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

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Objectives N, N, N', N'-tetrakis (2-pyridylmethy1) 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 (Ca^{2+}) 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 Ca^{2+} 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 Ca^{2+} 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 μmol/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 exhibites protective effects in atrial fibrillation cells, especially in circumstances of oxidative stress.