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THE STUDY ON THE EFFECT OF ENDOPLASMIC RETICULUM STRESS IN APOPTOSIS OF RAT DETRUSOR SMOOTH MUSCLE CELLS STIMULATED BY HIGH GLUCOSE IN VITRO

Hypothesis / aims of study
To explore the involvement of ERS-associated detrusor muscle apoptosis in the high-glucose stimulated DSMCs.

Study design, materials and methods
The detrusor tissue from healthy SD rats were isolated and purified. The expression of α-smooth muscle actin was detected and was observed under laser confocal microscope to identify the DSMCs. MTT colorimetry was used for the evaluation of DSM cell proliferation. The optical densities (OD) in each group at four different timepoints were detected. The Annexin V-FITC/PI double-staining and flow cytometry were used for the evaluation of DSM cell apoptosis. The early, late and total apoptotic rates were calculated. The expressions of GRP78, CHOP, and Caspase12 protein and mRNA in the DSMCs from HG groups at four different timepoints as well as NG group,were analyzed using Western blotting and qRT-PCR respectively.

Results
Four time-pointed detections of the ODs revealed the significant lower levels of DSM cell proliferation in HG group than in NG group at 24h,48 h and 72 h timepoints ($P<0.05$). Within single group, the NG group showed a significant elevated curve of cell proliferation level. In contrast, the HG group presented a significant declined trend.Meanwhile, the significantly higher early, late and total apoptotic rates were observed in HG group at four timepoints($P<0.05$). The densitometric analysis of the Western blotting bands for all three observed proteins revealed the significant increases in relative abundance in HG group at all four time points. The upregulated expression of GRP78 protein reached its peak at 24 h and then showed a relative declined trend, while both CHOP and Caspase12 expressions showed a uniform increase over time. The mRNA expressions of GRP78, CHOP, and Caspase12 in HG group were significantly increased at all four time points. The mRNA expression of GRP78 peaked at 24 h, while CHOP and Caspase12 still maintained the same climbing trend, in parallel with their enhanced protein expressions. Caspase12 and CHOP positively correlated with the apoptotic rate.

Interpretation of results
The high-glucose stimulation in vitro has the significant effect of proliferation suppression and apoptosis induction, and this effect became augmented with the time of stimulation extended. Both of the increasingly enhanced ERS-associated apoptosis and the compensated-decompensated transition of UPR are two distinctive pathophysiological characters within the duration of high-glucose stimulation to DSMCs in vitro.

Concluding message
Hyperglycemia can induce ERS-associated apoptosis in the progression of DCP directly.

Disclosures
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