Valala JVZ, Achcar JA, Montrezor LH (2022) Effects on Endothelin-1 on Irisin Production By Myocytes Derived From C2C12 Cells, **FASEB JOURNAL**, v. 36, p. R2610, 2022, John Wiley and Sons https://doi.org/10.1096/fasebj.2022.36.S1.R2610

Abstract

Skeletal muscle is one of the most metabolically active tissues. Consequently, it is highly susceptible to hormonal variations, whether by endocrine, paracrine, or autocrine pathways. This muscle is recognized as an endocrine organ that releases myokines, including irisin. Irisin acts as a signal that induces energy consumption, communicating directly with the adipose tissue, modulating the metabolic profile and increasing energy expenditure. Endothelin-1 (ET-1), a peptide with pro-inflammatory activity, is a potent vasoconstrictor that interacts with receptors on the vascular smooth muscle membrane, stimulating its contraction. However, the functional relationship between ET-1 and skeletal muscle remains unclear. The objective of this work was to study the effects of ET-1 on cell count, cell viability, and irisin production in C2C12 cell culture. The cells were cultured for 3 and 5 days, at an initial density (day 0) of 1 x 10^3 cells/well (48-well culture plates), in 200 µL of culture medium containing equine serum, antibiotics, and antifungal, in the absence of ET-1and in the presence of ET-1 at two concentrations (10^{-10} M and 10^{-6} M). The assays were performed in triplicates, with exchange of the medium every 48 h. Cell counting was performed with a Bio-Rad TC20™ cell counter. The MTT reduction assay was used to determine cell viability. Irisin in the culture medium was determined using an ELISA kit. The results were presented as mean ± SEM. Statistical analysis of the data employed ANOVA and Fisher's test for multiple comparisons, adopting significance level of 5% ($p<0.05$). Both time- and dose-dependent effects on cell counts were observed. Culturing for 5 days led to a higher number of cells (111,367 ± 42,813 cells), compared to 3-day culture (22,422 ± 9,141 cells). Furthermore, stimulation by ET-1 at 10^{-6} M resulted in higher numbers of cells for culture times of both 3 days (64,971 ± 41,841 cells) and 5 days (174,478 ± 49,152 cells), compared to the same culture times in the absence of ET-1. Cell viability was higher for the 5-day cultures of the control group (0.1984 ± 0.04) and the 10^{-10} M ET-1 group (0.2633 ± 0.0311), compared to the 3-day cultures (control group: 0.1681 ± 0.003; 10^{-10} M ET-1 group: 0.1421 ± 0.0135). Biologically, in the absence of ET-1, there was a time-dependent reduction in irisin production (3-day culture: 0.106 ± 0.0085 ng/mL; 5-day culture: 0.097 ± 0.09 ng/mL). Therefore, the findings suggested that the multiplication activity of the C2C12 cells cultivated according to the proposed study model was modulated by time and by ET-1, and that cell viability was modulated by the culture time. The time-dependent decreased in irisin production suggested that there were negative effects on cell multiplication and viability, and that irisin could modulate the intracellular activity pathways for the mechanisms of cell differentiation.

Keywords: Endothelin, Cell culture, Irisin, Biotechnology, Skeletal muscle

Funding: FAPESP (Grant #2019/27364-9)