EXPERIMENTAL WORK AND RESEARCH

Effects of Serum Containing Xinlikang (心力康) on Angiotensin II Induced Hypertrophy in Cultured Neonatal Rat Cardiomyocytes*

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ABSTRACT Objective: To evaluate the effects of Xinlikang (心力康, XLK) on angiotensin II (Ang II) induced hypertrophic cultured neonatal rat's cardiomyocyte (CMC). Methods: Primary cultured neonatal rat’s CMCs with the purity certified by immunohistochemical technique, were divided into three groups. Rats in the normal control group were untreated; those in the model group were established into hypertrophic models but underwent no treatment; and those in the XLK group were established to hypertrophic models and treated with XLK containing serum obtained from rats with aorta coarctation after 8 days of feeding with XLK. MTT and phase-contrast microscope were used to evaluate the effect of XLK on cell activity, pulsating rhythm and surface area; Atrial natriuretic peptide (ANP) expression was determined by radioimmunoassay; Protein content was determined by Bradford method; and DNA synthesis was detected by flow cytometric assay. Results: Immunohistochemistry results showed that more than 90% of the cells were actin stained positive cells. No significant effect of XLK on normal CMC was found. Ang II could significantly induce hypertrophy in CMCs, and XLK could significantly decrease the increased surface area and the accelerated pulsating rate in them. ANP expression was 780 ± 38 pg/L in the model group, and 430 ± 23 pg/L in the control group, and the elevated expression of ANP in model rats was significantly decreased in the XLK group; The DNA content in the G0/G1 and G2/M phases was significantly enhanced and at the same time it was accompanied with increase of total protein content in the model rats after being stimulated by Ang II for 24 h, showing that serum-containing XLK could also significantly suppress total protein synthesis (P < 0.05). Conclusion: XLK could improve Ang II mediated pathological growth of CMCs without influencing the growth of normal CMCs, suggesting that XLK is probably an effective drug for treatment of myocardial hypertrophy and heart failure.

KEY WORDS Xinlikang, angiotensin II, cardiomyocyte, hypertrophy

METHODS

Preparation of XLK Containing Serum

XLK, composed of Ginseng, Astragalus, Red Sage, Lepidium Seed, Acanthopanax Bark and Bitter Orange, was prepared into decoction at the concentration of 100 g original herbal medicine per 100 ml, by the Pharmaceutical Department of the Hospital Affiliated to Hubei College of TCM, batch No. 0010209.

Partial aorta coarctating operation was carried out in 36 Wistar rats aged 3 months, 23 of them survived 20 days later and were established into models of myocardial hypertrophy (confirmed by HE staining, Figure 1). They were fed with XLK 1 ml/100 g body weight, twice per day for 8 days, and then blood was collected from these model rats to prepare serum-containing XLK and the content of XLK was determined by thin-layer chromatography.

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Cell Culture
CMCs were dissociated from the hearts of 20 neonatal (2-day-old) Wistar rats, in reference to Delyani’s method and incubated on gelatin-coated glass dishes or 6-well plates at 1000–2000 cells/mm² of density, to incubate with DMEM/F12 (GIBCO, USA) medium containing 10% fetal calf serum (FCS, GIBCO, USA), 100 U/ml penicillin, 100 mg/ml streptomycin and 1 mmol/L 5-bromo-2'-deoxyuridine (Brdu, Sigma, USA) for 72 h. Before starting the experiment, the cultured cells were incubated with the medium replaced by serum-free opti-MEM (Life Technology, USA), with 1% fetal calf serum for over 24 h. Immunohistochemistry and phase-contrast microscope were used to observe the purity, vitality and morphology of the CMCs.

Cell Grouping and Treatment
The experiment was carried out according to the manufacturer’s instruction on CMCs when the confluence reached 30%–50%. The cells harvested were divided into three groups and treated respectively as follows. Cells in the model group were treated by adding Ang II (1 μmol/L) to the medium alone; to the XLK group, Ang II (1 μmol/L) in combination with XLK added to the medium, while the normal control group was untreated, that is, only 1% FCS was added into the culture medium. The incubation for all continued for 24 h at 37°C in a CO₂ incubator.

Identification of Cells
CMCs harvested were fixed, with the endogenous peroxidase quenched with 3% H₂O₂ in methanol and the nonspecific binding sites blocked with 10% normal goat serum. Then, they were incubated with rabbit anti-α-sarcometin actin (1:100 diluted, Santa Cruz, USA) overnight at 4°C, and, after the secondary antibody was added in accordance to the instruction, incubated again with strep-avilin-biotin immuno-peroxidase reacting solution (Histostain™-plus SP Kit) under 37°C, colorated by dianamobenzidine (Fast DAB tablets, Sigma), and observed under light microscopy. Image analysis was carried out with HPLAS-2000 analysis software.