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TAURINE INHIBITS OSTEOBLASTIC DIFFERENTIATION OF AORTIC VALVE INTERSTITIAL CELLS INDUCED BY BETA-GLYCEROPHOSPHATE, DEXAMETHASONE AND ASCORBIC ACID VIA THE ERK PATHWAY

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Purpose Aortic valve calcification (AVC) has been recognised as an active process characterised by osteoblastic differentiation of the aortic valve interstitial cells (AVICs). Taurine is a free β -amino acid and has protective effect on cardiovascular events. In previous studies, we demonstrated that taurine transporter (TAUT) is expressed in vascular smooth muscle cells (VSMCs) and taurine prevents β -glycerophosphate (β -GP)-induced osteoblastic differentiation of VSMCs through the extracellular signal-regulated protein kinase (ERK) pathway. But little is known about the effect of taurine on AVC. We hypothesised that taurine suppresses AVC by inhibiting the osteoblastic differentiation of AVICs, and ERK pathway which modulates cell differentiation takes part in the protective effect of taurine.

Methods To test this hypothesis, we isolated and cultured human AVICs from 4 patients of acute aortic dissection (type A) and minute lesions were excluded by pathological examination. The phenotype of cultured cells were confirmed by the expression of SM α -actin, vimentin and TAUT and no expression of CD31 (endothelial cells) or SM-myosin/desmin (muscle cells) by immunohistochemistry. To establish the osteoblastic differentiation model of AVICs, cultured cells were induced by pro-calcific medium (PCM) containing β -GP (10 mM), dexamethasone (10 nM) and ascorbic acid (50 μ g/ml). On the 14th day of PCM induction, two osteoblastic markers-core binding factor α 1 (Cbf α 1) and alkaline phosphate (ALP) were tested by western blot and BCIP/NBT staining, respectively. Calcified nodules were tested by Von Kossa staining on the 21th day. To test the protective effect of taurine, different concentrations of taurine (1 mM, 3 mM, 5 mM, 10 mM, 20 mM) were added into the PCM. On the 14th day of intervention, ALP activity was detected on a spectrophotometer by p-nitrophenol method (modified by total protein content measured by BCA method) and the expression of ALP and Cbf α 1 was analysed by western blot. To explore the intracellular mechanism of taurine, the activation of ERK pathway (as the ratio of p-ERK/total ERK) was tested at 0 min, 5 min, 10 min, 30 min, 60 min and 120 min after the intervention of taurine (10 mM) by western blot. An ERK inhibitor PD98059 (10 μ M) was added into the PCM with taurine (10 M), and the ALP activity/expression and Cbf α 1 expression were tested on the 14th day.

Results Cultured AVICs express SM α -actin, vimentin and TAUT but no CD31, SM-myosin or desmin. AVICs induced by PCM expressed Cbf α 1 (6.3 $\pm$ 2.3 fold of control) and were positive for ALP on the 14th day, and calcified nodules were identified on the 21th day. Taurine attenuated the PCM-induced osteoblastic differentiation of AVICs as a dose-dependent manner on the 14th day, reaching the maximum protective effect at 10 mM with 31.8 $\pm$ 5.6% decreasing for ALP activity, 36.3 $\pm$ 6.2% decreasing for ALP expression and 41.6 $\pm$ 7.3% decreasing for Cbf α 1 expression (p <0.05). Taurine elevated the content of p-ERK (4.22 $\pm$ 0.45 fold of control) after 30 min. PD98059 abolished the effect of taurine on ALP activity/expression and Cbf α 1 expression significantly.

Conclusion These results suggest that cultured AVICs express SM α -actin, vimentin and TAUT and have the potential to differentiate to osteoblastic cells. Taurine inhibits the osteoblastic differentiation of AVICs via the ERK pathway in vitro.