S-NITROSOGLUTATHIONE REDUCTASE AND S-NITROSOCYSTEINE MODULATE MYOBLAST PROLIFERATION AND FUSION

Yamashita, A.M.S.¹, Figueiredo-Freitas, C.¹, Possidonio, A.C.B.², Soares, C.P.², Nogueira, L.¹, Mermelstein, C.S.², Sorenson, M.M.¹

¹Instituto de Bioquímica Médica Leopoldo de Meis - IBqM, Universidade Federal do Rio de Janeiro, Brazil; ²Instituto de Ciências Biomédicas Universidade Federal do Rio de Janeiro, Brazil.

Introduction and objectives: During myogenesis, myoblasts proliferate and fuse into multinucleated myotubes. This process is dependent on the production of several factors, including nitric oxide (NO). One target for NO and its derivatives is the thiol group of cysteines to form S-nitrosothiols (RSNO). Recently, it has been shown that increasing intracellular RSNO enhances proliferation of different cell types, particularly when the denitrosylating enzyme S-nitrosothiol reductase (GSNOR) is inhibited. Although it was reported that adult skeletal muscles express GSNOR, the role of intracellular RSNO content and its regulation by GSNOR, during myogenesis is not known. The aim of this work was to investigate whether GSNOR activity interferes with the progress of proliferation and fusion of an in-vitro primary culture of myoblasts.

Materials and Methods: Primary cultures from pectoral muscle of chicken embryo were grown for 24 h and treated for 48 h with either DMSO, the GSNOR inhibitor C3, S-nitrosocysteine (CysNO) or L-NAME. Immunofluorescence of desmin and α-actinin (muscle proteins) and DAPI (nuclear dye) was used to quantify cell proliferation and fusion. GSNOR and NO synthase (NOS) were determined by immunoblotting. Total RSNO and GSNOR activity were determined from cell lysates by Saville’s method and spectrophotometrically by GSNO consumption rates, respectively.

Results and conclusions: GSNOR activity increased up to 2.4-fold following cell plating. The treatment with C3 or CysNO increased the intracellular RSNO levels (5.5- and 2-fold, respectively), which were correlated to an increase in the number of nuclei from myoblasts and also (less markedly) from fibroblasts. C3 or CysNO also produced a decrease in the fusion index (~20%). L-NAME treatment to block NOS reversed the effect of C3 and increased the thickness of the myotubes. Thus the activity of GSNOR negatively regulates myoblast fusion by increasing cell proliferation in cultured myoblasts.

Acknowledgements: CNPq, CAPES, FAPERJ; Key Words: GSNOR, S-nitrosylation, myogenesis.