (NOS) in mice reduces ventricular hypertrophy [20]. Using the phosphodiesterase inhibitor sildenafil to raise cardiac cGMP demonstrated to reduce hypertrophic responses while exerting cardioprotection [8,24]. As a result, blocking the NO/cGMP pathway could be a useful therapeutic approach for treating or preventing cardiac hypertrophy. Extracellular signal-regulated kinases (ERK)1/2 have been shown to be crucial components of numerous cellular and physiological signaling reactions. Cardiac hypertrophy may arise as a result of ERK1/2 stimulation mediated by β-adrenoceptors. Significant diseases like hypertrophy, proliferation, and oncogenesis are partially caused by aberrant ERK1/2 signaling [21]. Numerous studies have examined the signaling mechanisms that govern

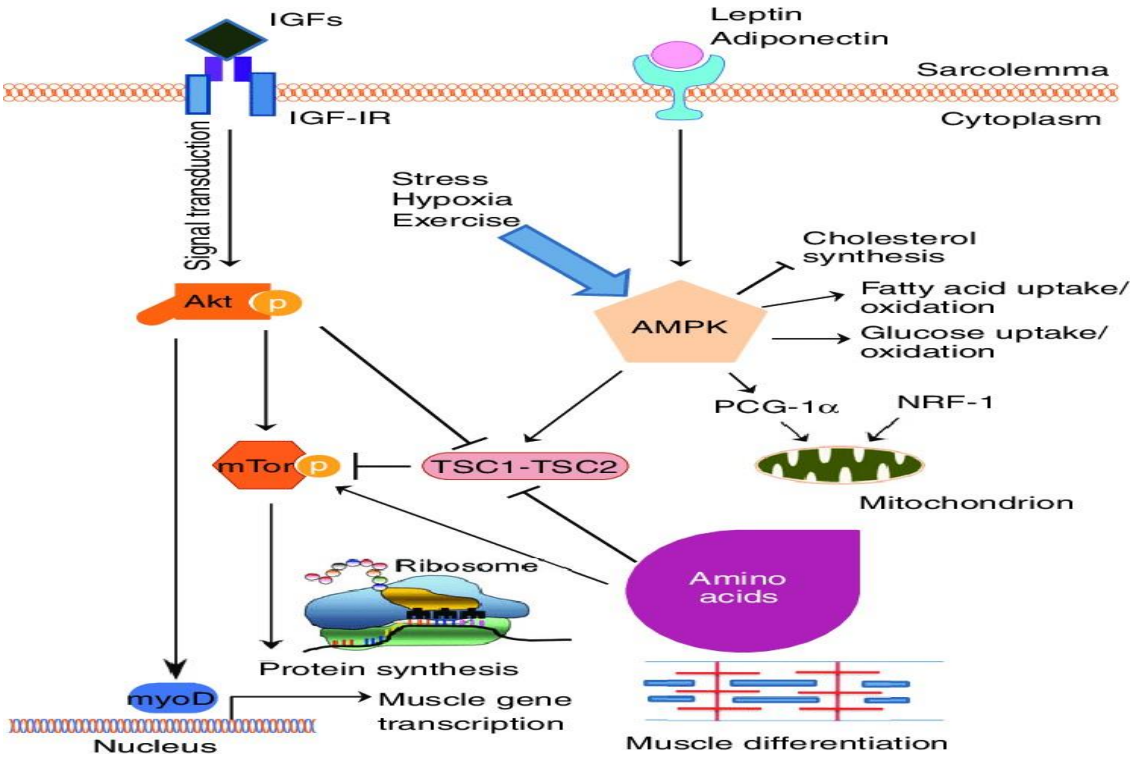

Figure 2: Signaling pathways controlling protein synthesis and master-gene transcription in skeletal muscle.

The diagram is a non-comprehensive introduction based mainly on mammalian literature, with many important intermediate proteins excluded. Thus, the reader is directed to dedicated reviews to learn more about these complex pathways, which have not been studied in detail in teleosts. Briefly, IGF hormones form complexes with IGFBPs, mediating their interaction with the IGF-IR at the cell membrane, an event that serves to initiate a signalling cascade involving activation or inhibition of several proteins via phosphorylation/dephosphorylation events. A key protein is Akt, which phosphorylates TOR, culminating in increased levels of protein synthesis by regulating essential proteins controlling mRNA translation. Akt also enhances the transcriptional activities of myoD to increase the transcription of myogenic genes. Other hormones (leptin and adiponectin) and cellular energy stressors (including exercise and hypoxia) stimulate the AMP-activated protein kinase pathway (AMPK). Activation of AMPK promotes catabolic pathways including the uptake and oxidation of glucose and fatty acids to provide ATP. Conversely, AMPK activation inhibits anabolic processes requiring ATP, including cholesterol and protein synthesis. The inhibition of translation by AMPK is mediated by TSC1-TSC2, an inhibitor of TOR.

cardiac hypertrophy, such as calcineurin A and Ca2+/calmodulin-activated phosphatase [5]. According to reports, phosphorylation of calcineurin A is induced by Ca2+ activation, and calcineurin-nuclear factor is subsequently translocated as a result activating transcription for gene expression into the nucleus, which in turn promotes cardiomyocyte hypertrophy or death [17]. Our group created KMUP-1, a novel derivative of piperazine and xanthine. According to our most recent research, KMUP-1 can inhibit phosphodiesterases, raise cGMP, and activate soluble guanylate cyclase (sGC). These actions cause the smooth muscles of the aorta, vena carvenosa, and trachea to relax [13,27-28]. It has been demonstrated that KMUP-1 inhibits the expression of inducible nitric oxide synthase (iNOS) in tracheal smooth muscle cells when tumour necrosis factor-α is present, and this effect is related to the sGC/cGMP/protein kinase G (PKG) expression pathway [29]. Additionally, it has been discovered that KMUP-1 exhibits anti-proliferation action in prostate epithelial cells and suppress the development of tumors in prostate cancer [14-15]. It is unknown, though, if it can also cause the heart's NO/cGMP pathway to become active, which would have cardioprotective effects. Therefore, the purpose of this work was to determine whether KMUP-1 has an anti-hypertrophic impact in cardiac hypertrophy, both in vivo and in vitro, and if so, whether the NO pathway mediates this effect.

Methods

Experimental animals

Male Wistar rats weighing 250 to 300 g were purchased from the National Laboratory Animal Breeding and Research Centre (Taipei, Taiwan). They were housed under conditions of constant temperature and controlled illumination (light on between 7 h 30 min and 19 h 30 min). Food and water were available ad libitum.

Experimental groups and treatment

Rats were randomly divided into five groups. (i) Control group (n= 10): normal saline was given s.c. for 10 days; (ii) isoprenaline (ISO) group (n= 20): to induce cardiac hypertrophy, ISO 5 mg·kg−1·day−1 s.c. was administered for 10 days; (iii) ISO/KMUP-1 group (n= 10): KMUP-1 was injected i.p. for 10 days at a dose of 0.5 mg·kg−1·day−1, prior to ISO 5 mg·kg−1·day−1; (iv) ISO/L-NNA (Nω-nitro-L-arginine) 10/KMUP-1 group (n= 10): drugs given to this group were the same as the ISO/KMUP-1 group, except that the NOS inhibitor, L-NNA at a dose of 10 mg·kg−1·day−1 was added to the drinking water of this group; and (v) ISO/L-NNA 20/KMUP-1 group (n= 10): drugs given to this group were the same as the ISO/L-NNA 10/KMUP-1 group, except that a higher dose of L-NNA (20mg·kg−1·day−1) was administered. After 10 days of treatment, under pentobarbitone anaesthesia, the hearts were removed for assessment. Myocardial interstitial fibrosis was stained by Masson's trichrome. All hearts were sectioned across the ventricles for histological analyses. The extent of fibrosis in both the viable and infarcted region of the left ventricle was quantified using Masson's trichrome stained heart sections.

Determination of survival and heart weight indices

The number of survivors in each group was recorded daily until the end of study. After the defined treatment period, the rats were killed by an i.p. injection of 40 mg·kg−1 pentobarbitone sodium. The heart weight and body weight were both recorded. The heart weight index was calculated by dividing the heart weight by the body weight.

Measurement of plasma nitrite and nitrate (NOx)

Plasma NOx was measured by Griess reaction. In brief, blood was sampled from the aorta and was centrifuged at 370×g for 20 min at 4°C. The supernatants were extracted three times. A total of 150 μL of the samples were incubated with 150 μL of the Griess reagent (part I: 1% sulphanilamide; part II: 0.1% naphthylethylene diamide dihydrochloride and 2% phosphoric acid) at room temperature. Ten minutes later, the absorbance was measured at 540 nm using an automatic plate reader, and the NOx concentrations were expressed as μmol·L−1 and calculated using a standard curve of NOx.

cGMP determination

Intracellular cGMP concentrations were assayed as previously described [27]. In brief, the left ventricle was sonicated in ice-cold 5–10% trichloroacetic acid and then centrifuged at 13 000×g for 60 min at 4°C. Then the protein content was determined using Bio-Rad protein assay. The cGMP level was determined by a commercially available radioimmunoassay kit subsequently (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Culture of neonatal rat ventricular cardiomyocytes

Cardiomyocytes were obtained from 1–3-day-old neonatal Wistar rat ventricles as previously described [18]. Briefly, the ventricular cells were dispersed by digestion with trypsin and collagenase II. The cell supernatant was collected by centrifugation, and the pellet was resuspended in fetal bovine serum. The above steps were repeated 7–10 times until the ventricle was completely digested. Cells were plated onto collagen-coated culture dishes or cover slips and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 2 mmol·L−1 L-glutamate and 100 μmol·L−1 5-bromodeoxyuridine. To induce hypertrophy, cardiomyocytes were cultured in serum-free medium for at least 24 h and then treated with 1 μmol·L−1 ISO for 48 h.

Preparation of cytosolic and nuclear protein extracts

Separation and preparation of cytoplasmic and nuclear extracts were performed using NE-CER Nuclear and Cytoplasmic Extraction Reagents (Pierce Biotechnology, Rockford, IL, USA). In brief, cardiomyocytes were harvested and resuspended in 100 μL of ice-cold cytoplasmic extraction reagent (CER) I containing protease inhibitor, and incubated on ice for 10 min. Then 5.5 μL of ice-cold CER II was added to the cell suspension. After being vortexed for another 5 s, the cell extract was centrifuged at 4°C for 5 min at maximum speed, and the supernatant, which embraces the cytoplasmic fraction, was transferred to a fresh, prechilled tube and stored at −80°C. The pellet was resuspended in 50 μL of ice-cold nuclear extraction reagent for a total of 40 min. The insoluble fraction was precipitated by centrifugation at 4°C for 10 min at maximum speed, and the supernatant was used for nuclear fraction.

The protein concentration was determined using the Bio-Rad Protein Assay. For subcellular location determination, the protein extracts were reacted with antibodies against lamin B (ProteinTech Group, Chicago, IL, USA), the nuclear marker, and β-actin, the cytoplasmic marker respectively.

Western blot analysis

Lysates of cardiomyocytes and cardiac tissue were prepared as described previously [15]. In brief, cardiomyocytes were made quiescent for 24 h, followed by incubation in the absence or presence of KMUP-1 for 1 h and then stimulated with ISO (1 μmol·L−1). Reactions were terminated by washing twice with cold phosphate-buffered saline, and the cells were then harvested. Left ventricle was homogenized in ice-cold lysis buffer and protease inhibitor (Sigma). The homogenate was centrifuged at 830×g for 60 min at 4°C, and supernatant was recovered as the total cellular protein. Total protein from each sample was separated by SDS- PAGE on 10% acrylamide gels, transferred to a polyvinylidine difluoride membrane and then blocked with 5% non-fat dry milk in Tris-buffered saline. Membrane was subsequently incubated with a 1:1000 dilution of endothelial nitric oxide synthase (eNOS), iNOS, PKG, phospho-ERK1/2, calcineurin A and NFATc3 antibodies. Proteins were detected with horseradish peroxidase conjugated second antibody (1:1000 dilution, Chemicon). The immunoreactive bands detected by chemiluminescence reagents (PerkinElmer Life Sciences Inc., Waltham, MA, USA) were developed by Hyperfilm (Kodak, Rochester, NY, USA).

Calcineurin phosphatase activity assay

Calcineurin activity was measured using a calcineurin cellular assay kit plus (BIOMOL, Plymouth Meeting, PA, USA; catalogue number AK-816) according to the manufacturer's protocol. Briefly, neonatal rat ventricular myocytes were collected in 400 μL of lysis buffer (50 mmol·L−1 Tris, pH 7.5, 0.1 mmol·L−1 EDTA, 0.1 mmol·L−1 EGTA, 1 mmol·L−1 dithiothreitol, 0.2% Nonidet P-40). Free phosphate was removed by passing the lysates through a desalting column before assaying. The RII phosphopeptide was used as a specific substrate for calcineurin

Statistical analysis

The survival curves were generated using the Kaplan–Meier analysis, and the log-rank test was used to detect any significant difference between survival curves. The values of other parameters were expressed as mean ± SE. Statistical significance was estimated by Student's t-test or one-way analysis of variance (anova) followed by Dunnett's t-test. A P-value <0.05 was considered to indicate statistical significance.

Drugs

Isoprenaline and L-NNA were obtained from Sigma-Aldrich Inc. (St. Louis, MO, USA). KMUP-1 was synthesized in our laboratory and was dissolved in normal saline or water. Anti-eNOS and ERK1/2 antibodies were obtained from Upstate Biotechnology, Inc. (Lake Placid, New York, NY, USA); anti-PKG antibodies were purchased from Calbiochem Co. (San Diego, CA, USA); anti-iNOS, calcineurin A and β-actin antibodies were obtained from Sigma-Aldrich Inc.; Anti-phospho ERK1/2 antibody was obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA); anti-NFATc3 antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other reagents used were of analytical grades or higher and were obtained from standard commercial sources.

Outcomes

KMUP-1's effects on survival

Initially, we looked into KMUP-1's potential to increase rats' lifespan following long-term ISO treatment. As demonstrated in Figure 1A, the control group's survival rate was 100% up until the study's 10-day conclusion. At the conclusion of the trial, the ISO group's survival rate dropped dramatically to 60% when compared to the control group; however, KMUP-1 kept the ISO/KMUP-1 group's survival rate at 90%. Conversely, in the ISO/L-NNA 10/KMUP-1 and ISO/L-NNA 20/KMUP-1 groups, L-NNA tended to lessen the protective effect of KMUP-1 by lowering the survival rate from 90% to 70%; however, the changes were not statistically significant.

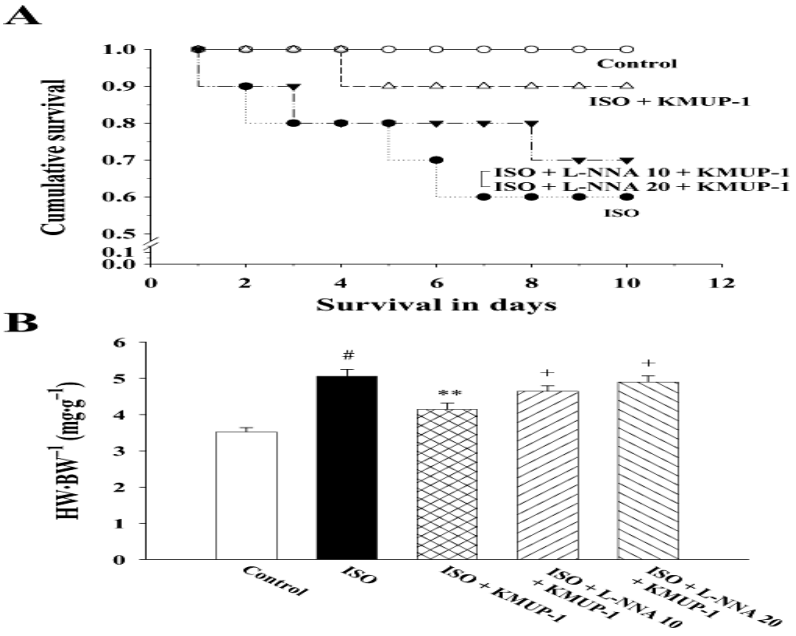

Figure 3: Effects of KMUP-1 on the survival rate (A) and heart weight to body weight ratio (B) in rats. #P < 0.05 versus control group; **P < 0.01 versus ISO group; +P < 0.05 versus ISO/KMUP-1 group. HW/BW, heart weight/body weight; ISO, isoprenaline; L-NNA, Nω-nitro-L-arginine.

Consequences for cardiac fibrosis and hypertrophy

We also looked at KMUP-1's potential anti-fibrotic and anti-hypertrophic properties. The increased heart weight/body weight ratio in Figure 1B

indicates that ISO produced myocardial hypertrophy. KMUP-1 was administered concurrently to reduce the hypertrophy. As seen in the ISO/L-NNA 10/KMUP-1 and ISO/L-NNA 20/KMUP-1 groups, L-NNA inhibited the anti-hypertrophic action of KMUP-1 in a dose-dependent way. Significant myocardial fibrosis was produced by ISO, as seen in Figure 2. In a similar vein, fibrosis was lessened by KMUP-1 pretreatment, and L-NNA blocked KMUP-1's anti-fibrotic activity.

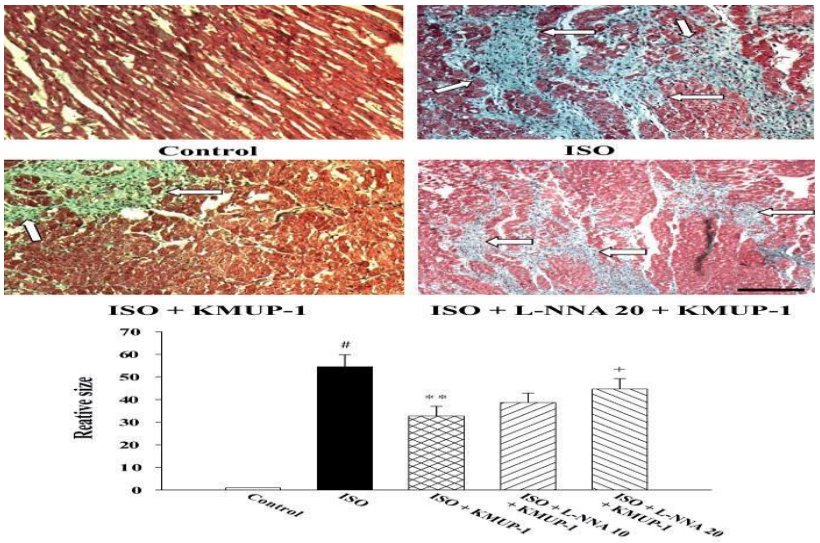

Figure 4: Effects of KMUP-1 on cardiac fibrosis. Representative photomicrographs of heart slices dyed with Masson's trichrome stain (original magnification ×100) were shown with white arrows indicating fibrotic tissue. Quantification of fibrotic area was shown in the bottom panel. Data are shown as mean ± SE. #P < 0.05 versus control group; **P < 0.01 versus ISO group; +P < 0.05 versus ISO/KMUP-1 group. The size of the bar is 100 μm. ISO, isoprenaline; L-NNA, Nω-nitro-L-arginine.

Effects of KMUP-1 on plasma NOx (nitrite/nitrate) and expression of cardiac eNOS and iNOS

Endogenous NO was found to be an important negative modulator for the hypertrophic responses. Therefore, we next investigated the effects of KMUP-1 on the endogenous NO system. As shown in Figure 3A, plasma NOx was increased by ISO when compared with control and was further increased by pretreatment with KMUP-1 when compared with the ISO group. The NOx-enhancing effect of KMUP-1 was blocked by L-NNA. The expression of eNOS and iNOS in rat hearts is shown in Figure 3B and C respectively. In heart tissues, ISO decreased eNOS expression and increased iNOS expression. However, pretreatment with KMUP-1 resulted in an up-regulation of eNOS expression and down-regulation of iNOS (P < 0.01 vs. ISO group). L-NNA significantly decreased the concentrations of both eNOS and iNOS.

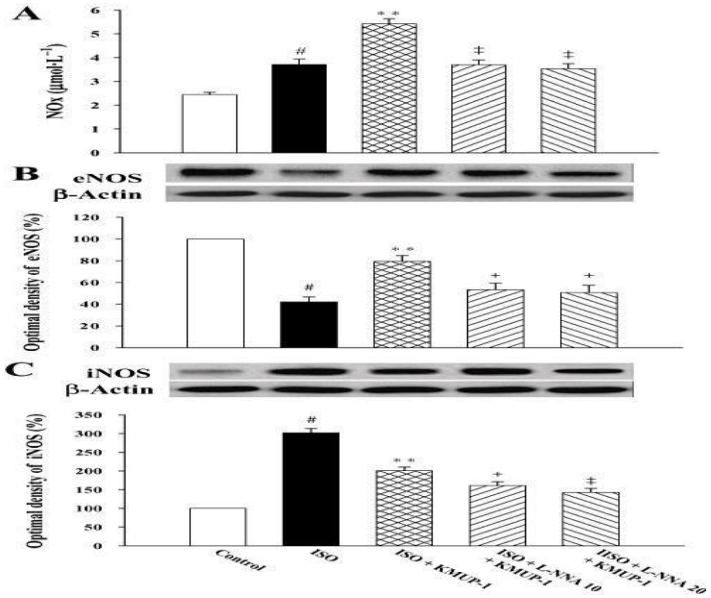

Figure 5: Effects of KMUP-1 on serum nitric oxide (NOx) content and protein expression of cardiac eNOS and iNOS. The NOx production was quantified by measurements of nitrite and nitrate in rat serum (A). Effects of KMUP-1 on eNOS (B) and iNOS (C) protein expression in rat hearts were determined by Western blot analysis and densitometry. #P < 0.05 versus control group; **P < 0.01 versus ISO group; +P < 0.05, ‡P < 0.01 versus ISO/KMUP-1 group. eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; ISO, isoprenaline; L-NNA, Nω-nitro-L-arginine.

Effects of KMUP-1 on cGMP and PKG in rat hearts

We then investigated how KMUP-1 affected myocardial cGMP and PKG, the second messenger of NO and downstream cGMP-dependent protein kinase respectively. ISO decreased cardiac cGMP and PKG concentrations as shown in Figure 4A and B. Pretreatment with KMUP-1 increased both cGMP and PKG concentrations when compared with ISO alone. These effects of KMUP-1 on cGMP and PKG were both attenuated by L-NNA.

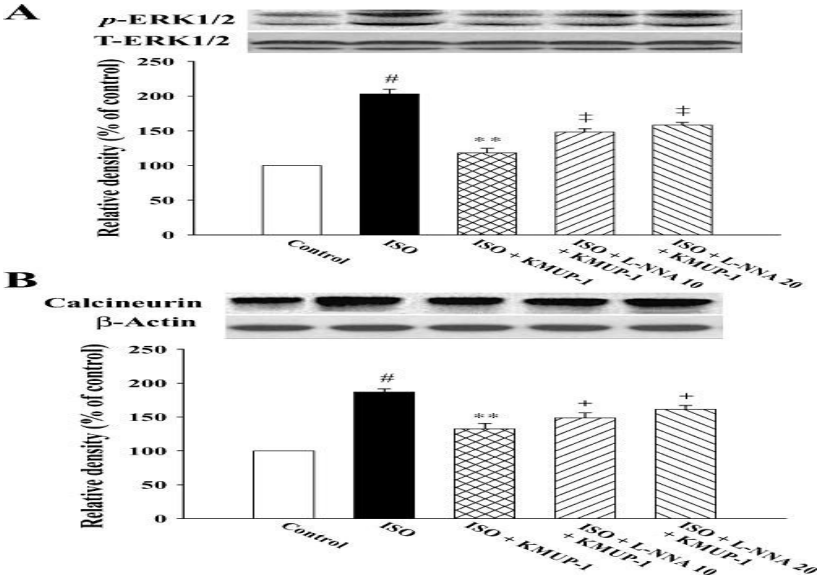

Figure : 6 Effects of KMUP-1 on the activation of calcineurin A (A) and ERK1/2 (B) protein expression in rat hearts determined by Western blot analysis and densitometry. #P < 0.05 versus control group; **P < 0.01 versus ISO group; +P < 0.05, ‡P < 0.01 versus ISO/KMUP-1 group. ERK, extracellular signal-regulated kinase; ISO, isoprenaline; L-NNA, Nω-nitro-L-arginine.

Effects of KMUP-1 on isoprenaline-induced hypertrophy of cultured neonatal cardiomyocytes

Exposure of neonatal rat ventricular cardiomyocytes to ISO (1 μmol·L−1) for 48 h resulted in a robust hypertrophic response as evidenced by significant increases in cell surface area. When KMUP-1 (0.1–10 μmol·L−1) was given 1 h before ISO treatment, the ISO- induced cellular hypertrophy was significantly inhibited in a dose- dependent manner.

Effects of KMUP-1 on activations of ERK1/2 and calcineurin A in cultured neonatal cardiomyocytes

The phosphorylation of ERK1/2 in neonatal rat cardiomyocytes was induced by ISO (1 μmol·L−1) incubation within 2 min, peaking at 5 min. Pretreatment with KMUP-1 for 1 h inhibited ISO-induced ERK1/2 phosphorylation in a concentration-dependent manner (Figure 7B). Similarly, ISO induced the expression of calcineurin A in cardiomyocytes (Figure 7C), peaking at 30 min, whereas pretreatment with KMUP-1 attenuated these effects dose-dependently.

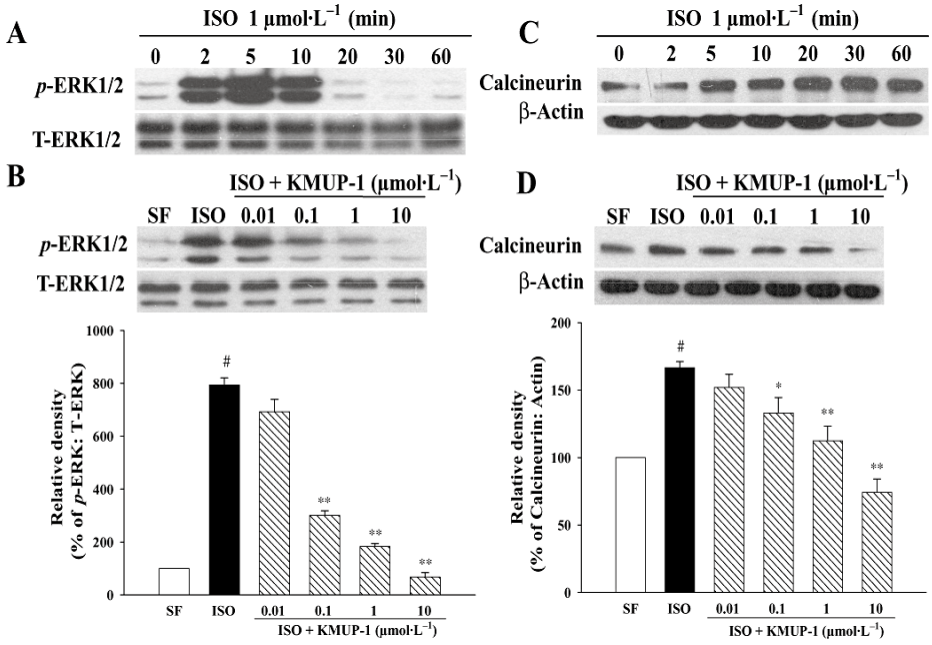

Figure: 7 Effects of KMUP-1 on ISO-induced ERK1/2 phosphorylation and calcineurin A protein expression in neonatal rat cardiomocytes. Western blots show time courses of ISO-induced ERK1/2 phosphorylation (A) and calcineurin expression (C). Quiescent neonatal

cardiomocytes were pretreated with different concentrations of KMUP-1, followed by incubation with ISO (1 μmol·L−1). KMUP-1 attenuated ISO-induced ERK1/2 phosphorylation (B) and calcineurin A activation (D). Representative Western blots and densitometric quantification of all experiments (n= 5) for each group are shown. #P < 0.05 versus serum-free (SF) group. *P < 0.05, **P < 0.01 versus ISO group. ERK, extracellular signal-regulated kinase; ISO, isoprenaline.

Effects of KMUP-1 on calcineurin activity and NFATc3 translocation in cultured neonatal cardiomyocytes

To investigate effects of KMUP-1 on ISO-induced activation of calcineurin–NFAT (nuclear factor of activated T cell) signalling further, we examined calcineurin phosphatase activity and translocation of NFATc3, an important NFAT subtype involved in hypertrophic signalling (Wilkins et al., 2002). We first found that ISO conferred a twofold increase in calcineurin phosphatase activity (Figure 8A). However, the increase was significantly attenuated by KMUP-1.

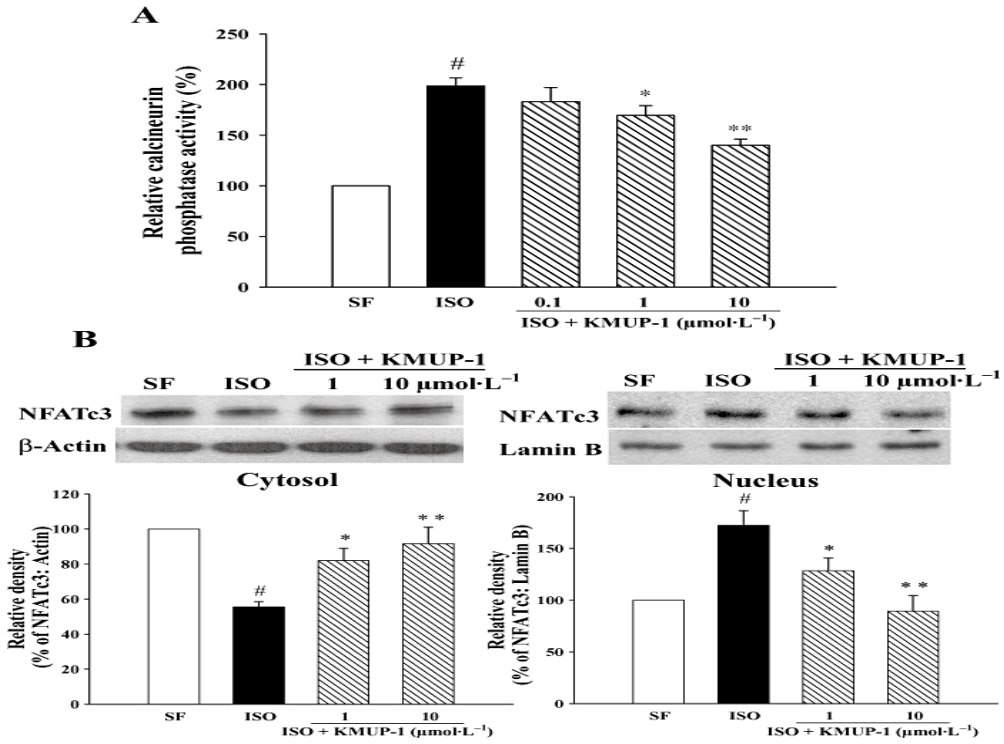

Figure:8 Effect of KMUP-1 on calcineurin activity and NFATc3 translocation in cardiomyocytes. Neonatal rat cardiomocytes were treated for 1 h in the presence or absence of KMUP-1, followed by incubation with ISO (1 μmol·L−1) for 24 h. (A) Cell lysates were subjected to calcineurin phosphatase activity assays (n= 3). (B) Immunoblotting studies on cytosolic and nuclear fractions of NFATc3 were performed separately. The quantifications of NFATc3 protein are shown in the bottom panels (n= 3). #P < 0.05 versus serum-free (SF) group. *P < 0.05, **P < 0.01 versus ISO group. ISO, isoprenaline; NFAT, nuclear factor of activated T cell.

Next, we used immunoblotting for NFATc3 using the cytosolic and nuclear fractions of cardiomyocytes independently to investigate the translocation of NFATc3 from the cytosolic compartment to the nuclear compartment (Figure 8B). Our findings show that in cardiomyocytes, ISO led to a drop in cytosolic NFATc3 protein and an increase in nuclear NFATc3 protein, indicating that ISO induces NFATc3 to translocate from the cytosol into the nucleus. In a similar vein, KMUP-1 prevented the translocation brought on by ISO.

Discussion :

The current study's findings suggest that KMUP-1, a new derivative based on xanthine, can reduce the physiological, morphological, and molecular aspects of ISO-induced heart hypertrophic responses. We showed that rats given ISO had higher survival rates when they were given KMUP-1. Furthermore, in rats given KMUP-1 beforehand, the effects of ISO-induced cardiac hypertrophy, fibrosis, and activation of hypertrophic signaling pathways (calcineurin A and ERK1/2) were all reduced. Moreover, KMUP-1 increased the NO/cGMP/PKG pathway, and NOS inhibition attenuated KMUP-1's cardioprotective effects. When combined, these findings suggest that NOS activation plays a role in mediating the anti-hypertrophic effect of KMUP-1. According to newly available data, the NO system is crucial in controlling cardiac hypertrophy [16,20]. Heart failure and NO have a complicated and contentious role in one another. The goal of the current investigation was to ascertain how KMUP-1 controls the NO system in this animal model of cardiac hypertrophy as well as the function of the NO system in this model. Our findings showed that ISO enhanced plasma NOx, an indirect measure of total endogenous NO generation. On the other hand, KMUP-1 significantly raised the plasma levels of NOx. Interestingly, we discovered that KMUP-1 counteracted the discrepancy in eNOS and iNOS expression caused by ISO in cardiac tissue by increasing eNOS and decreasing iNO expression. Furthermore, the results we obtained indicate that the primary reason of the increased endogenous generation of NO in rats with ISO-induced cardiac hypertrophy was cardiac iNOS expression, but cardiac eNOS expression may be the primary cause of the larger rise in endogenous NO production brought on by KMUP-1. The functions of both eNOS and iNOS in cardiac pathologies are intricate. Recent research has demonstrated that NO, depending on whether it comes from eNOS or iNOS, may have either positive or negative effects on the myocardium. For instance, cytokine-induced production of iNOS in chick embryonic cardiac myocytes was correlated with a transitory decrease in cytolosic calcium that resulted in a drop of basal myocardial shortening [10]. Furthermore, myocardial apoptosis was induced in vitro by iNOS activation, partially due to reactive oxygen species production [9]. In fact, transgenic mice with persistent cardiac-specific iNOS overexpression

showed elevated According to [19], there is concomitant cardiac fibrosis, hypertrophy, and dilatation along with the generation of peroxynitrite, bradyarrhythmia, and sudden death. Conversely, bradykinin-induced cardiac oxygen consumption reduction was eliminated in eNOS mutant mice [16]. Additionally, recent research revealed that decreased eNOS activity precedes the development of myocardial hypertrophy and increased cytokine expression, and that overexpression of eNOS attenuates cardiac hypertrophy in mice [20]. [25]. In line with previous investigations, our findings showed that KMUP-1 increased eNOS and decreased iNOS protein, both of which helped to lower myocardial oxygen consumption, counteract cardiac hypertrophy, and increase survival.

A substantial amount of recent research suggests that cGMP has a cardioprotective function agent that prevents hypertrophy. For instance, hypertrophy can be prevented by NO-induced cGMP through the sGC receptor [4]. Furthermore, the same anti-hypertrophic action is observed when adult cardiac myocytes are stimulated with a cGMP analogue or natriuretic pepetides [4,22]. On the other hand, cardiac fibrosis and hypertrophy were brought on by interruption of the cardiac guanylate cyclase-A gene [11]. Although the exact mechanism by which elevated cGMP production inhibits cardiac hypertrophy is yet unknown, it most likely includes the serine/threonine kinase cGMP-dependent protein kinase (PKG), which is activated by cGMP. According to [6], PKG blocks L-type Ca2+ channels, which lowers the transient amplitude of Ca2+ and prevents calcineurin-mediated activation of NFAT, a transcription factor required for hypertrophy. Thus, in the current investigation, elevated cardiac cGMP and How PKG can act as a brake against ISO-induced cardiac hypertrophy is explained by the down-regulation of calcineurin/NFAT signaling brought on by KMUP-1. Similar to what we found, it has been demonstrated that protecting effects against ISO-induced cardiac hypertrophy are conveyed by increased intracellular concentration of cGMP within cardiomyocytes generated by activation of atrial natriuretic peptide receptor in the mouse heart [31]. Interest in the possible clinical application of natriuretic peptides has grown due to the fact that cGMP is the common second messenger between NO and natriuretic peptides. Natriuretic peptides also exhibit anti-hypertrophic, vasodilatory, and diuretic effects. The US FDA has approved nesiritide, a human B-type natriuretic peptide, as the only natriuretic peptide that can be used to treat heart failure. But according to a recent meta-analysis, there might be some overlooked Aaronson and Sackner-Bernstein (2006) discuss safety concerns. Thus, as a potential superior treatment option in the future for the control of cardiac hypertrophy, medication aimed at the NO/cGMP pathway may be used. In the current investigation, we showed that L-NNA suppressed the cGMP-enhancing effect generated by KMUP-1 in a dose-dependent manner, suggesting that the NO pathway activation, rather than natriuretic peptides, mediates the action of KMUP-1 on cGMP. Like calcineurin A, ERK1/2 is crucial to the hypertrophic signaling pathway. In fact, nearly all known hypertrophic agonists cause ERK1/2 to become active [26], and this activation itself contributes to hypertrophy [3]. In this investigation, we also looked at how KMUP-1 affects ERK1/2 They discovered that KMUP-1 inhibited the activation of ERK1/2 by ISO. Nevertheless, L-NNA reduced but did not completely eliminate KMUP-1's deactivating effect on ERK1/2, which is comparable to calcineurin A. This suggests that KMUP-1 may perhaps target a different pathway besides NOS activation, and it calls for more research.

Conclusion:

Finally, KMUP-1 reduces the growth of the heart in rats that is caused by ISO. At minimum, NOS activity plays a mediating role in these outcomes. Cardiac hypertrophy and related cardiovascular morbidity may be avoided with the use of this innovative medication that inhibits the NO/cGMP pathway.

Conflict of interest:

None of our authors have conflicts of interests.

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