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ORIGINAL ARTICLE

Basic Study

# Contribution of mammalian target of rapamycin in the pathophysiology of cirrhotic cardiomyopathy

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## Abstract

**AIM:** To explore the role of mammalian target of rapamycin (mTOR) in the pathogenesis of cirrhotic cardiomyopathy and the potential of rapamycin to improve this pathologic condition.

**METHODS:** Male albino Wistar rats weighing 100-120 g were treated with tetrachloride carbon ($CCl_4$) for 8 wk to induce cirrhosis. Subsequently, animals were administered rapamycin (2 mg/kg per day). The $QT_c$ intervals were calculated in a 5-min electrocardiogram. Then, the left ventricular papillary muscles were

isolated to examine inotropic responsiveness to β-adrenergic stimulation using a standard organ bath equipped by Powerlab system. Phosphorylated-mTOR localization in left ventricles was immunohistochemically assessed, and ventricular tumor necrosis factor (TNF)-α was measured. Western blot was used to measure levels of ventricular phosphorylated-mTOR protein.

**RESULTS:** Cirrhosis was confirmed by hematoxylin and eosin staining of liver tissues, visual observation of lethargy, weight loss, jaundice, brown urine, ascites, liver stiffness, and a significant increase of spleen weight ($P < 0.001$). A significant prolongation in $QT_c$ intervals occurred in cirrhotic rats exposed to $CCl_4$ ($P < 0.001$), while this prolongation was decreased with rapamycin treatment ($P < 0.01$). $CCl_4$-induced cirrhosis caused a significant decrease of contractile responsiveness to isoproterenol stimulation and a significant increase in cardiac TNF-α. These findings were correlated with data from western blot and immunohistochemical studies on phosphorylated-mTOR expression in left ventricles. Phosphorylated-mTOR was significantly enhanced in cirrhotic rats, especially in the endothelium, compared to controls. Rapamycin treatment significantly increased contractile force and myocardial localization of phosphorylated-mTOR and decreased cardiac TNF-α concentration compared to cirrhotic rats with no treatment.

**CONCLUSION:** In this study, we demonstrated a potential role for cardiac mTOR in the pathophysiology of cirrhotic cardiomyopathy. Rapamycin normalized the inotropic effect and altered phosphorylated-mTOR expression and myocardial localization in cirrhotic rats.

**Key words:** Cirrhotic cardiomyopathy; Rat; Mammalian target of rapamycin; Rapamycin; Inotropic effect



**Core tip:** Enhanced levels of cardiac phosphorylated mammalian target of rapamycin (mTOR) contribute to impairment of electrophysiological and mechanical function induced by cirrhosis, called "cirrhotic cardiomyopathy". Here, we find that the mTOR inhibitor rapamycin normalized the impaired inotropic responsiveness to β-adrenergic stimulation and prolonged Q-T interval in tetrachloride carbon ($CCl_4$)-induced cirrhotic rats. Cardiac ventricular expression of phosphorylated-mTOR (p-mTOR) was increased in rats with cirrhosis, and this effect was ameliorated by rapamycin. $CCl_4$-induced cirrhosis was associated with an increase in cardiac proinflammatory cytokine tumor necrosis factor-α, and this increase was reversed by rapamycin as well.

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## INTRODUCTION

For a long time, cardiac dysfunction in liver cirrhosis, termed "cirrhotic cardiomyopathy", was thought to be a common occurrence in patients suffering from alcoholic cirrhosis[1,2]. During the last decade, however, non-alcoholic cirrhotic patients have also been reported to demonstrate these cardiac abnormalities[3]. Cardiovascular dysfunction is observed in cirrhosis, but the underlying mechanisms are not still well understood. Despite the hyperdynamic systemic circulation and the absence of coronary artery or valvular disease and hypertension, cardiac hypertrophy and cardiomyocyte edema are observed in cirrhotic patients[3-7]. Furthermore, there is evidence for a concomitant decrease of inotropic effect along with impaired myocardial contractility[6]. Previous studies have shown that both portal hypertension and cirrhosis contribute to cardiomyopathy[1,8]. Cardiomyopathy is characterized by latent heart failure with impaired contractile responsiveness to pharmacological or physiological stress and/or altered diastolic relaxation with electrophysiological abnormalities, without any diagnosed cardiac disease and causes of cirrhosis[4,6].

A variety of mechanisms are responsible for the pathogenesis of cirrhotic cardiomyopathy. The major predisposing factors of cardiac contractility include alteration in ventricular receptor signal transduction (*i.e.*, β-adrenergic, muscarinic, and cannabinoid receptors)[9-12] and ionic channel function (*i.e.*, $K^+$ and L-type voltage-gated $Ca^{2+}$)[13-15], cardiomyocyte plasma membrane fluidity changes[5,6], and complex alterations of carbon monoxide and nitric oxide (NO)[16,17]. Moreover, a rise in pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) is observed in this condition, resulting in stimulation of inducible nitric oxide synthase (iNOS) and NO overproduction[18].

Mammalian target of rapamycin (mTOR), a serine/threonine kinase component downstream of phosphotidylinositide 3-kinase (PI3K)/Akt signaling pathway[19,20], is a key regulator of mRNA translation and cell growth in cardiomyocytes[21,22]. Protein synthesis, a major factor for cardiac hypertrophic growth, is regulated by the PI3K/Akt/mTOR signaling pathway through inactivation of eukaryotic translation initiation factor 4E-binding proteins (4E-BPs)[23], leading to stimulation of polymerase Ⅰ and Ⅲ transcription[24], control of ribosome biogenesis and mitochondrial metabolism[25], and suppression of autophagy[26-28]. Zhang *et al*[29] found that mTOR knockout mice had improved baseline cardiomyocyte survival, decreased dilated cardiac hypertrophy, and less heart failure than control mice. Moreover, it was shown that activation of PI3K/Akt/mTOR signaling may lead to the development of cardiac

hypertrophy[30]. Indeed, the mTOR inhibitor rapamycin appeared to block the development of cardiomyocyte hypertrophy[29], and cohort studies have shown that rapamycin has cardioprotective effects in patients after liver transplantation[31,32].

Although our current knowledge of the predisposing factors of cirrhotic cardiomyopathy is somewhat understood, the role of other pathophysiological mechanisms underlying cardiac dysfunction induced by cirrhosis remains to be clarified. To this purpose, we examined the hypothesis that tetrachloride carbon ($CCl_4$)-induced cardiac inotropic dysfunction in response to adrenergic stimulation is associated with altered expression of cardiac p-mTOR in a rat model of cirrhotic cardiomyopathy. In this study, we demonstrate for the first time the positive inotropic effect of mTOR suppression by rapamycin and its ability to normalize cardiac levels of p-mTOR and the pro-inflammatory factor TNF-α in cirrhotic cardiomyopathy.

## MATERIALS AND METHODS

### *Chemicals and reagents*

The following compounds and reagents were applied in this investigation: rapamycin (Wyeth, Kildare, United Kingdom/Ireland), isoproterenol hydrochloride (Sigma, St. Louis, MO, United States), carbon tetrachloride (Merck, Darmstadt, Germany); TNF-α assay kit, polyclonal p-mTOR antibody (pSer2448), and horseradish peroxidase (HRP)-conjugated rabbit anti-rat Immunoglobulin G antibody (Biorbyt Co. Ltd., Cambridge, United Kingdom).

### *Animal model of cirrhosis*

Male albino Wistar rats weighing 100-120 g were used with housing facilities (environment temperature at 21 ℃-23 ℃, 12-h regular light/dark cycle). Animals had unlimited access to food and water except for a brief time during injection and during the surgical procedure. The rats were divided into four main groups: control/drinking water, control/rapamycin, cirrhotic/drinking water, and cirrhotic/rapamycin. All experiments and manipulations were conducted in Prof. Dehpour's Hepatological Research Laboratory in accordance with the institutional animal care and use committee (Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences) guidelines. This study was approved by the Ethics Committee of Tehran University of Medical Sciences.

To induce cirrhosis, $CCl_4$ (0.4 g/kg; a solution of 1:6 in mineral oil) was intraperitoneally injected to the animals three times a week for 8 wks until the appearance of ascites[33]. Rapamycin (2 mg/kg per day) was freshly dissolved in normal saline and daily administered in drinking water in a constant volume of 14 mL/100 g body weight during the 8-wk period[34,35]. Twenty-four hours after cessation of $CCl_4$, animals were sacrificed by guillotine decapitation. The liver was removed, sectioned, and stained with hematoxylin-eosin (HE). Light microscopy of stained liver sections confirmed the induction of cirrhosis in rats[4].

Twenty-four hours after the last administration of either $CCl_4$ or N/S, a lead Ⅱ electrocardiogram (ECG) was recorded for 15 min using three stainless steel subcutaneous electrodes attached to a bioamplifier (ADInstrument, Sydney, Australia) from the anesthetized rats. The signals were digitized at a sampling rate of 10 kHz by a Powerlab system and were displayed using Lab Chart 7 software (ADInstrument). The Q-T intervals, presented as corrected Q-T ($QT_c$), were calculated in a 5-min ECG. The $QT_c$ was presented using Bazett's formula ($QT_c = QT/\sqrt{R\text{-}R}$)[36].

### *Preparation of isolated papillary muscle*

Briefly, animals' hearts were excised following decapitation and left ventricular papillary muscles were dissected in cold oxygenated physiological salt solution (PSS) containing (in mmol/L) NaCl, 112; KCl, 5; $CaCl_2$, 1.8; $MgCl_2$, 1; $NaH_2PO_4$, 0.5; $KH_2PO_4$, 0.5; $NaHCO_3$, 25; glucose, 10; and EDTA, 0.004[37,38]. The isolated papillary muscles were suspended in a 25-mL organ bath chamber containing PSS buffer solution bubbled with a gas mixture of 95% $O_2$: 5% $CO_2$ at 37 ℃ for 90 min to reach equilibrium. The contractility was induced by electrical field stimulation (Grass 88 Stimulator; Grass Instruments, West Warwick, RI, United States) at 1 Hz and 30 V, 20% higher than the threshold. After achievement of baseline contractile force, the muscle contraction was stimulated by addition of cumulative concentrations of isoproterenol ($10^{-10}$ to $10^{-5}$ mol/L). The contractile force induced by the highest concentration of isoproterenol ($10^{-5}$ mol/L) was considered as maximal contractility[16]. The resulted contractile forces were expressed as a percentage of the baseline papillary muscle contractility.

### *Immunohistochemistry*

The ventricle samples were immediately fixed in freshly prepared 10% formalin and paraffin-embedded blocks. After deparaffinizing in xylene and rehydrating in decreasing concentrations of ethanol, 3% hydrogen peroxidase was added for 5 min to block dual endogenous peroxidase activity. Then, the immunohistochemical staining was performed based on the Avidin-Biotin peroxidase method. Polyclonal p-mTOR antibody (pSer2448) (1:50 dilution) was reacted for 1 h at room temperature followed by secondary HRP-conjugated rabbit anti-rat Immunoglobulin G antibody (1:50 dilution) for 30 min at room temperature. The sections were washed three times with Tris (pH 7.4), incubated with diaminobenzidine (DAB) solution for 10 min, and then incubated with 5% $CuSO_4$ for 5 min. Ultimately, the slides were washed and counterstained with H&E to obtain brown-colored precipitation for examination under light microscopy.

### *Ventricular TNF-α quantification*

To measure tissue TNF-α, the left ventricles were excised, rinsed in PSS, snap-frozen in liquid nitrogen, and stored at -80 ℃ for further analysis. The samples were then homogenized in ice-cold phosphate-buffered saline (PBS) and centrifuged at 14200 *g* for 30 min. Fifty microliters of the samples and standards were pipetted into a 96-well plate precoated with rat TNF-α specific antibody. Following addition of 50 μL of biotinylated anti-TNF-α solution, the plate of the enzyme linked immunosorbent assay kit was incubated for 90 min at room temperature. The wells were washed, exposed to 100 μL of streptavidin-peroxidase, incubated for 45 min at room temperature, and washed four times with PBS. Finally, 100 μL of both stabilized chromogen and stop solution were respectively added in two stages and incubated for 20 min for spectrophotometrically analysis at λ = 450 nm[16].

### *Western blot analysis*

The dissection and snap-freezing of left ventricles were performed as described in the above section. Briefly, left ventricles were homogenized in buffer (20 mmol/L Tris-HCl (pH 7.2), 0.2 mmol/L phenylmethylsulfonyl fluoride, and 1 mmol/L dithiothreitol), centrifuged at 40000 *g*, and resuspended in Tris buffer containing proteinase inhibitor. Thirty micrograms of protein samples were loaded and separated on sodium dodecyl sulfate-10% polyacrylamide gel (SDS-PAGE) by electrophoresis and were wet electroblotted onto nitrocellulose membrane at 4 ℃ for 12 h[39,40]. The blots were blocked for 1 h at room temperature with 2% bovine serum albumin in 0.1% Tween Tris-buffered saline (TBS-T) (pH 7.5). Then, the membranes were washed and incubated overnight at 4 ℃ with polyclonal p-mTOR primary antibody (pSer2448) (1:100 dilution). After washing, these blots were exposed to HRP-conjugated anti-rat secondary antibody (1:1000 dilution). Detection of blots was performed using enhanced chemiluminescence (ECL kit, Amersham, Chalfont St. Giles, United Kingdom) method. The levels of p-mTOR in cirrhotic, control, and rapamycin-treated animals were semi-quantified using ImageJ software (National Institutes of Health, Bethesda, MD, United States), which was defined as the p-mTOR/glyceraldehyde 3 phosphate dehydrogenase (GAPDH) densitometric ratio (%).

### *Statistical analysis*

All data are expressed as mean ± SD and analyzed using GraphPad Prism software (version 5.0, GraphPad Software, Inc., La Jolla, CA, United States). To examine the differences between three or more experimental groups, one-way analysis of variance (ANOVA) followed by a Tukey's post test was used. For two-group comparisons, Student's *t*-test was applied. Evaluation of the effects of two variables (cirrhosis *vs* control and type of treatment) was performed using two-way ANOVA followed by a Bonferroni post test. A value of $P < 0.05$ was considered to be statistically significant.

## RESULTS

Presence of $CCl_4$-induced cirrhosis was confirmed by visual observation of lethargy, weight loss, jaundice, brown urine, and ascites along with liver stiffness and a significant increase in spleen weight (1.52 ± 0.13 g *vs* 2.74 ± 0.41 g in control *vs* cirrhotic rats, $P < 0.001$), which contributed to the development of portal hypertension. H&E staining of liver tissues sampled from cirrhotic rats demonstrated focal hepatocellular necrosis and apoptotic cells as well as enhanced inflammatory cell infiltration into the portal tract. Fatty degeneration areas and central vein dilation were also seen histologically (Figure 1). Moreover, cirrhosis model animals had significantly prolonged $QT_c$ intervals compared to controls ($P < 0.001$; Figure 2). The prolonged $QT_c$ interval in cirrhotic rats was decreased by rapamycin (2 mg/kg) ($P < 0.01$; Figure 2).

### *Effect of rapamycin on papillary muscle contractility*

As shown in Figure 3A, baseline papillary muscle inotropic responses to isoproterenol stimulation in cirrhotic rats were significantly decreased compared to controls ($P < 0.001$). The order was in agreement with the maximum response ($R_{max}$) to isoproterenol (76.46% ± 10.08% *vs* 117.36% ± 8.25%, $P < 0.001$; Figure 3A). Rapamycin did not significantly alter $R_{max}$ in control rats. Likewise there was no significant difference in the $EC_{50}$ of isoproterenol ($4.08 \pm 1.35 \times 10^{-8}$ and $6.59 \pm 1.29 \times 10^{-8}$ in N/S- and rapamycin-treated non-cirrhotic control groups, respectively; $P > 0.05$; Figure 3B). In cirrhotic rats, there was a significant rise in papillary muscle contractility and a significant enhancement of $R_{max}$ following chronic treatment with rapamycin (2 mg/kg) compared to cirrhotic rats treated with N/S ($P < 0.001$; Figure 3C). There were no significant differences in the $EC_{50}$ of isoproterenol among all four studied groups ($P > 0.05$; Figure 3D).

### *Effect of rapamycin treatment on ventricular TNF-α concentration*

As shown in Figure 4, there was a significant increase in ventricular levels of TNF-α in cirrhotic rats compared to controls ($P < 0.001$). Treatment with rapamycin (2 mg/kg) for 8 wk caused no marked enhancement in tissue TNF-α concentration in the control group ($P > 0.05$). In addition, rapamycin significantly decreased the elevation in tissue TNF-α concentration in animals with cirrhosis ($P < 0.05$).

### *Ventricular p-mTOR expression*

As shown in Figure 5, expression of p-mTOR in the left

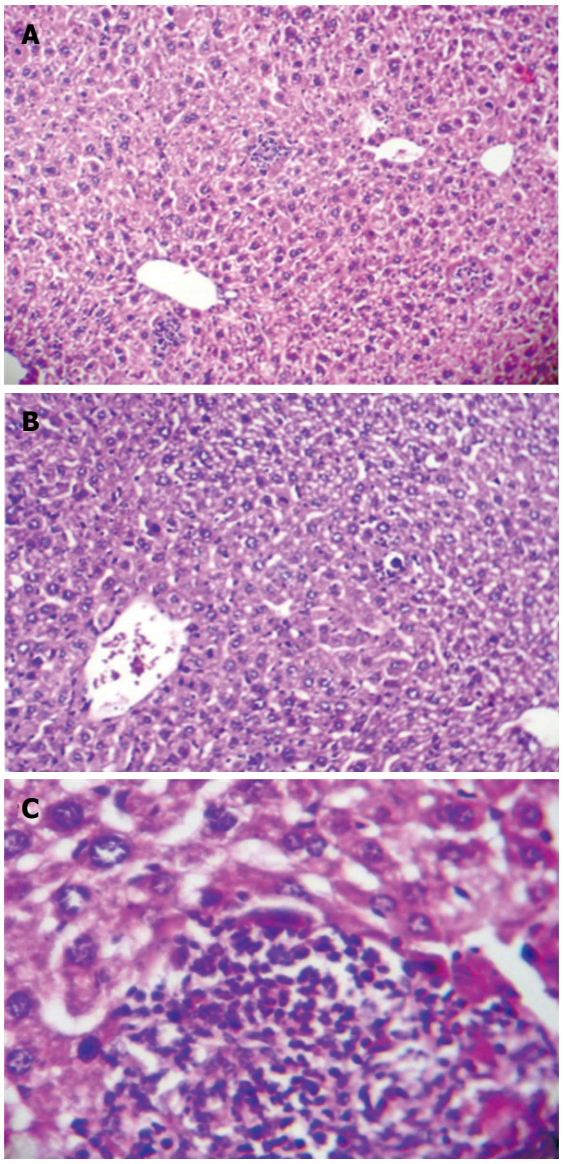


**Figure 1 Histological change in liver tissue of $CCl_4$-induced cirrhotic rats (hematoxylin and eosin; magnification × 100 and × 400).** A: Focal hepatocellular necrosis, apoptotic cells, and patchy inflammatory cell infiltration along with central vein dilation are observed; B: Fatty degeneration areas are clearly seen; C: Inflammatory cell infiltration into the portal tract.

ventricles of cirrhotic rats was increased compared to controls ($P < 0.001$). Rapamycin treatment reversed this increase in p-mTOR level in animals with cirrhosis ($P < 0.001$). Moreover, treatment of cirrhotic rats with rapamycin decreased p-mTOR protein expression to the level of control animals ($P > 0.05$).

To explore which cells express p-mTOR, immunohistochemical analysis was performed. Although almost no immunostaining was observed in the ventricular myocytes and endothelial cells in the control group (Figure 6A), p-mTOR immunostaining was markedly stronger in endothelial cells, but not in myocardial layer, of cirrhotic rats (Figure 6B). In the cirrhosis group, rapamycin could decrease p-mTOR immunostaining and induce mTOR phosphorylation in ventricular myocytes, as shown in Figure 6D.

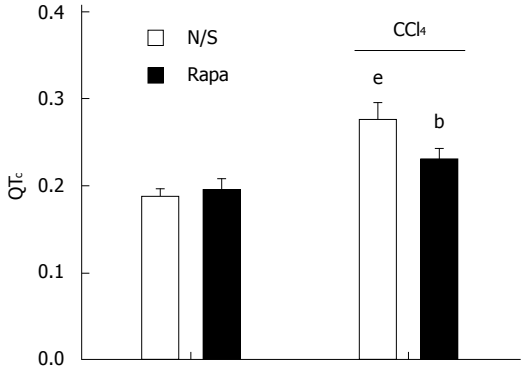


**Figure 2 QT interval in control and $CCl_4$-induced cirrhotic rats treated with normal saline or rapamycin (2 mg/kg).** QT intervals were defined as corrected QT ($QT_c$) using Bazett's formula. The data are expressed as the mean ± SD. $^eP < 0.001$ *vs* control/normal saline group; $^bP < 0.01$ *vs* control/rapamycin and cirrhotic/saline group.

## DISCUSSION

The main finding of the present study is the demonstration that cardiac mTOR expression and protein levels are increased in rats with cirrhotic cardiomyopathy. For the first time we showed that altered expression of p-mTOR in cirrhotic heart contributed to cardiac contractile suppression. This effect was confirmed by immunohistochemical assay, which showed a strong p-mTOR signal in cirrhotic left ventricles, especially in endothelial cells. Interestingly, the data from an *in vitro* papillary muscle study suggested that the enhanced expression of p-mTOR caused cardiac dysfunction. Consistent with that finding, we found a relationship between changes of mTOR activity and hypertrophic cardiomyopathy and heart failure[20,30,40-44]. Moreover, an increase in cardiac tissue TNF-α was observed in cirrhotic animals, which was accompanied by cardiomyocyte contractile dysfunction. Recently, several studies have investigated the role of TNF-α in the pathogenesis of heart failure and impaired cardiac contractility and have demonstrated that increased NO synthesis, an underlying mechanism for cirrhosis, in cardiac tissues of cirrhotic mice is attributed to elevated TNF-α level[4].

We also showed that repeated treatment with rapamycin normalized the cardiac contractile force defect in cirrhotic rats. To our knowledge, this is the first investigation to examine the hypothesis that rapamycin, *via* mTOR suppression, improves cardiac inotropic responsiveness to isoproterenol β-adrenergic stimulation and shortens the prolonged $QT_c$ in rats with cirrhosis. Since mTOR phosphorylation was not obviously detectable in ventricular cardiomyocytes taken from $CCl_4$-induced cirrhotic rats, rapamycin caused significantly greater increases in p-mTOR protein level in cardiomyocytes than endothelial cells. Interestingly, despite the abundant expression of p-mTOR in cardiomyocytes, but not in endothelial cells, of rapamycin-treated rats with cirrhosis, total

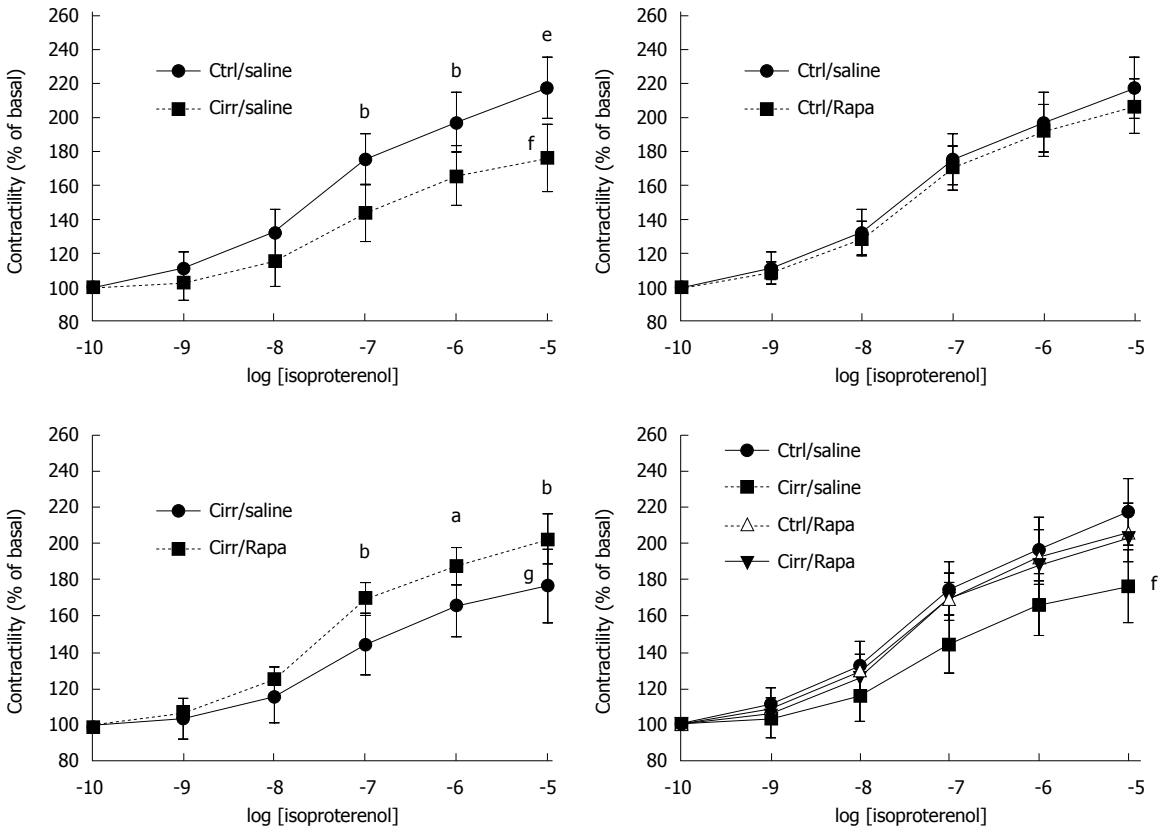


**Figure 3 Contractile force in response to β-adrenergic stimulation in cirrhotic and control rats treated with normal saline or rapamycin (2 mg/kg).** Inotropic responsiveness to β-adrenergic stimulation with isoproterenol in the isolated papillary muscle from cirrhotic and control rats treated with normal saline or rapamycin (2 mg/kg) was analyzed to determine the contractile force (% of basal). The data are expressed as the mean ± SD. Maximal response ($R_{max}$) in the $CCl_4$-induced cirrhotic rats was significantly lower than the other groups. There were no significant differences in $EC_{50}$ values among the four studied groups. $^{f}P < 0.001$ *vs* the control group receiving normal saline; $^{g}P < 0.001$ *vs* the cirrhosis group receiving normal saline; $^{a}P < 0.05$, $^{b}P < 0.01$, $^{e}P < 0.001$ *vs* the cirrhotic group receiving normal saline in that concentration.

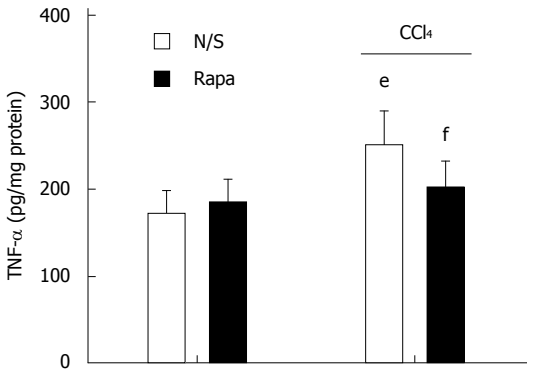


**Figure 4 Left ventricular tumor necrosis factor-α levels in control and $CCl_4$-induced cirrhotic rats treated with normal saline or rapamycin (2 mg/kg).** The data are expressed as the mean ± SD. $^{e}P < 0.001$ *vs* control/normal saline group; $^{f}P < 0.001$ *vs* cirrhosis/normal saline group. TNF-α: Tumor necrosis factor-α.

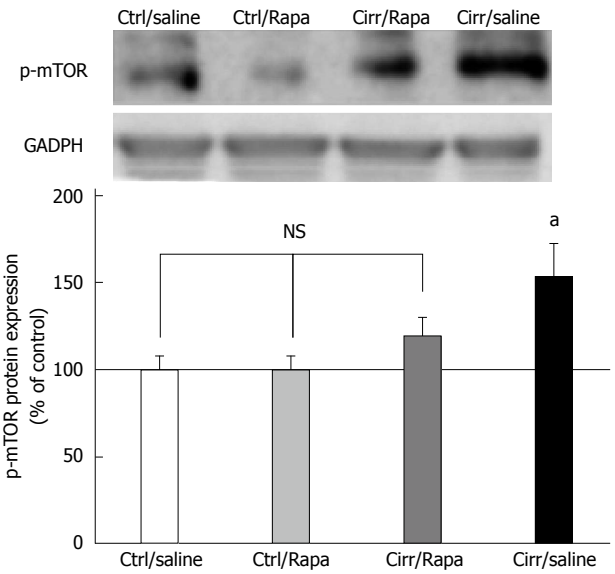


**Figure 5 Western blot analysis of p-mammalian target of rapamycin protein in the left ventricles of control and $CCl_4$-induced cirrhotic rats treated with normal saline or rapamycin (2 mg/kg).** The upper panels demonstrate the representative immunoblots of p-mTOR and glyceraldehyde 3 phosphate dehydrogenase (GAPDH) proteins in the control, control + rapamycin, cirrhotic and cirrhotic + rapamycin. The lower panel shows the densitometric analysis after normalization with GAPDH. Values are expressed as p-mTOR/GAPDH ratio (%) and normalized to the control group receiving normal saline (mean ± SD). $^{a}P < 0.05$ *vs* the other three groups; NS: Non-significant.

cardiac p-mTOR protein was reduced in comparison with cirrhotic rats receiving N/S. This finding was correlated with the positive inotropic effects of rapamycin in this paradigm. Decreased tissue levels of TNF-α after treatment with rapamycin confirmed the hypothesis that reduction in overproduced cytokines, such as TNF-α and interleukin-1β, from hepatic and systemic reticuloendothelial cells can reverse

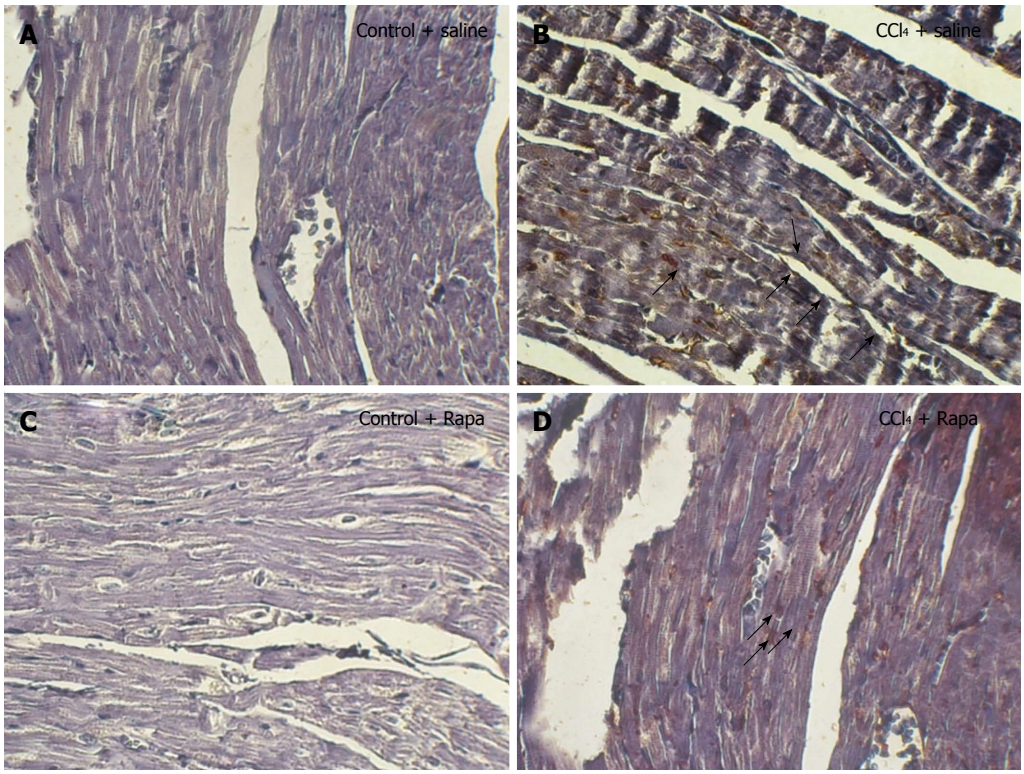


**Figure 6 Immunohistochemical staining for p-mTOR in the ventricles of the rats in the following groups: control, cirrhotic, control + rapamycin and cirrhotic + rapamycin (× 400 magnification).** Human gastric tissue was used as the positive control. Note the increased immunostaining of p-mTOR in the myocytes of the rats with cirrhosis. No significant immunostaining was localized to the cardiomyocytes of the untreated cirrhotic rats. In contrast, treatment with rapamycin caused significant immunostaining in the cardiomyocytes of the cirrhotic rats. The black arrows indicate to the p-mTOR immunoblots in rat ventricles.

their negative inotropic effects[16,45,46]. Evidence has shown that rapamycin acts as an effective agent, like isoproterenol, to raise intracellular cyclic adenosine monophosphate by reducing the expression and release of the pro-inflammatory cytokine TNF-α from human heart tissue[47]. Also, rapamycin may inhibit nuclear factor-kappa B (NFκB) activation and TNF-α, a potent inducer of in vascular smooth muscles[48].

During the last two decades, many investigations have been performed to explore the possible manifestations and potential mechanisms underlying cirrhotic cardiomyopathy. For instance, a decrease in isolated papillary muscle contractile force was observed in response to adrenergic stimulation in bile duct-ligated rats[12,36,49-51]. These results were similar to our observation that negative inotropic responsiveness to adrenergic stimulation is a result of $CCl_4$-induced cirrhosis. Although most of the studies are based on the hypothesis that defects of cardiac contractile force may result from downregulation of β-adrenergic receptors[10,37] as well as increased cardiac NO synthesis[16], we tried to investigate the role of mTOR inhibition in a rat model of cirrhosis to attenuate the impairment in cardiac contractile performance. Previous studies have reported the protective effects of rapamycin on the development of left ventricle hypertrophy and ischemia/reperfusion injury after myocardial infarction[21,22,42-44,52]. Blockade of NFκB and PI3K/Akt/mTOR signaling pathway may play an essential role in ameliorating myocardial hypertrophy induced by p70S6K, a main component downstream of mTOR, activation in the infarcted hearts[21,22,30,43]. In addition to the role of mTOR in cardiomyocyte hypertrophy, p-mTOR played a role in the impairment of cardiac survival and structure and also myocardial contractile dysfunction[53]. Inhibition of mTOR activated 4E-BP1, another downstream target of mTOR, resulting in inhibition of protein synthesis, pathogenesis of cardiomyopathy, and subsequent complications of cardiac hypertrophy[29,43].

Moreover, increment of autophagy and autophagosome formation upon mTOR inhibition with rapamycin is considered to be other protective mechanisms in heart failure[43,54]. Regarding the requirement of the ubiquitin proteasome system for activation of NFκB, rapamycin can restrict the myocardial infarction size and remodeling by inhibiting the ubiquitin proteasome and subsequent NFκB activity[43,55,56].

In addition to the observed positive effect of rapamycin on electrophysiological and mechanical cardiac function in cirrhosis, it is noteworthy that rapamycin has protective effects on human liver fibrosis and inhibits the progression of fibrosis, especially at early stages[35,57,58]. Rapamycin exerts this effect by inhibiting cell proliferation, deposition of extracellular matrix, and the profibrogenic pathway and factors[59-62]. Additionally, cohort studies have reported that patients receiving rapamycin after liver

transplantation had no cardiovascular problems. They showed that rapamycin not only did not increase the risk of congestive heart failure and myocardial infarction but plays a role as a cardioprotective agent[31,32]. In our study, the positive role of rapamycin on cirrhotic cardiomyopathy was attributed to a direct effect on cirrhotic heart, and it was assumed that a part of this phenomenon was associated with the anti-fibrogenic effect of this drug. This assumption is strongly amplified since cardiac and liver diseases share a common etiology[6]. Although experimental and clinical investigations on cirrhotic patients revealed latent heart failure with impaired response to provocations and subsequent mortality, no effective treatment has been found for improving cardiac contractility in patients with cirrhotic cardiomyopathy and evident ventricular failure[6]. As the prolongation in QT interval is considered an important life-threatening element in patients with cirrhotic cardiomyopathy, early identification and treatment of patients are necessary. Therefore, due to the anti-cytokine and beneficial role of rapamycin in correcting the abnormal cardiac contractile force and QT interval, rapamycin is expected to be the subject for further clinical investigations in patients with cirrhotic cardiomyopathy.

The present study has provided evidence that an increase in p-mTOR is responsible for the impaired cardiac contractility in animals with $CCl_4$-induced cirrhosis. Moreover, mTOR blockade corrected the cardiac contractile dysfunction in liver cirrhosis, highlighting the possible therapeutic potential for the mTOR antagonist rapamycin in this condition. This treatment may increase survival in cirrhosis-associated heart failure until a transplant becomes available. In addition, our utilization of an experimental model of cirrhotic cardiomyopathy and its translation to clinical benefits may guide future research studies.

## COMMENTS

### Background

"Cirrhotic cardiomyopathy" has been recognized as cardiac dysfunction in liver cirrhosis, which commonly occurs in patients suffering from cirrhosis. Unfortunately, the responsible mechanisms underlying the pathophysiology of cirrhotic cardiomyopathy are not well understood. Therefore, understanding these mechanisms may help to develop possible treatments for this disease.

### Research frontiers

To date, a variety of mechanisms have been described that are responsible for the pathogenesis of cirrhotic cardiomyopathy. The major predisposing factors of cardiac contractility include alterations in ventricular receptor signal transduction and ionic function, cardiomyocyte plasma membrane fluidity changes, and complex alterations in carbon monoxide and nitric oxide.

### Innovations and breakthroughs

Although the current knowledge of the mechanisms underlying cirrhotic cardiomyopathy is somewhat understood, the role of other pathophysiological mechanisms remains to be clarified. To this purpose, the authors examined the hypothesis that $CCl_4$-induced cardiac inotropic dysfunction in response to adrenergic stimulation is associated with altered expression of cardiac phosphorylated-mammalian target of rapamycin (mTOR) in a rat model of cirrhotic cardiomyopathy. Therefore, this study is the first to demonstrate the positive inotropic effect of mTOR suppression by rapamycin and its ability to normalize cardiac levels of phosphorylated-mTOR as well as the pro-inflammatory factor TNF-$\alpha$ in cirrhotic cardiomyopathy.

### Applications

mTOR blockade corrected the cardiac contractile dysfunction in liver cirrhosis, highlighting the therapeutic potential of the mTOR antagonist rapamycin in this condition. Treatment with rapamycin may increase survival in those with cirrhosis-associated heart failure until a transplant becomes available. This study may guide researches to utilize the experimental model of cirrhotic cardiomyopathy translating to clinical benefits.

### Peer-review

This is an interesting study about the role of mTOR in the pathogenesis of cirrhotic cardiomyopathy and the potential use of rapamycin for improving cardiac dysfunction.

## REFERENCES


1 **Zardi EM**, Abbate A, Zardi DM, Dobrina A, Margiotta D, Van Tassell BW, Afeltra A, Sanyal AJ. Cirrhotic cardiomyopathy. *J Am Coll Cardiol* 2010; **56**: 539-549 [PMID: 20688208 DOI: 10.1016/j.jacc.2009.12.075]

2 **Shorr E**, Zweifach BW, Furchgott RF, Baez S. Hepatorenal factors in circulatory homeostasis. IV. Tissue origins of the vasotropic principles, VEM and VDM, which appear during evolution of hemorrhagi and tourniquet shock. *Circulation* 1951; **3**: 42-79 [PMID: 14792729 DOI: 10.1161/01.CIR.3.1.42]

3 **Gould L**, Shariff M, Zahir M, Di Lieto M. Cardiac hemodynamics in alcoholic patients with chronic liver disease and a presystolic gallop. *J Clin Invest* 1969; **48**: 860-868 [PMID: 4180971 DOI: 10.1172/JCI106044]

4 **Bortoluzzi A**, Ceolotto G, Gola E, Sticca A, Bova S, Morando F, Piano S, Fasolato S, Rosi S, Gatta A, Angeli P. Positive cardiac inotropic effect of albumin infusion in rodents with cirrhosis and ascites: molecular mechanisms. *Hepatology* 2013; **57**: 266-276 [PMID: 22911662 DOI: 10.1002/hep.26021]

5 **Ma Z**, Lee SS. Cirrhotic cardiomyopathy: getting to the heart of the matter. *Hepatology* 1996; **24**: 451-459 [PMID: 8690419 DOI: 10.1002/hep.510240226]

6 **Møller S**, Henriksen JH. Cirrhotic cardiomyopathy: a pathophysiological review of circulatory dysfunction in liver disease. *Heart* 2002; **87**: 9-15 [PMID: 11751653 DOI: 10.1136/heart.87.1.9]

7 **Lunseth JH**, Olmstead EG, Abboud F. A study of heart disease in one hundred eight hospitalized patients dying with portal cirrhosis. *AMA Arch Intern Med* 1958; **102**: 405-413 [PMID: 13570726 DOI: 10.1001/archinte.1958.00030010405009]

8 **Donovan CL**, Marcovitz PA, Punch JD, Bach DS, Brown KA, Lucey MR, Armstrong WF. Two-dimensional and dobutamine stress echocardiography in the preoperative assessment of patients with end-stage liver disease prior to orthotopic liver transplantation. *Transplantation* 1996; **61**: 1180-1188 [PMID: 8610415 DOI: 10.1097/00007890-199604270-00011]

9 **Battarbee HD**, Farrar GE, Spears RP. Responses to hypotension in conscious rats with chronic portal venous hypertension. *Am J Physiol* 1990; **259**: G48-G55 [PMID: 2142583]

10 **Ma Z**, Meddings JB, Lee SS. Membrane physical properties determine cardiac beta-adrenergic receptor function in cirrhotic rats. *Am J Physiol* 1994; **267**: G87-G93 [PMID: 8048535]

11 **Jaue DN**, Ma Z, Lee SS. Cardiac muscarinic receptor function in rats with cirrhotic cardiomyopathy. *Hepatology* 1997; **25**: 1361-1365 [PMID: 9185753 DOI: 10.1002/hep.510250610]

12 **Gaskari SA**, Liu H, Moezi L, Li Y, Baik SK, Lee SS. Role of endocannabinoids in the pathogenesis of cirrhotic cardiomyopathy in bile duct-ligated rats. *Br J Pharmacol* 2005; **146**: 315-323 [PMID: 16025138 DOI: 10.1038/sj.bjp.0706331]

13 **Henriksen JH**, Fuglsang S, Bendtsen F, Christensen E, Møller S. Dyssynchronous electrical and mechanical systole in patients with

cirrhosis. *J Hepatol* 2002; **36**: 513-520 [PMID: 11943423 DOI: 10.1016/S0168-8278(02)00010-7]
14 **Ward CA**, Ma Z, Lee SS, Giles WR. Potassium currents in atrial and ventricular myocytes from a rat model of cirrhosis. *Am J Physiol* 1997; **273**: G537-G544 [PMID: 9277435]
15 **Ward CA**, Liu H, Lee SS. Altered cellular calcium regulatory systems in a rat model of cirrhotic cardiomyopathy. *Gastroenterology* 2001; **121**: 1209-1218 [PMID: 11677214 DOI: 10.1053/gast.2001.28653]
16 **Liu H**, Ma Z, Lee SS. Contribution of nitric oxide to the pathogenesis of cirrhotic cardiomyopathy in bile duct-ligated rats. *Gastroenterology* 2000; **118**: 937-944 [PMID: 10784593 DOI: 10.1016/S0016-5085(00)70180-6]
17 **Liu H**, Song D, Lee SS. Role of heme oxygenase-carbon monoxide pathway in pathogenesis of cirrhotic cardiomyopathy in the rat. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G68-G74 [PMID: 11123199]
18 **Kumar A**, Paladugu B, Mensing J, Kumar A, Parrillo JE. Nitric oxide-dependent and -independent mechanisms are involved in TNF-alpha -induced depression of cardiac myocyte contractility. *Am J Physiol Regul Integr Comp Physiol* 2007; **292**: R1900-R1906 [PMID: 17234961 DOI: 10.1152/ajpregu.00146.2006]
19 **Dazert E**, Hall MN. mTOR signaling in disease. *Curr Opin Cell Biol* 2011; **23**: 744-755 [PMID: 21963299 DOI: 10.1016/j.ceb.2011.09.003]
20 **Laplante M**, Sabatini DM. mTOR signaling in growth control and disease. *Cell* 2012; **149**: 274-293 [PMID: 22500797 DOI: 10.1016/j.cell.2012.03.017]
21 **McMullen JR**, Sherwood MC, Tarnavski O, Zhang L, Dorfman AL, Shioi T, Izumo S. Inhibition of mTOR signaling with rapamycin regresses established cardiac hypertrophy induced by pressure overload. *Circulation* 2004; **109**: 3050-3055 [PMID: 15184287 DOI: 10.1161/01.CIR.0000130641.08705.45]
22 **Shioi T**, McMullen JR, Tarnavski O, Converso K, Sherwood MC, Manning WJ, Izumo S. Rapamycin attenuates load-induced cardiac hypertrophy in mice. *Circulation* 2003; **107**: 1664-1670 [PMID: 12668503 DOI: 10.1161/01.CIR.0000057979.36322.88]
23 **Gingras AC**, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, Aebersold R, Sonenberg N. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. *Genes Dev* 1999; **13**: 1422-1437 [PMID: 10364159 DOI: 10.1101/gad.13.11.1422]
24 **Kantidakis T**, Ramsbottom BA, Birch JL, Dowding SN, White RJ. mTOR associates with TFIIIC, is found at tRNA and 5S rRNA genes, and targets their repressor Maf1. *Proc Natl Acad Sci USA* 2010; **107**: 11823-11828 [PMID: 20543138 DOI: 10.1073/pnas.1005188107]
25 **Cunningham JT**, Rodgers JT, Arlow DH, Vazquez F, Mootha VK, Puigserver P. mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. *Nature* 2007; **450**: 736-740 [PMID: 18046414 DOI: 10.1038/nature06322]
26 **Chan EY**. mTORC1 phosphorylates the ULK1-mAtg13-FIP200 autophagy regulatory complex. *Sci Signal* 2009; **2**: pe51 [PMID: 19690328 DOI: 10.1126/scisignal.284pe51]
27 **Shioi T**, Kang PM, Douglas PS, Hampe J, Yballe CM, Lawitts J, Cantley LC, Izumo S. The conserved phosphoinositide 3-kinase pathway determines heart size in mice. *EMBO J* 2000; **19**: 2537-2548 [PMID: 10835352 DOI: 10.1093/emboj/19.11.2537]
28 **Shioi T**, McMullen JR, Kang PM, Douglas PS, Obata T, Franke TF, Cantley LC, Izumo S. Akt/protein kinase B promotes organ growth in transgenic mice. *Mol Cell Biol* 2002; **22**: 2799-2809 [PMID: 11909972 DOI: 10.1128/MCB.22.8.2799-2809.2002]
29 **Zhang D**, Contu R, Latronico MV, Zhang J, Rizzi R, Catalucci D, Miyamoto S, Huang K, Ceci M, Gu Y, Dalton ND, Peterson KL, Guan KL, Brown JH, Chen J, Sonenberg N, Condorelli G. MTORC1 regulates cardiac function and myocyte survival through 4E-BP1 inhibition in mice. *J Clin Invest* 2010; **120**: 2805-2816 [PMID: 20644257 DOI: 10.1172/JCI43008]
30 **Ha T**, Li Y, Gao X, McMullen JR, Shioi T, Izumo S, Kelley JL, Zhao A, Haddad GE, Williams DL, Browder IW, Kao RL, Li C. Attenuation of cardiac hypertrophy by inhibiting both mTOR and NFkappaB activation in vivo. *Free Radic Biol Med* 2005; **39**: 1570-1580 [PMID: 16298682 DOI: 10.1016/j.freeradbiomed.2005.08.002]
31 **Weick A**, Chacra W, Kuchipudi A, Elbatta M, Divine G, Burmeister C, Moonka D. Incidence of cardiovascular and cerebrovascular events associated with sirolimus use after liver transplantation. *Transplant Proc* 2015; **47**: 460-464 [PMID: 25769591 DOI: 10.1016/j.transproceed.2014.11.036]
32 **McKenna GJ**, Trotter JF, Klintmalm E, Ruiz R, Onaca N, Testa G, Saracino G, Levy MF, Goldstein RM, Klintmalm GB. Sirolimus and cardiovascular disease risk in liver transplantation. *Transplantation* 2013; **95**: 215-221 [PMID: 23232369 DOI: 10.1097/TP.0b013e318279090c]
33 **Pérez-Vargas JE**, Zarco N, Vergara P, Shibayama M, Segovia J, Tsutsumi V, Muriel P. l-Theanine prevents carbon tetrachloride-induced liver fibrosis via inhibition of nuclear factor κB and down-regulation of transforming growth factor β and connective tissue growth factor. *Hum Exp Toxicol* 2016; **35**: 135-146 [PMID: 25852135 DOI: 10.1177/0960327115578864]
34 **Parada B**, Reis F, Figueiredo A, Nunes P, Teixeira-Lemos E, Garrido P, Sereno J, Pinto R, Cunha MF, Neto P, Santos P, Velada I, Mota A, Teixeira F. Inhibition of bladder tumour growth by sirolimus in an experimental carcinogenesis model. *BJU Int* 2011; **107**: 135-143 [PMID: 20367636 DOI: 10.1111/j.1464-410X.2010.09326.x]
35 **Kim YJ**, Lee ES, Kim SH, Lee HY, Noh SM, Kang DY, Lee BS. Inhibitory effects of rapamycin on the different stages of hepatic fibrosis. *World J Gastroenterol* 2014; **20**: 7452-7460 [PMID: 24966615 DOI: 10.3748/wjg.v20.i23.7452]
36 **Jazaeri F**, Tavangar SM, Ghazi-Khansari M, Khorramizadeh MR, Mani AR, Dehpour AR. Cirrhosis is associated with development of tolerance to cardiac chronotropic effect of endotoxin in rats. *Liver Int* 2013; **33**: 368-374 [PMID: 23311391 DOI: 10.1111/liv.12039]
37 **Ma Z**, Miyamoto A, Lee SS. Role of altered beta-adrenoceptor signal transduction in the pathogenesis of cirrhotic cardiomyopathy in rats. *Gastroenterology* 1996; **110**: 1191-1198 [PMID: 8613009 DOI: 10.1053/gast.1996.v110.pm8613009]
38 **Otani H**, Otani H, Das DK. Alpha 1-adrenoceptor-mediated phosphoinositide breakdown and inotropic response in rat left ventricular papillary muscles. *Circ Res* 1988; **62**: 8-17 [PMID: 2826043 DOI: 10.1161/01.RES.62.1.8]
39 **Markwell MA**, Haas SM, Bieber LL, Tolbert NE. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal Biochem* 1978; **87**: 206-210 [PMID: 98070 DOI: 10.1016/0003-2697(78)90586-9]
40 **Festuccia WT**, Laplante M, Brûlé S, Houde VP, Achouba A, Lachance D, Pedrosa ML, Silva ME, Guerra-Sá R, Couet J, Arsenault M, Marette A, Deshaies Y. Rosiglitazone-induced heart remodelling is associated with enhanced turnover of myofibrillar protein and mTOR activation. *J Mol Cell Cardiol* 2009; **47**: 85-95 [PMID: 19397913 DOI: 10.1016/j.yjmcc.2009.04.011]
41 **Lassaletta AD**, Elmadhun NY, Zanetti AV, Feng J, Anduaga J, Gohh RY, Sellke FW, Bianchi C. Rapamycin treatment of healthy pigs subjected to acute myocardial ischemia-reperfusion injury attenuates cardiac functions and increases myocardial necrosis. *Ann Thorac Surg* 2014; **97**: 901-907 [PMID: 24266948 DOI: 10.1016/j.athoracsur.2013.09.059]
42 **Das A**, Salloum FN, Durrant D, Ockaili R, Kukreja RC. Rapamycin protects against myocardial ischemia-reperfusion injury through JAK2-STAT3 signaling pathway. *J Mol Cell Cardiol* 2012; **53**: 858-869 [PMID: 22999860 DOI: 10.1016/j.yjmcc.2012.09.007]
43 **Buss SJ**, Muenz S, Riffel JH, Malekar P, Hagenmueller M, Weiss CS, Bea F, Bekeredjian R, Schinke-Braun M, Izumo S, Katus HA, Hardt SE. Beneficial effects of Mammalian target of rapamycin inhibition on left ventricular remodeling after myocardial infarction. *J Am Coll Cardiol* 2009; **54**: 2435-2446 [PMID: 20082935 DOI: 10.1016/j.jacc.2009.08.031]
44 **Proud CG**. Ras, PI3-kinase and mTOR signaling in cardiac hypertrophy. *Cardiovasc Res* 2004; **63**: 403-413 [PMID: 15276465 DOI: 10.1016/j.cardiores.2004.02.003]

45 **Tilg H**, Wilmer A, Vogel W, Herold M, Nölchen B, Judmaier G, Huber C. Serum levels of cytokines in chronic liver diseases. *Gastroenterology* 1992; **103**: 264-274 [PMID: 1612333]

46 **Napoli J**, Bishop GA, McCaughan GW. Increased intrahepatic messenger RNA expression of interleukins 2, 6, and 8 in human cirrhosis. *Gastroenterology* 1994; **107**: 789-798 [PMID: 8076766 DOI: 10.1016/0016-5085(94)90128-7]

47 **Adkins JR**, Castresana MR, Wang Z, Newman WH. Rapamycin inhibits release of tumor necrosis factor-alpha from human vascular smooth muscle cells. *Am Surg* 2004; **70**: 384-387; discussion 384-387 [PMID: 15156944]

48 **Giordano A**, Avellino R, Ferraro P, Romano S, Corcione N, Romano MF. Rapamycin antagonizes NF-kappaB nuclear translocation activated by TNF-alpha in primary vascular smooth muscle cells and enhances apoptosis. *Am J Physiol Heart Circ Physiol* 2006; **290**: H2459-H2465 [PMID: 16428340 DOI: 10.1152/ajpheart.00750.2005]

49 **Møller S**, Henriksen JH. Cirrhotic cardiomyopathy. *J Hepatol* 2010; **53**: 179-190 [PMID: 20462649 DOI: 10.1016/j.jhep.2010.02.023]

50 **Mani AR**, Ippolito S, Ollosson R, Moore KP. Nitration of cardiac proteins is associated with abnormal cardiac chronotropic responses in rats with biliary cirrhosis. *Hepatology* 2006; **43**: 847-856 [PMID: 16557556 DOI: 10.1002/hep.21115]

51 **Inserte J**, Perelló A, Agulló L, Ruiz-Meana M, Schlüter KD, Escalona N, Graupera M, Bosch J, Garcia-Dorado D. Left ventricular hypertrophy in rats with biliary cirrhosis. *Hepatology* 2003; **38**: 589-598 [PMID: 12939585 DOI: 10.1053/jhep.2003.50369]

52 **Khan S**, Salloum F, Das A, Xi L, Vetrovec GW, Kukreja RC. Rapamycin confers preconditioning-like protection against ischemia-reperfusion injury in isolated mouse heart and cardiomyocytes. *J Mol Cell Cardiol* 2006; **41**: 256-264 [PMID: 16769083 DOI: 10.1016/j.yjmcc.2006.04.014]

53 **Zhang B**, Turdi S, Li Q, Lopez FL, Eason AR, Anversa P, Ren J. Cardiac overexpression of insulin-like growth factor 1 attenuates chronic alcohol intake-induced myocardial contractile dysfunction but not hypertrophy: Roles of Akt, mTOR, GSK3beta, and PTEN. *Free Radic Biol Med* 2010; **49**: 1238-1253 [PMID: 20678571 DOI: 10.1016/j.freeradbiomed.2010.07.020]

54 **Nakai A**, Yamaguchi O, Takeda T, Higuchi Y, Hikoso S, Taniike M, Omiya S, Mizote I, Matsumura Y, Asahi M, Nishida K, Hori M, Mizushima N, Otsu K. The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress. *Nat Med* 2007; **13**: 619-624 [PMID: 17450150 DOI: 10.1038/nm1574]

55 **Palombella VJ**, Rando OJ, Goldberg AL, Maniatis T. The ubiquitin-proteasome pathway is required for processing the NF-kappa B1 precursor protein and the activation of NF-kappa B. *Cell* 1994; **78**: 773-785 [PMID: 8087845 DOI: 10.1016/S0092-8674(94)90482-0]

56 **Pye J**, Ardeshirpour F, McCain A, Bellinger DA, Merricks E, Adams J, Elliott PJ, Pien C, Fischer TH, Baldwin AS, Nichols TC. Proteasome inhibition ablates activation of NF-kappa B in myocardial reperfusion and reduces reperfusion injury. *Am J Physiol Heart Circ Physiol* 2003; **284**: H919-H926 [PMID: 12424098 DOI: 10.1152/ajpheart.00851.2002]

57 **Biecker E**, De Gottardi A, Neef M, Unternährer M, Schneider V, Ledermann M, Sägesser H, Shaw S, Reichen J. Long-term treatment of bile duct-ligated rats with rapamycin (sirolimus) significantly attenuates liver fibrosis: analysis of the underlying mechanisms. *J Pharmacol Exp Ther* 2005; **313**: 952-961 [PMID: 15769867 DOI: 10.1124/jpet.104.079616]

58 **Bridle KR**, Popa C, Morgan ML, Sobbe AL, Clouston AD, Fletcher LM, Crawford DH. Rapamycin inhibits hepatic fibrosis in rats by attenuating multiple profibrogenic pathways. *Liver Transpl* 2009; **15**: 1315-1324 [PMID: 19790156 DOI: 10.1002/lt.21804]

59 **Neef M**, Ledermann M, Saegesser H, Schneider V, Reichen J. Low-dose oral rapamycin treatment reduces fibrogenesis, improves liver function, and prolongs survival in rats with established liver cirrhosis. *J Hepatol* 2006; **45**: 786-796 [PMID: 17050028 DOI: 10.1016/j.jhep.2006.07.030]

60 **Zhu J**, Wu J, Frizell E, Liu SL, Bashey R, Rubin R, Norton P, Zern MA. Rapamycin inhibits hepatic stellate cell proliferation in vitro and limits fibrogenesis in an in vivo model of liver fibrosis. *Gastroenterology* 1999; **117**: 1198-1204 [PMID: 10535884 DOI: 10.1016/S0016-5085(99)70406-3]

61 **Gäbele E**, Reif S, Tsukada S, Bataller R, Yata Y, Morris T, Schrum LW, Brenner DA, Rippe RA. The role of p70S6K in hepatic stellate cell collagen gene expression and cell proliferation. *J Biol Chem* 2005; **280**: 13374-13382 [PMID: 15677443 DOI: 10.1074/jbc.M409444200]

62 **McKenna GJ**, Trotter JF, Klintmalm E, Onaca N, Ruiz R, Jennings LW, Neri M, O'Leary JG, Davis GL, Levy MF, Goldstein RM, Klintmalm GB. Limiting hepatitis C virus progression in liver transplant recipients using sirolimus-based immunosuppression. *Am J Transplant* 2011; **11**: 2379-2387 [PMID: 21967703 DOI: 10.1111/j.1600-6143.2011.03767.x]


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