Thymosin beta 4 and its bioactive fragments as preconditioning agents for skeletal myoblasts

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Tissue engineering of skeletal muscle represents a potentially powerful technique to enhance recovery and rehabilitation after injury, and the implantation of myoblasts in tissue engineering chambers *in vivo* has produced encouraging results in terms of promoting vascularised tissue constructs (Lokmic *et al.*, 2007). A major obstacle for growing tissue constructs in implanted tissue chambers is the initial period of hypoxia and/or elevated ROS that myoblasts are subjected to before vascularisation (Lokmic *et al.*, 2007). Thymosin β4 (Tβ4) is a small, ubiquitously expressed peptide that possesses a broad range of biological activities, and has been shown to reduce inflammation, enhance angiogenesis, and improve regeneration in a number of tissues (Goldstein *et al.*, 2012). In addition to the full-length peptide (residues 1-43) there are three naturally occurring biologically active peptide fragments of Tβ4 - Tβ4[1-4], Tβ4[1-15] and Tβ4[17-23] - each with their own characteristic biological activities (Sosne *et al.*, 2010). The aim of this study was to examine whether pre-treatment with Tβ4 or its fragment peptides could protect skeletal myoblasts against hypoxia and elevated ROS, with a view to using Tβ4 as a preconditioning agent for C2C12 myoblasts before chamber implantation. To examine this, we examined the ability of Tβ4 and its fragment peptides to protect myoblasts against either i) hypoxic insult consisting of 48 hours in a GENbox atmospheric generator (Biomerieux), or ii) oxidative stress induced by exposure to 100μM H_2O_2 for 24hrs. We found that full-length Tβ4 and two of its active peptides (Tβ4[1-4] and Tβ4[1-15]) increased myoblast survival following either hypoxia or exposure to H_2O_2, while Tβ4[17-23] provided no protection against either insult. In addition, we found that Tβ4 treatment increased mRNA expression of signalling pathways involved in angiogenesis, suggesting that Tβ4 treatment may also potentially aid in vascularisation of tissue constructs after implantation. Taken together, these results suggest that pre-treatment with Tβ4 may encourage both myoblast survival and vascularisation of tissue constructs in implanted chambers, and that further studies using *in vivo* tissue engineering chambers are warranted.

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