LONG-TERM ENHANCED EXTERNAL COUNTERPULSATION DOWNREGULATES THE MIF EXPRESSION OF VASCULAR ENDOTHELIAL CELLS IN ATHEROSCLEROTIC PIGS

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Objectives  Mechanisms underlying the beneficial effect of Enhanced External Counterpulsation (EECP) in atherosclerotic diseases are not well defined. Since Migration Inhibitory Factor (MIF), as a novel pro-inflammatory and immuno-regulatory factor, has recently been believed to play a pivotal role in the pathogenesis of atherosclerosis, we hypothesised that long-term EECP could downregulate the expression of MIF in vascular endothelial cells (VECs) of atherosclerotic lesions, contributing to its clinical outcomes. We studied this hypothesis in a porcine model of atherosclerosis.

Methods  Eighteen 20-day-old male domestic pigs were randomly assigned into three groups: the normal control group (normal group, n=6), the hypercholesterolemic control group (HC group, n=6) and the hypercholesterolemic+EECP group (EECP group, n=6). Pigs in normal group were fed with normal diet, while the pigs in the other two groups were fed with cholesterol-rich diet in order to induce atherosclerosis. Six pigs in EECP group were anesthetised by intramuscular injection of 846 mixture and intravenous infusion of pentobarbital sodium. The EECP procedures were performed on them for 2 h every 2 days with 0.035 MPa/cm² pressure, summed total 36 h. After the end of EECP, all the pigs were sacrificed by injecting overdose of 10% potassium chloride into the heart. For each animal, the thoracic aorta was isolated for harvesting VECs with collagenase. One half of the harvested VECs were fixed with 4% paraformaldehyde and further embedded with paraffin. The remained VECs were prepared for extracting their total mRNA. Immunocytochemical staining for MIF was performed on paraffin-embedded VECs, while RT-PCR was applied for detecting the transcriptional expression of MIF, respectively.

Results  The staining-positive rate of MIF of aortic VECs in EECP group was much lower than that in HC group ((211±14)% vs (358±26)%), p<0.05), but still higher than that in normal group.
significantly ((211±14)% vs (168±22)%), p<0.05. In consistence with the result of immunochemical staining analysis, the relative ratio of RT-PCR products of MIF were lower in EECP group than that in HC group ((1.26±0.15) vs (1.89±0.22), p<0.05), but still higher than that in normal group significantly ((1.26±0.15) vs (0.65±0.11), p<0.05). The immunocytochemical expression of MIF correlated positively to its relative ratio of RT-PCR products (r=0.662, p<0.05).

**Conclusions** MIF in VECs may play an important role in atherogenesis. Transcriptional down regulation of MIF in VECs may be one of the molecular mechanisms contributing to the clinical outcomes following EECP performation.