GW23-e1622

TPEN REDUCES CALCIUM OVERLOAD, OXIDATIVE STRESS AND EXHIBITS PROTECTIVE EFFECTS IN PACED HL-1 CELLS

doi:10.1136/heartjnl-2012-302920b.15

Zhou Mo, Xu Wen-jing, Sun Li-ping, Lin Chao-lan, Zhang Ying-ying, Wang Xing, Chang Hui-ying, Han Wei, Han Wei. *Department of Cardiology, The First Affiliated Hospital of Harbin Medical University, Harbin, 150001, China*

Objectives N, N, N', N'-tetrakis (2-pyridylmethyl) Ethylenediamine (TPEN), a membrane-permeable zinc chelator, has been demonstrated to modify the intracellular level of calcium and to exhibit antioxidative effects. The present study was to investigate whether TPEN could provide protective effects in atrial fibrillation cells, especially under circumstances of oxidative stress.

Methods Cell models of atrial fibrillation were established by rapid paced HL-1 cells with 600 times per minute (10 Hz/5ms, 1.0 V/cm). These cells were divided into five groups: paced group, in which HL-

1 cells were paced for 2 h with 600 times per minute; control group, in which HL-1 cells were never paced as sinus rhythm control; ONOO⁻ group, in which HL-1 cells were pretreated with ONOO⁻ and then paced; TPEN group, in which HL-1 cells were pretreated with TPEN and then paced, and ONOO⁻+TPEN group, in which cells were pretreated with ONOO⁻ and TPEN before they were paced. The changes of fluorescence intensity of intracellular calcium ion (Ca²⁺) were observed with laser scanning confocal microscope. The protein expression of 3-nitrotyrosine (3-NT; a biological marker of peroxynitrite) was detected by Western blot. The occurrence of cardiomyocytic apoptosis was determined by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labelling (TUNEL). Additionally, MTT assay was used to measure the viability of HL-1 cells under different concentrations of ONOO⁻ and TPEN.

Results The fluorescence intensity of Ca²⁺ within HL-1 cells increased significantly when cells were rapidly paced ($p<0.05$), and this intensity further increased when paced cells were pretreated with ONOO⁻ ($p<0.05$). TPEN treatment could significantly reduce the increased level of intracellular Ca²⁺ induced by rapid pacing ($p<0.05$) and ONOO⁻ stimulation ($p<0.05$). Western blot analysis showed that the expression of 3-NT increased dramatically in the paced group as compared to control group ($p<0.05$) and the same increase was observed in the ONOO⁻ group compared with that in the paced group ($p<0.05$). Noticeably, the expression of 3-NT decreased prominently in the TPEN group and the ONOO⁻+TPEN group as compared to paced group ($p<0.05$) and ONOO⁻ group ($p<0.05$). MTT assay revealed that ONOO⁻ reduced the viability of HL-1 cells in a dose dependent manner ($p<0.01$), and 1 $\mu\text{mol/l}$ TPEN could significantly ameliorate the damage caused by ONOO⁻ ($p<0.05$). Additionally, TPEN significantly reduced the apoptosis index of HL-1 cells induced by rapid pacing ($p<0.01$) and ONOO⁻ stimulation ($p<0.01$).

Conclusions TPEN exhibits protective effects in atrial fibrillation cells, especially in circumstances of oxidative stress.