行政院國家科學委員會專題研究計畫成果報告
一氧化氮合成酵素及過氧化氫酵素基因經由腺病毒媒介傳送入心臟細胞對內皮素所誘發細胞肥大的抑制作用
Inhibitory effects of adenovirus-mediated nitric oxide synthase and catalase genes transfer on rat cardiomyocyte hypertrophy induced by endothelin-1

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一、中文摘要

大多數心血管方面的疾病，都會造成心臟的肥大，初期的心臟肥大可以代償心臟功能，然而持續性的心臟肥大，便會導致心臟的衰竭。在心臟細胞，由於機械應力或內生性生長因子的刺激作用下，會造成心臟細胞的肥大，心臟細胞的過度肥大有可能導致心臟機能受損，進一步引起心臟衰竭。內皮素(endothelin-1; ET-1)有造成細胞肥大的作用，但對於此作用其細胞內的機轉目前還不是很清楚，已知內皮素可增加許多迅即基因如 c-fos、c-jun 及 egr-1 等的基因表現；另外，對一些與心臟細胞的增大有關的基因如心房利鈉(atrial natriuretic peptide)、肌凝蛋白重鏈(myosin heavy chain)、骨骼肌肉肌動蛋白(actin)等，也有增加它們基因表現的作用。近來有許多研究報告指出活性氧族群(reactive oxygen species ; ROS)及一氧化氮(nitric oxide; NO)可於細胞內扮演一訊號傳遞者的角色，所以在本研究中，我們進一步觀察一氧化氮合成酵素及過氧化氫酵素基因經由腺病毒媒介傳送入心臟細胞是否對內皮素所誘發細胞肥大產生抑制的作用，並進而闡釋其可能的細胞內機轉。由本報告一氧化氮合成酵素基因經由腺病毒媒介傳送入心臟細胞可抑制內皮素所誘發心臟細胞肥大，可推測一氧化氮合成酵素基因經由腺病毒媒介傳送入心臟細胞，在臨床上相關心臟肥大或心臟衰竭疾病的預防治療上具有運用潛力。

關鍵詞：一氧化氮、基因傳送、內皮一氧化氮合成酵素、腺病毒、心臟細胞、細胞肥大

Abstract

Cardiomyocyte hypertrophy play a transition step in the pathogenesis of heart failure. The objectives of this study were to determine whether transfer of recombinant endothelial nitric oxide synthase (eNOS) gene to neonatal rat cardiomyocytes would result in expression of a functional enzyme and to assess the effect of expression of eNOS on cardiomyocyte hypertrophy. Cardiomyocytes were transduced in vitro with adenoviral vectors encoding cDNA for eNOS (AdeNOS) and PGK empty vector. In contrast to AdPGK-transduced cells, cardiomyocytes transduced with AdeNOS increased calcium-dependent NOS activity (measured by the conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline) and produced increased amounts of nitrite. Cardiomyocytes transduced with AdeNOS showed diminished endothelin-1 (ET-1)-stimulated protein synthesis as measured by [^{3}H]-leucine uptake. The present study demonstrates that adenovirus-mediated gene transfer of eNOS to cardiomyocytes results in the expression of a functional enzyme. Expression of recombinant eNOS in cardiomyocytes results in inhibition of ET-1-stimulated protein synthesis. These findings imply that eNOS gene transfer to cardiomyocytes may be a unique mode of increasing local NO production in the heart.

Keywords: nitric oxide • gene transfer • endothelial nitric oxide synthase • adenovirus • cardiomyocyte • hypertrophy

二、緣由與目的

Nitric oxide (NO), a redox-active molecule, has been identified as an important potential regulator of certain signaling events. NO acts by stimulating soluble guanylate cyclase, leading to enhanced production of intracellular cyclic GMP, an intracellular second messenger that can activate cyclic GMP-dependent protein kinases (Schmidt, 1993). NO also is capable of reacting with oxygen radicals such as superoxide anion (Akaike et al.,1998) as well as directly modulating the activity of signaling molecules (Lander et al., 1997). The interaction with superoxide anion was suggested to be important in mechanisms where NO was implicated in modulating cytotoxic mechanisms, presumably by influencing oxidative stress (Lander et al.,1997).

Endothelin-1 (ET-1) is a 21-amino acid peptide produced primarily in vascular endothelium (Yanagisawa et al., 1988) which induces myocardial hypertrophy associated with increased protein synthesis and the induction of a fetal gene program (Choukroun et al., 1998; Wang et al., 1992; Yamazaki et al., 1996). ET-1 has been shown to activate many signaling pathways through its Gq-linked ETA receptor. In cultured cardiac cells, the activation of the ETA receptor, leading to the activation of phospholipase C and protein kinase C, and cross-talk between ETA receptor with other growth factor receptors having intrinsic tyrosine kinase activity leading to activation of mitogen-activated protein (MAP) kinases have been documented (Clerk et al., 1994).

ET-1 has been shown to cause hypertrophy of cardiac myocytes in culture (Wang et al., 1993; Yamazaki et al., 1996). Expression of endothelial NO synthase (eNOS) and NO production are present in cardiac myocytes (Balligand et al., 1993), and cardiac NO production may be increased due to induction of inducible NO synthase (iNOS) in pathological states such as myocardial failure (Haywood et al., 1996). It has also been shown the ability of NO to antagonize the actions of Et-1 in many cell types (Rizvi and Myers, 1997). Furthermore, Calderone et al (1998) observed that NO inhibits the growth-promoting effects of norepinephrine in cardiac myocytes. To better understand the role that NO might have in influencing ET-1 action in cardiac myocytes, we will examine the effects of adenovirus-mediated eNOS gene transfer on ET-1-induced cardiomyocyte hypertrophy.

The major goal of the present study was to test the hypothesis that eNOS transfer into cardiomyocytes inhibits endothelin-1-stimulated cell hypertrophy. The data presented in this report show that infection of cardiomyocytes with replication-deficient adenovirus vector containing human eNOS (AdeNOS) produces a dose [1-100 plaque-forming units (PFU) per cell]- and time of incubation (1-12 hr)-dependent increase in the expression of eNOS protein, NOS enzyme activity, and the production of NO. We have shown that eNOS expression in cardiomyocytes inhibited the ET-1-stimulated protein.

三、結果與討論、

RESULTS

eNOS Gene Transfer

Western analysis demonstrated expression of eNOS protein in AdeNOS-transfected cells (Figure 1). Calcium-dependent NOS activity in eNOS gene–transfected cells was increased as compared with AdPGK–transfected cells (Figure 2). The NOS activity of eNOS gene–transfected cells was completely calcium-dependent and was inhibited by 1 mmol/L LNA. eNOS gene transfer increased NO production by 4- to 8-fold over AdPGK-transfected cells (Figure 2). These results demonstrate that transfected eNOS gene in neonatal rat cardiomyocytes is functional and can be used to study the effect of NO on cardiomyocyte hypertrophy.

Figure 1. Adenovirus-mediated transfer of eNOS gene in cardiomyocytes. Cells were infected with AdeNOS vector carrying human eNOS gene. Western blot analysis after gene transfer showed increase expression of eNOS protein, in eNOS gene–transfected cells vs none in cells transfected with control vector (PGK reporter gene).

Figure 2. Effects of adenovirus-mediated transfer of eNOS gene on NO production and NOS activity in cardiomyocytes. Cells were infected with AdeNOS vector carrying human eNOS gene. Nitrite assay (A, B) showing increased production of nitrite in eNOS gene–transfected cells compared with the control group. Citrulline assay (C, D) demonstrates calcium-dependent NOS activity (pmol citrulline · mg-1· min-1) and is inhibited by 1 mmol/L LNA in eNOS gene–transfected cells compared with PGK-transfected cells. *Significant ($P<0.05$) increase in NOS activity and NO production in eNOS gene–transfected cells vs PGK-transfected cells.

Effect of eNOS Gene Transfer on cardiomyocyte hypertrophy

We have demonstrated earlier that ET-1-induced cardiomyocyte hypertrophy. Therefore, we used ET-1 (10 μmole/L) to test the effect of eNOS gene transfer on cardiomyocyte hypertrophy. eNOS gene transfer inhibited ET-1–increased protein synthesis. There was a 20 % decrease in protein synthesis in eNOS gene–transfected cells compared with PGK–transfected cells under ET-1 treatment. Treatment of eNOS gene–transfected cardiomyocytes with 1 mmol/L LNA, a specific inhibitor of eNOS, completely reversed this inhibitory effect, showing that inhibition of protein synthesis is due to increased NO production in these cells. Treatment of cells with control vector carrying the PGK in place of the eNOS gene had no effect on ET-1-increased protein synthesis, indicating that inhibition of cardiomyocyte hypertrophy in eNOS gene–transfected cells is not due to the viral vector used. In addition, the extent of inhibition of ET-1-increased protein synthesis was comparable to that produced by 100 μmol/L SNAP treatment of cardiomyocyte. These results would suggest that eNOS gene transfer inhibits ET-1-induced hypertrophy by increasing local NO production in cardiomyocytes.

DISCUSSION

Cardiac hypertrophy marked by cell enlargement without proliferation is a compensatory mechanism in response to a variety of cardiovascular diseases. Although the initial hypertrophic response may be beneficial, sustained hypertrophy often undergoes a transition to heart failure. The extra-stimuli that induce hypertrophy include mechanical overload and neurohumoral factors such as ET-1. These stimuli bind to heterotrimeric G protein-coupled receptors and rapidly activate immediate-early genes that encode transcription factors such as c-fos, c-jun and egr-1, etc. via protein kinase C (PKC) pathway and mitogen-activated protein (MAP) kinase phosphorylation cascade. Subsequently, late target genes such as atrial natriuretic factor (ANF), β-myosin heavy chain (β-MHC), skeletal α-actin and myosin light chain 2, etc. (normally expressed in the embryonic ventricle) are induced, ultimately leading to cardiomyocyte hypertrophy. Recently, reactive oxygen species (ROS) and nitric oxide (NO) have been demonstrated to be involved in the pathogenesis of atherosclerosis. However, the role of these free radicals in the development of cardiac hypertrophy remains unclear. Using ET-1-induced cultured cardiomyocyte hypertrophy as a model, we have previously demonstrated that ET-1 treatment to cardiomyocytes increases intracellular ROS generation which subsequently modulates c-fos gene expression via Ras pathway. In this study, we further investigate the role of NO in ET-1-induced cardiomyocyte hypertrophy.

ET-1, a 21-amino acid and a terminal tryptophan residue, was originally

characterized from the supernatant of cultured endothelial cells (Yanagisawa et al., 1988). It is the most potent and long-lasting vasoconstrictor and also exerts inotropic and chronotropic effects on cardiomyocytes and induces cardiomyocyte hypertrophy. The free radical gas, NO, is synthesized from L-arginine and O_2 by NOSs that require NADPH and tetrahydrobiopterin as cofactors. These NOS isoforms have been cloned and characterized. At least, two of the three isoenzymes (inducible, Ca^{++}-insensitive iNOS and constitutive, Ca^{++} -sensitive eNOS) are now known to be present in cardiomyocytes and play an important role in the regulation of cardiac contractile function. (Ballingand et al., 1993) The inducible isoform of NOS is not normally expressed in the myocardium, but is synthesized de novo in response to inflammatory cytokines. The constitutive eNOS is distinguished from inducible iNOS by their calcium sensitivity for the binding of activator calmodulin and enzymatic activity within the physiological range of intracellular calcium. These results indicate that a constitutive Ca^{++}-dependent NOS is activated that make NO formation.

Recent evidence indicates that NO activates guanylyl cyclase with a resulting increase in cGMP accumulation which subsequently activates the receptor protein cGMP-dependent protein kinase (PKG). This NO/cGMP signaling pathway plays an important role in regulating contractile properties of cardiac muscle *in vitro* and *in vivo*. These results clearly indicate that adenovirus-mediated nitric oxide synthase gene transfer inhibits ET-1-induced cardiomyocyte hypertrophic response. These findings further support that NO plays an important role as a negative regulator in ET-1-induced cardiomyocyte hypertrophy. In summary, increase of NOS activity and NO formation *via* adenovirus-mediated nitric oxide synthase gene transfer in cardiomyocytes could inhibit ET-1-stimulated protein synthesis. Thus, our findings support the inhibitory effect of adenovirus-mediated nitric oxide synthase gene transfer on ET-1-induced cardiac hypertrophy and may allow the development of new strategies for the treatment of hypertrophy and heart failure.

四、計畫成果自評

由本報告一氧化氮合成酵素基因經由腺病毒媒介傳送入心臟細胞可抑制內皮素所誘發心臟細胞肥大，可推測一氧化氮合成酵素基因經由腺病毒媒介傳送入心臟細胞，在臨床上相關心臟肥大或心臟衰竭疾病的預防治療上具有運用潛力。

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附件：封面格式

行政院國家科學委員會補助專題研究計畫成果報告

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