OPTIMIZATION AND APPLICATION OF CELL-BASED ASSAYS FOR RAPID, ACCURATE AND SENSITIVE DETERMINATION OF IGF-I BIOACTIVITY

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Growth factors play a major role in cell growth and proliferation and are thus used to enrich cell growth media. Insulin-like growth factor-I (IGF-I) is a potent mitogen which is an attractive alternative to the controversial use of serum and insulin in cell culture. Recent biotechnological advances in South African research have led to the development of novel technologies to produce IGF-I of high purity at low cost, thereby enabling the widespread availability of IGF-I on the commercial market. However, little is known about the bioactivity of this product as well as how it fared against commercial IGF-I and insulin. Thus, this study was aimed at determining the bioactivity of this novel IGF-I product using the UptiBlue™ assay and to critically compare this assay with other traditional cell proliferation assays as indirect measurement of IGF-I bioactivity. The bioactivity of recombinant IGF-I, commercial IGF-I and insulin were determined using three cell proliferation assays namely; the crystal violet, MTS and UptiBlue™ assays. Each of the assays were optimized with regard to cell number and dye incubation time for Balb/c 3T3 fibroblast, C2C12 mouse myoblast and CHO cell lines. The data obtained demonstrated that the UptiBlue™ assay was more sensitive than the other two traditional assays for rapid indirect measurement of IGF-I bioactivity. In addition, the locally produced recombinant IGF-I displayed significantly higher bioactivity (EC_{50} = 8-21 ng/ml), compared to insulin (EC_{50}= 10-20 µg/ml) and also compared favourably well with commercially available IGF product (EC_{50}= 3-4 ng/ml). Thus, it can be concluded that this locally produced South African recombinant IGF-I is a potent promoter of cell growth based on the observed high bioactivity obtained at low concentrations in serum free media. It could therefore be used as, a suitable and cost effective alternative growth factor for supplementation of serum free media for culturing mammalian cells.