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Cardioprotective Potential of Thymosin s4 in a Preclinical Pig Model of Ischemia/Reperfusion Injury


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Prolonged myocardial ischemia results in myocardial dysfunction, even after successful revascularisation. We have reported that retrograde application of embryonic EPCs, derived from murine d 7.5 embryos (Tie-2+, c-Kit+, Sca-1+, flk-1 low, MHC-1-), provide rapid cardioprotection via soluble factors. Now, we investigated the role of Thymosin beta 4 (TB4) as paracrine factor mediating the cardioprotective potential of eEPCs.

Methods:

In vitro, neonatal rat cardiomyocytes (NRCMs) were subjected to hypoxia (4h)/reoxygenation (1h) in the absence or presence of eEPCs with or without TB4 shRNA transfection, which decreased of TB4 mRNA levels to 20% of control cells. In vivo, pigs (n=6 per group) underwent percutaneous LAD occlusion for 60 min. After 55 min of ischemia either saline solution or eEPCs (5 * 10 6 Dil labeled cells) ± TB4 shRNA, or TB4 protein (15mg/animal) were applied via selective pressure-regulated retroinfusion into the anterior interventricular vein. Infarct size and functional reserve (sonomicrometry at 150/min atrial pacing) were determined after 24h reperfusion. Myeloperoxidase (MPO) levels were obtained to analyze inflammatory cell invasion.

Results:

In vitro, survival of NRCMs was increased from 32 ± 4 to 90 ± 2%, when eEPC were present. TB4 shRNA, but not control shRNA, abolished this effect (45 ± 7%), whereas TB4 protein restored it (85±3%). In vivo, eEPCs significantly decreased infarct size compared to control group (36±3% vs. 58±5% of AAR, p<0.05), but not in the presence of TB4-shRNA (48 ± 7% of AAR). Moreover, TB4 protein retroinfusion alone was as effective as eEPC application (36±4%). Functional reserve of the infarcted area was enhanced after eEPC (33±4 SES, % of RCx) or TB4 protein application (39±5% SES) compared to control (10±3%), except eEPCs transfected with TB4 shRNA (19±5% SES). Retroinfusion of eEPCs or TB4 protein significantly reduced MPO levels in the ischemic tissue (1996±546,
1455±197 U/g tissue MPO) compared to control (3323±388 U/g tissue MPO) or TB4 shRNA treated eEPCs (5449±829 U/g tissue MPO).

**Conclusion:**

Our findings reveal that early cardioprotection after ischemia may be achieved by embryonic EPC retroinfusion, unless TB4 levels are largely reduced. Consistently, TB4 protein suffices to provide significant cardioprotection after ischemia and reperfusion.