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PRECLINICAL STUDY

Progression of Heart Failure Was Suppressed by Inhibition of Apoptosis Signal-Regulating Kinase 1 Via Transcoronary Gene Transfer

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Objectives We examined whether the inhibition of apoptosis signal-regulating kinase 1 (ASK1) would attenuate the progression of heart failure in TO-2 hamsters with hereditary dilated cardiomyopathy.

Background Heart failure remains the leading cause of mortality and requires novel therapies targeting the biologically relevant processes within cardiomyocytes that lead to cell death. Apoptosis signal-regulating kinase 1 is a key signaling molecule for cardiomyocyte death.

Methods We generated recombinant adeno-associated virus (rAAV) expressing an N-terminal truncated form of the dominant-negative mutant of ASK1 (ASKΔN(KR)). TO-2 hamsters were subjected to an in vivo rAAV transcoronary transfer.

Results ASKΔN(KR) retained its dominant-negative activity in vitro. The rAAV expressing ASKΔN(KR) treatment inhibited ASK1 activation in the hamster hearts and suppressed progression of ventricular remodeling such as chamber dilation, impairment of contractile and relaxation functions, and fibrosis. Inhibition of ASK1 reduced the number of apoptotic cells and selectively attenuated c-Jun NH_2-terminal kinase activation. Although the deficiency of δ-sarcoglycan, a genetic defect in the hamster, leads to the degradation of dystrophin, the treatment significantly protected hearts from this degradation, probably by inhibiting calpain activation.

Conclusions Apoptosis signal-regulating kinase 1 is involved in the pathogenesis of heart failure progression, mediated through c-Jun NH_2-terminal kinase-mediated apoptosis and calpain-dependent dystrophin cleavage, and may be a therapeutic target to treat patients with heart failure. (J Am Coll Cardiol 2007;50:453–62) © 2007 by the American College of Cardiology Foundation

Left ventricular (LV) remodeling is generally accepted as a determinant of the clinical course of heart failure (1). Neurohumoral factors and inflammatory cytokines, which stimulate membrane-bound receptors and downstream multiple cytoplasmic signal transduction cascades, play an important role in the onset and progression of ventricular remodeling (1,2). Despite advances in pharmacological treatments targeting the membrane-bound receptors, prognosis for heart failure, especially severe heart failure, remains poor. A novel and effective drug targeting the cytoplasmic signal transduction cascades that lead to unfavorable cardiac remodeling is necessary to treat patients with heart failure.

Apoptosis signal-regulating kinase 1 (ASK1) is a mitogen-activated protein (MAP) kinase kinase kinase that activates MAP kinase kinase (MKK) 4/7-c-Jun NH_2-terminal kinase (JNK) and MKK3/6-p38 MAP kinase (3,4). We have

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Abbreviations and Acronyms

ANOVA = analysis of variance
ASK1 = apoptosis signal-regulating kinase 1
ERK = extracellular signal-regulated kinase
JNK = c-Jun NH_2-terminal kinase
LV = left ventricular
LVPWT = left ventricular posterior wall
MAP = mitogen-activated protein
MKK = mitogen-activated protein kinase kinase
rAAV = recombinant adeno-associated virus
TUNEL = terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling

previously reported that ASK1 is activated in pressure overloaded and postinfarct mouse hearts (5) and that the activation leads to not only apoptosis but also necrosis of cardiomyocytes depending on the stress conditions (5,6). Apoptosis signal-regulating kinase 1 knockout mice showed attenuated cardiac remodeling, suggesting that inhibition of ASK1 is beneficial for preventing heart failure (5). However, it remains unclear whether ASK1 inhibition after onset of the disease is therapeutically efficacious for preventing progression of cardiac remodeling and heart failure.

The TO-2 cardiomyopathic hamster is a widely used experimental model of progressive heart failure. The hamster possesses a mutation in the δ-sarcoglycan gene, a component of dystrophin associated protein complex (7), which also has been found in some human patients with dilated cardiomyopathy (8). Deficiency of δ-sarcoglycan causes a concomitant loss of the other sarcoglycan subunits (α, β, γ) and reduced expression level of the dystrophin-glycoprotein complex, leading to mechanical instability of the sarcolemmal membrane in the heart and skeletal muscles (9,10). The disruption of the dystrophin-sarcoglycan complex causes increased calcium influx and chronic elevation of intracellular calcium, resulting in cell damage in the δ-sarcoglycan-deficient hamsters (11). Abnormality in dystrophin is observed in human dilated or ischemic cardiomyopathic hearts (12).

In this study, we examined whether chronic inhibition of ASK1 activation by transcoronary gene transfer using recombinant adeno-associated virus (rAAV) (13) could attenuate progression of cardiac remodeling in TO-2 cardiomyopathic hamsters. ASK1 inhibition was remarkably effective for preventing progression of cardiac remodeling and heart failure. Thus, suppression of ASK1 may constitute a novel therapeutic strategy for the treatment of patients with heart failure.

Methods

Animals. Male F1B (normal hamster) and TO-2 hamster strains were obtained from Bio Breeders (Watertown, Massachusetts). All animal protocols were approved by the Yamaguchi University School of Medicine Animal Subject Committee.

Virus vectors. Recombinant adeno-associated virus type-2 was generated by 3 plasmid cotransfection method. ASKΔN(KR) was a 5′-deletion mutant of ASK(KR) starting at 1,945 bp downstream of the translation initiation site. Recombinant adenovirus vectors were constructed using ViraPower Adenoviral Expression System (Invitrogen, Carlsbad, California).

Animal procedures and hemodynamic measurement. Recombinant adeno-associated virus was delivered into the coronary arteries of 10-week-old TO-2 hamsters following a previously described protocol (13). A total of 1 to 4×10^{11} viral particles per 100 g of body weight was injected through the transcoronary route. Hamsters were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally), followed by echocardiographic analysis with an HDI-5000 ultrasound machine (Philips, Eindhoven, the Netherlands) equipped with a 15-MHz linear probe. Hamsters were underwent LV pressure measurement as previously described (13).

Evaluation of apoptosis. Triple staining with the terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) assay was performed using an in situ apoptosis detection kit (Takara, Otsu, Japan). The cells undergoing apoptosis were labeled with fluorescein-dUTP and observed under a confocal fluorescence microscope. Immunohistochemical staining using anti-activated caspase 3 antibody (Abcam Inc., Cambridge, Massachusetts) was performed with paraffin-embedded sections.

Immune complex kinase assay and Western blots. The activity of ASK1 or MKK6 was measured with an immune complex kinase assay as previously described (4). Protein homogenates (60 μg/lane) were subjected to Western blot analysis using the antibodies against JNK, p38 and extracellular signal-regulated kinase (ERK) (Santa Cruz Biotechnology, Santa Cruz, California), phospho-p38, phospho-JNK, and phosho-ERK (Cell Signaling Technology, Beverly, Massachusetts), α-, β-, and γ-sarcoglycans, and dystrophin (rod domain; Novocastra, Newcastle, United Kingdom).

Measurement of calpain activity and intracellular calcium level. Rat neonatal ventricular cardiomyocytes were prepared as previously reported (4) and recombinant adenovirus vectors expressing ASKΔN(KR) (AdV/ASKΔN(KR)) or LacZ (AdV/LacZ) were infected at a multiplicity of infection of 25. Twenty-four hours after adenovirus infection, intracellular calpain proteolytic activity was estimated by t-butoxycarbonyl-leucyl-methionine-7-amino-4-chloromethylcoumarin (Boc-Leu-Met-CMAC) (Molecular Probes, Eugene, Oregon) as we previously reported (14) using an excitation wavelength of 340 nm and an emission wavelength of 430 nm. Calcium overload was induced by changing an incubation solution to a sodium-free one. We measured at least 3 cardiomyocytes in each experiment. To measure intracellular calcium levels, 4 μmol/l fura-2 AM (Molecular Probes) was loaded into cardiomyocytes for 10 min. The fluorescent light emitted at 510 nm and excited at 340 and 380 nm after the change to the sodium-free solution was measured, and $R_{340/380}$ was calculated.