Influence of *myo*-inositol on protein turnover in C_2C_{12} myotubes

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Introduction: Muscle atrophy is a devastating condition present in numerous neuromuscular and chronic diseases (Skipworth *et al.*, 2006). Loss of muscle tissue leads to weakness, loss of independence, reduced response to chemotherapies and is used as a negative prognostic factor (Al-Majid & Mccarthy, 2001; Andreyev *et al.*, 1998; Tisdale, 2002). Identifying therapeutic targets which reverse muscular atrophy and maintain muscle mass remain key research priorities. Recently d-*myo*-inositol-1,2,6-trisphosphate (α-trinositol, AT) has been shown to attenuate the loss of body mass in a MAC-16 tumour murine cachexia model, attenuate protein degradation in C_2C_{12} myotubes and increase protein synthesis *via* upregulating the phosphorylation of mTOR and attenuating the autophosphorylation of eukaryotic initiation factor 2α (eIF-2α) (Russell *et al.*, 2009, Russell *et al.*, 2010). However, AT is not naturally present in mammalian tissue and furthermore its association with target molecules in the body has not yet been fully characterised. Naturally occurring *myo*-inositol (MI) is present in all mammalian and plant cells, is obtained from the diet and synthesised *de novo* by kidney cells (Clements & Darnell, 1980; Clements & Reynertson, 1977; Holub, 1986). To date, no study has looked at the role of natural MI on the regulation of protein turnover. Therefore the aims of the study were to investigate the role of MI on protein synthesis and degradation and Akt and eIF2α phosphorylation under catabolic conditions in C_2C_{12} myotubes. A secondary aim was to investigate the role of MI in C_2C_{12} myoblast cell proliferation.

Methods: C_2C_{12} myotubes were treated with the catabolic stimuli dexamethasone (DEX, 1 μM) for 24 h and TNFα (50 ng/ml) for 2 and 24 h, in the presence and absence of MI (100 μM). Protein synthesis was determined by the incorporation of ^{3}H-tyrosine into myotubes. Protein degradation was determined by the release of ^{3}H-tyrosine into the medium as a fraction of total ^{3}H-tyrosine. The expression of the muscle specific atrophy genes, muscle atrophy F box (MAFbx/atrogin-1) and muscle RING finger 1 (MuRF-1) was determined by PCR. The expression of phosphorylated Akt and eIF2α proteins, as well as atrogin-1 and MuRF-1 proteins was determined by western blot analysis. Myoblast proliferation was determined by the incorporation of 5-bromo-2-deoxyuridine (BrDU) into newly synthesised DNA during serum starvation, with and without MI (0-1000 μM), and in the presence of TNFα (0-250 ng/ml).

Results: DEX decreased protein synthesis by 15% ($p < 0.001$) and increased protein degradation by 34% ($p < 0.001$). MI, either alone, or when combined with DEX, did not influence protein synthesis or protein degradation. MI decreased MuRF1 mRNA by 18%, when compared to basal conditions ($p < 0.05$). DEX increased atrogin-1 mRNA expression by 47%, when compared to basal levels; an effect attenuated by 44% ($p = 0.05$) with MI. TNFα treatment did not influence protein synthesis or degradation. However, TNFα treatment for 2 h decreased MuRF-1 mRNA by 30% ($p = 0.04$) and increased atrogin-1 mRNA expression by 90% ($p = 0.09$). MI, either alone, or when combined with TNFα, had no effect MuRF-1 or atrogin-1 mRNA levels. TNFα and serum starvation decreased myoblast proliferation by 18% ($p < 0.01$) and 16% ($p < 0.001$), respectively. MI blocked the serum starvation ($p < 0.008$), but not the TNFα, reduction in proliferation.

Conclusion: These results show that natural MI does not influence protein synthesis or degradation. However, MI attenuates the DEX-induced up regulation of atrophy-gene expression in C_2C_{12} myotubes. The measurement of atrogin-1, MuRF1, Akt and eIF2α protein levels is currently being performed. MI may play a protective role in maintaining C_2C_{12} myoblast proliferation during serum starvation.

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