

## The inhibitory effect of Orengetokuto on human aortic valve calcification

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Aortic valve stenosis (AVS) is a common heart valve disease in elderly people, and is mostly accelerated by ectopic aortic valve calcification (AVC). We recently demonstrated that tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) induces calcification of human aortic valve interstitial cells (HAVICs) obtained from AVS patients. In this study, we investigated the inhibitory effect of Orengetokuto (OGT) on TNF- $\alpha$ -induced calcification of HAVICs. OGT is composed of four crude drugs (Coptodis rhizoma, Scutellariae radix, Phellodendri cortex, and Gardeniae fructus) and is used for hypertension, palpitation and others. HAVICs isolated from aortic valves were cultured in  $\alpha$ -MEM including 10% FBS, and calcification was induced by TNF- $\alpha$  (30 ng/ $\mu$ L). Gene expression of BMP2 and ALP activity as typical calcification markers, were significantly increased in response to TNF- $\alpha$ . To confirm the effect of OGT on TNF- $\alpha$ -induced calcification, HAVICs were cultured in medium containing OGT and TNF- $\alpha$ . We found that OGT inhibited TNF- $\alpha$  induced calcification confirmed by Alizarin Red S stain. Increasing BMP2 gene expression and ALP activity induced by TNF- $\alpha$  were also inhibited by OGT. These results suggest that OGT can be used for treatment of AVS by inhibiting acceleration of AVC.