OPTIMIZATION OF THE IN VITRO POTENCY ASSAY OF HUMAN GRANULOCYTE-COLONY STIMULATING FACTOR

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Key words: Optimization, Potency Assay, G-CSF

Introduction. Granulocyte-colony stimulating factor (G-CSF) is a glycoprotein that plays a central role in survival, proliferation and differentiation of granulocytes; it is useful in the treatment of neutropenia in cancer patients (1). To evaluate its potency, an in vitro bioassay based on the proliferation of murine myeloma cells (M-NFS-60) is performed (2). Such proliferation is determined indirectly from the mitochondrial reduction of tetrazolium salt MTT to formazan, which is then measured in a spectrophotometer (3).
The aim of this research was to study the effects of different G-CSF concentrations, different amounts of M-NFS-60 cells used per well, different incubation periods, and different MTT concentrations over the formazan production, in order to determine optimal conditions for the in vitro potency assay.

Methods. G-CSF was dissolved in RPMI-1640 medium at (UI/mL): 200, 100, 50, 25, 12.5, 6.2, 3.1, 1.6 and 0.78. The M-NFS-60 cell suspensions were prepared at (cells/well) :350, 3500, 35000, and 79000. MTT solutions were used at (mM): 0.5, 2.0, and 3.75. 50µl of each G-CSF supplemented medium were placed, in triplicate, in a microplate well, and then 50 µl of each cell suspension were added. Two 96 well microplate were incubated at 36°C $\pm$ 1 °C for 48 and 72 hours in a humidified incubator using 6 $\pm$ 1 % CO_2. 20 µl of each MTT solutions were added to the wells and the plates were reincubated for 4 hours. The formazan crystals were then solubilized with dimethylsulfoxide and their absorbance determined at 520 nm. Data analysis was performed on the Graphad PRISM software.

Results. An ANOVA determined that all four factors in the study were statistically significant (pvalue<0.05, 95% confidence). For the G-CSF concentration, the means plot shows the typical dose-response curve for a bioassay (Fig 1a), with a linear range between 0.78 and 25 UI/mL; a decrease in the response for greater concentration values indicates a probable G-CSF receptor saturation in the cells. There was a greater response with 35000 cells per well as observed in Fig. 1b. Incubation for 72 hours produced a greater response since the cell cultures reached a maximum cell proliferation (Fig.1c). Furthermore, MTT at 2.0 mM concentration provided better absorbance readings (Fig.1d) since it probably avoided the mitochondrial enzyme inhibition.

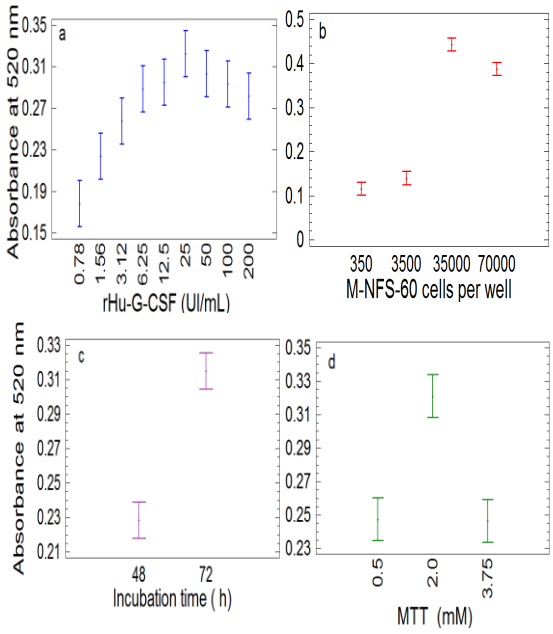

Fig.1 Means plot of biological response to different (a): G-CSF concentrations, (b) amount of cells per well, (c): incubation periods, and (d) MTT concentrations.

Conclusions. The conditions that optimize the potency in vitro assay for G-CSF due to the increase of formazan production are: 0.78-25 UI/mL G-CSF, 35000 cells per well, 72 h incubation period, and 2.0 mM MTT solution.

Acknowledgements. To Laboratorios Cryopharma, S.A de C.V. CONACYT's grant project number 185923.

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