RESEARCH OF TRIPOLIDE ON ANG II-INDUCED NEONATAL CARDIAC FIBROBLASTS PROLIFERATION AND COLLAGEN SYNTHESIS

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1Zhai Kang, 1Hasim Buaijaer, 1Yuan Binbin, 1Zhang Zhengang, 1Department of Cardiology, Nanjing Benq Hospital, The Affiliated Hospital of Nanjing Medical University; 2Department of Cardiology of The First Peoples Hospital of Yangzhou University

Objectives To observe whether TP can inhibit proliferation and collagen synthesis of cardiac fibroblasts and clarify the possible mechanisms.

Methods Differential attachment technique to obtain and cultivate neonatal SD rat cardiac fibroblasts. Experimental cells were randomly divided into five groups: (1) control (culture medium); (2) Ang II group (10-7 mol/l Ang II); (3) 1 μg/l TP group (1 μg/l TP+10-7 mol/l Ang II); (4) 10 μg/l TP group (10 μg/l TP+10-7 mol/l Ang II); (5) 100 μg/l TP group (100 μg/l TP+10-7 mol/l Ang II). Ang II are simultaneously added into the culture medium. MTT colorimetric determination of cell proliferation. Collagen synthesis, TGF-β1 secretion and phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) by Hydroxyproline, ELISA and Western Bloting.

Results 1. The proliferation of CFB is significantly promoted after adding Ang II, compared with the control group (p<0.05 or p<0.01). The 100 μg/l TP showed the effect of inhibiting the proliferation of CFB at the first 24 h (p<0.01), reached a peak within 48 h (p<0.001), started to diminish after 72 h, indicate the best time to exert effects were at 2 to 3 days. 2. With the time increase after adding Ang II, collagen synthesis increased, there is significant difference compared with the control group (p<0.05 or p<0.01). After 24 h, 48 h, 72 h of adding TP, the collagen content of each group compared with the Ang II group were significantly different. The effect of high concentration TP (100 μg/l) reached the peak (p<0.001) at 48 h (p<0.001). S. After 24 h of adding Ang II, TGF-β1 expression was significantly increased (p<0.01). After 24 h of adding different concentrations of TP, TGF-β1 expression were significantly decreased (p<0.05 or p<0.01). 4. After 30min of adding Ang II, ERK1/2 phosphorylation increased compared with the negative control (p<0.05). After 30min of adding 100μg/l TP, p-ERK1/2 expression increased compared with the Ang II group (p<0.05). And 1 μg/l, 10 μg/l TP did not inhibit ERK1/2 phosphorylation caused by Ang II. Positive control U0126 significantly inhibited the ERK1/2 phosphorylation (p<0.01).

Conclusions Ang II promote neonatal SD rat cardiac fibroblasts proliferation and collagen synthesis, the possible mechanism may be the MAPK signal transduction pathway. Ang II has the effect of promoting cardiac fibroblasts proliferation by increase phosphorylation of ERK1/2 expression, promoting collagen synthesis by increasing the expression of TGF-β1. Tripolidine significantly inhibited the Ang II-induced cardiac fibroblast proliferation and collagen synthesis in a dose-dependent manner, and the mechanism is probably by inhibiting the ERK1/2 phosphorylation and reducing the expression of TGF-β1.