

P-89

ESTABLISHMENT OF *IN VITRO* BIOASSAY FOR TRANSFORMING GROWTH FACTOR (TGF- β)

Mi-Sung Kim, Seong-Min Ahn and Aree Moon

College of Pharmacy, Duksung Womens University, Seoul 132-714, Korea

Transforming growth factor- β (TGF- β), a hormonally active polypeptide found in normal and transformed tissue, is a potent regulator of cell growth and differentiation. In this study, we wished to establish an *in vitro* bioassay system to seek the most sensitive method that can measure TGF- β activity. We have examined anti-proliferative activity of human TGF- interim standard (89/514) obtained from National Institute for Biological Standards and Control (NIBSC, UK) in three different cell lines: MCF10A human breast epithelial cells, H-ras transformed MCF10A human breast epithelial cells and CCL-64 mink lung epithelial cells. Among the cell lines tested, CCL-64 cell proliferation were the most sensitively inhibited by treatment of TGF- β in a dose-dependent manner. We then compared two commonly used assays for cytotoxicity: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide) assays. XTT assay, when the soluble product was detected at 490 nm, was more sensitive to the treatment of TGF- β dose-dependently. To seek the appropriate cell number for the TGF- β bioassay, 1104, 1105 and 1106 cells were plated in a 96-well plate. Cell number of 1105 gave the most desirable pattern for anti-proliferative activity of TGF- β . When the incubation time for TGF- β treatment was tested, 24 hr incubation at 37°C, 5 % CO₂ was suitable. Taken together, we have found the experimental protocol which gives the most sensitive quantitation of biological activity of TGF- β : 1105 CCL-64 cells were plated on a 96-well plate and the media was changed to serum free media (phenol red-free) containing various concentrations of TGF- β in pg/ml. Following 24 hr incubation, XTT was treated for 4 hr at 37°C, 5% CO₂, then absorbance at 490 nm was determined.