目的：研究 TNF 介导的 BCMEC 释放 PDGF 及药物对 BCMSMC 增殖的保护作用. 方法：体外培养 BCMEC 和 BCMSMC，用结晶紫染色法观察 TNF 引起的 BCMEC 释放 PDGF 继而促进 BCMSMC 增殖作用. 结果：TNF 不能促进无血清培养的 BCMSMC 增殖，但 TNF (5 - 20 μg·L^{-1}) 剂量依赖地促进 BCMEC 释放 PDGF. TNF (20 μg·L^{-1}) 促进 BCMSMC 增殖的百分率为 34±4 %. 药物 Imperatorin，iso-imperatorin 和 PMDP 不影响 TNF 引起 BCMEC 释放 PDGF，但能剂量依赖地 (1 - 100 μmol·L^{-1}) 抑制 PDGF 促 BCMSMC 增殖的作用. 结论：TNF 促进 BCMEC 释放 PDGF 继而引起 BCMSMC 的增殖. 药物能抑制 PDGF 引起的 BCMSMC 增殖.

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Inhibitory effects of nifedipine on DNA and protein synthesis in cultured cardiac nonmyocytes of neonatal rats[1]

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KEY WORDS cultured cells; nifedipine; angiotensin Ⅱ; heart hypertrophy; heart

AIM: To observe the inhibitory effects of nifedipine (Nif) on cardiac nonmyocytes growth and proliferation. **METHODS**: Using nonmyocytes in culture as a model, [^{3}H]thymidine and [^{3}H]leucine incorporation were measured. **RESULTS**: There was a significant decrease in cell number and in total cellular protein after 72-h exposure to Nif 1 μmol·L^{-1} in the presence of angiotensin Ⅱ (Ang Ⅱ). A 48-h exposure to 1, 10, 100, 1000 nmol·L^{-1} of Ang Ⅱ caused a 19 %, 35 %, 46 %, 48 % increase in protein synthesis and 27 %, 46 %, 56 %, 57 % increase in DNA synthesis. Nif 1, 3, and 10 μmol·L^{-1} were able to reduce the Ang Ⅱ 100 nmol·L^{-1}-induced increase of protein synthesis and DNA synthesis. **CONCLUSION**: Nif had a direct inhibitory action on the growth of nonmyocytes, which was related to the regression of cardical hypertrophy.

The cardiac nonmyocytes are important in the patheophysiology of left ventricular hypertrophy (LVH)[1]. The renin-angiotensin system represents a cascade of biochemical events leading to the generation of angiotensin Ⅱ (Ang Ⅱ). Local generation of autocrine or paracrine actions of Ang Ⅱ have been shown in the heart[2]. It has been proposed that Ang Ⅱ participates in the mechanisms of structural cellular changes by enhancing cell growth in LVH[3]. Nifedipine (Nif), an dihydropyridine calcium antagonist, showed a conspicuous LVH regression[4]. Potential explanation for the mechanism of action of Nif in this situation included the effect of blood pressure reduction and direct effects on the myocardium. In this experiment, we observed the effects of Nif on the growth of cardiac nonmyocytes by using cardiac nonmyocytes in culture as a model, which may partly explain the benefical effects of Nif in LVH.

MATERIALS AND METHODS

Tissue culture Monolayer cultured cardiac nonmyocytes of neonatal rats were prepared as previously described[5]. Briefly, fragments of ventricles of 2 - 4 d old neonatal rats were aseptically removed and tissue fragments were placed in Hanks' solution. The cells were isolated with 0.06 % trypsin at 37 ℃. Cultures of cardiac nonmyocytes were prepared passing twice the cells adherent to the culture dish during the preplating procedure. Until the second passage,

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the cell suspension was diluted to $1 \cdot 10^9$ cells·L^{-1} and placed in 3.5 cm dishes and 24-well tissue culture plates in humidified 5 % CO_2, 95 % air atmosphere at 37 ℃. The culture medium was consisted of 15 % heat-inactivated fetal serum, 75 % medium 199 and 0.1 % benzylpenicillin-streptomycin solution. After 24 h, the culture medium was replaced with defined serum-free media. The medium contained $NaSeO_4$ 30 nmol·L^{-1}, human transferrin (Sigma) 5 mg·L^{-1}, and insulin 5 mg·L^{-1}. The media was replaced every 3 d with fresh media.

DNA, protein synthesis Following another 24-h incubation in quiescent medium, the various concentrations of Nif and Ang Ⅱ were added to the medium. The medium from quiescent cultured cardiac nonmyocytes grown in 24-well plates was aspirated and replaced with serum-free medium containing [^{3}H] thymidine 3.7 MBq·L^{-1} plus the various concentration of Ang Ⅱ or/and Nif for 48 h. The medium was aspirated and cells were washed rapidly 3 times with cold Hanks' solution, cells were then lysed by addition of 1 mL/well 1 % sodium dodecylsulphate (SDS); Lysates were collected and precipitated by addition of 20 % trichloroacetic acid (TCA) 1 mL and then applied to GF/C filters. After washing with Hanks' solution 5 mL 3 times, filters were dried and transferred to vials containing 4 mL scintillation fluor, and [^{3}H]thymidine incorporation was determined by liquid scintillation method. The radioactivity measurement which represented the [^{3}H]thymidine incorporated into newly synthesized DNA was expressed per 10^5 cell number. For analysis of protein synthesis, the same procedure was followed substituting [^{3}H]leucine 3.7 MBq·L^{-1} for [^{3}H]thymidine.

Total cellular protein determination Dishes were washed rapidly 3 times with Hanks' solution, the cells were dissolved in 1 % SDS and each dish was measured by a colorimetric method[6].

Cell number determination Two distinct methods were used for determining cell number. Cells were counted with haemocytometer or the cell number was measured by crystal violet staining[7]. The absorbance (A) in each well was then measured with spectrophotometer at wavelength of 590 nm.

Nif (Sigma) was added from stock solutions in Me_2SO and a final concentration of 0.01 % Me_2SO had no effect on cellular growth; Ang Ⅱ was the product of Sigma, and [^{3}H]thymidine (814 TBq·mol^{-1}) and [^{3}H]leucine (2.04 PBq·mol^{-1}) were from Shanghai Institute of Nuclear Research

RESULTS

Effects of Nif on proliferation of cells Cells were cultured for 1 – 7 d using Nif 1 μmol·L^{-1} in the presence and absence of Ang Ⅱ 100 nmol·L^{-1}. Cells in the presence of Ang Ⅱ grew more rapidly than that in the absence of Ang Ⅱ. Nif caused more significant decrease in cell number in the presence of Ang Ⅱ than in the absence of Ang Ⅱ (Fig 1). Thus, Nif inhibited Ang Ⅱ-induced proliferation of cultured cardiac nonmyocytes.

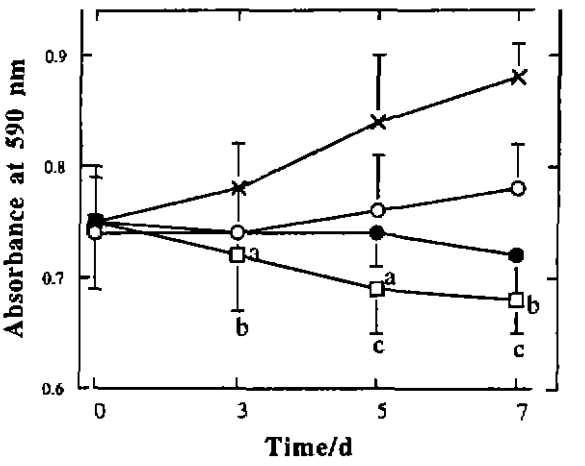

Fig 1. Effects of Nif on proliferation of cells in cultured cardiac nonmyocytes of neonatal rats. (○) **control;** (●) **Nif;** (×) **Ang Ⅱ;** (□) **Nif + Ang Ⅱ.** $n = 6$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$, **Nif** *vs* **control; Nif + Ang Ⅱ** *vs* **Ang Ⅱ.**

Effects of Nif on total cell protein Cells were cultured for 72 h using Nif 1 μmol·L^{-1} in the presence and absence of Ang Ⅱ 100 nmol·L^{-1}. The total cell protein were inhibited significantly in Nif-treated cells. Nif 1 μmol·L^{-1} reduced the total cell protein from 121 ± 9 and 151 ± 12 to 109 ± 10 ($P < 0.05$ *vs* control) and 129 ± 16 ($P < 0.01$ *vs* control), respectively in the absence and presence of Ang Ⅱ.

Effects of Ang Ⅱ on DNA and protein synthesis To investigate the effects of Ang Ⅱ on cell growth, cells were cultured for 48 h in fresh serum-free media containing various concentrations of Ang Ⅱ. Ang Ⅱ induced a significant increase in [^{3}H]leucine incorporation and [^{3}H]thymidine incorporation in concentration dependent manner. Ang Ⅱ 1, 10, 100, 1000 nmol·L^{-1} increased the protein synthesis by 19 %, 35 %, 46 %, 48 % and the DNA synthesis by 27 %, 46 %, 56 %, 57 %, respectively (Fig 2).

Effects of Nif on DNA and protein synthesis Cells were incubated for 48 h in serum-free media

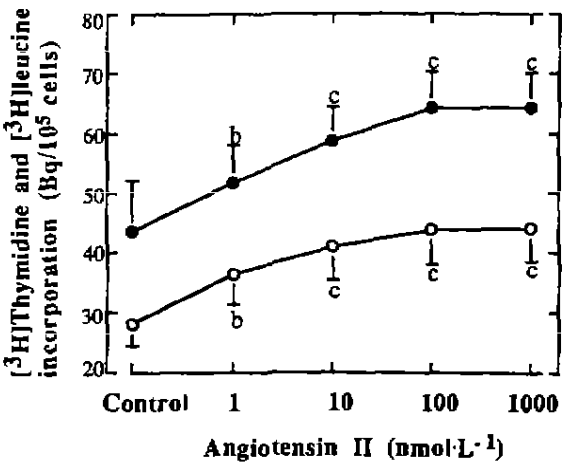

Fig 2. Effects of Ang Ⅱ on [^{3}H] thymidine (●) and [^{3}H]leucine (○) incorporation in cultured cardiac nonmyocytes of neonatal rats. $n = 6$, $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

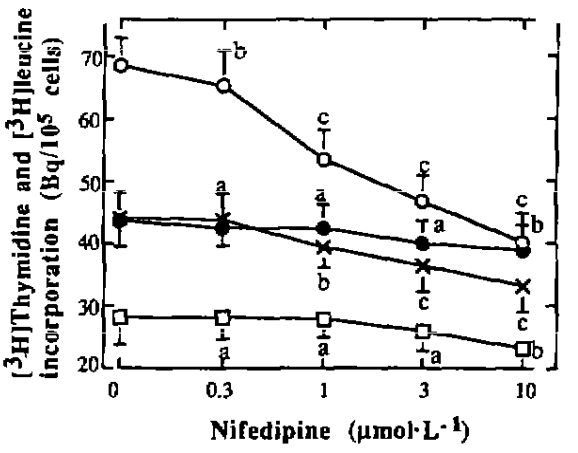

Fig 3. Effects of Nif on [^{3}H]thymidine (○, ●) and [^{3}H] leucine (×, □) incorporation in cultured cardiac nonmyocytes of neonatal rats. (○, ×) Nif + Ang Ⅱ; (●, □) Nif. $n = 6$, $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* 0 nmol·L^{-1}.

containing various concentrations of Nif in the presence and absence of 100 nmol · L^{-1} Ang Ⅱ. Nif was able to inhibit the [^{3}H]thymidine incorporation significantly in the precence of Ang Ⅱ. Nif 0.3, 1, 3, and 10 μmol · L^{-1} inhibited Ang Ⅱ-induced [^{3}H]thymidine incorporation from 4 109 ± 274 to 3 920 ± 338, 3 209 ± 292, 2 795 ± 259, 2 405 ± 262 dpm/10^5 cells. Nif was able to inhibit the [^{3}H]leucine incorporation significantly in the precence of Ang Ⅱ. Nif 1, 3, and 10 μmol·L^{-1} inhibited Ang Ⅱ-induced [^{3}H]leucine incorporation by 11 %, 18 %, 25 % (Fig 3).

DISCUSSION

Calcium antagonists are well established in the treatment of LVH[8], but the mechanism is not well known. Verapamil significantly reduced serum-stimulated cultured cardiomyocyte hypertrophy in a stereoselective manner[9]. In the present study the effects of Nif on Ang Ⅱ-induced cardiac nonmyocytes growth were examined. The results showed that Nif was able to inhibit Ang Ⅱ-induced cell growth in a dose-dependent manner. A toxic effect could be excluded by determination of cell viability with trypan blue.

LVH takes place by enlargement or hypertrophy of the cardiac myocytes and hyperplasia of cardiac nonmyocytes. Cardiac nonmyocytes (fibroblasts), which accont for as many as 50 % of the total cell number of the heari[10]. Ang Ⅱ causes fibrosis and increases collagen deposition in cardiac interstitius, which will induce the increase of fibrillar collagen and myocardial stiffness in the intact hypertrophied left ventricle[1,11]. The experiments showed that Nif inhibited the DNA and protein synthesis in cultured nonmyocardial cells. The results led us to the conclusion that Nif may directly influence the cardiac hypertrophy.

Ang Ⅱ stimulates the phosphoinositide signalling system, generating inositol 1,4,5-triphosphate which induced Ca^{2+} release from the sarcoplasmic reticulum. It has been reported that calcium antagonists inhibit the Ang Ⅱ-induced increase in intracellular Ca^{2+} concentration by the mechanisms that are not yet known[12]. The inhibitory effect of Nif on cell growth may depend on a specific blockade of the long-lasting type of calcium channel, but, some other intracellular mechanism, as yet not known, may be responsible for those strong antiproliferative effects. Several studies have shown that calcium antagonists in the concentration range $(1-8) \times 10^{-6}$ mol·L^{-1}, exert non-specific effects such as α-adrenoceptor blockade, inhibition of cyclic AMP phosphodiesterase and stimulation of Na, K-ATPase[13,14]. In general, such interac-

tions may promote the transition of nonmyocytes from an active to a quiescent growth state. Thus, the inhibitory effects of Nif on cell growth remain to be elucidated.

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硝苯地平对培养乳鼠非心肌细胞 DNA 及蛋白合成的抑制作用[1]

R972

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关键词 培养的细胞; 硝苯地平; 血管紧张素 II; 心脏肥厚; 心脏 DNA 蛋白合成

目的: 观察硝苯地平(Nifedipine, Nif)对非心肌细胞生长和增殖的抑制作用. **方法**: 以培养乳鼠非心肌细胞作为模型, 用[^{3}H]胸腺嘧啶核苷结合及[^{3}H]亮氨酸结合的方法. **结果**: 在血管紧张素 II 存在的情况下, Nif 1 μmol·L^{-1}作用细胞 72 h, 细胞的总蛋白及细胞数目明显减少. 测得血管紧张素 II 1, 10, 100, 1000 nmol·L^{-1}在 48 h 内, 增加 DNA 合成及蛋白合成. Nif 1 - 10 μmol·L^{-1}均能够减少 100 nmol·L^{-1}血管紧张素 II 诱导的细胞 DNA 合成及蛋白合成. **结论**: Nif 可以直接抑制非心肌细胞的生长, 该种作用与其抑制心肌肥厚有关.