OPTIMIZATION OF THE HUMAN MACROPHAGE-COLONY STIMULATING FACTOR EXPENDITURE IN A M-NFS-60 CELL CULTURE

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Key words: Optimization, M-CSF, M-NFS-60

Introduction. The murine myeloblastic leukemia cell line (M-NFS-60) is used in various cytokine in vitro potency assays. Their proliferation and viability are dependent of interleukins and growth factors. The ATCC® suggest a 62 ng/mL macrophage-colony stimulating factor (M-CSF) concentration in the M-NFS-60 cells culture medium, and its exchange or the addition of fresh media every 48 hours (1). M-CSF is an effective high-cost glycoprotein, whose mean effective dose is less than 2 ng/mL (2), suggesting that a lower concentration could be used for the proliferation of M-NFS-60 cells.
The aim of this study was to optimize the concentration of M-CSF in the cell culture medium used for the growth of the M-NFS-60 cell line while reducing the assays costs.

Methods. RPMI-1640 medium, containing 10% FBS and 0.05 mM 2-mercaptoethanol was supplemented with M-CSF (Sigma) at the following concentrations (ng/mL): 1.5, 5.4, and 11.6. M-NFS-60 cells were cultivated with each one of the supplemented media in T-flask at 37°C and 5% CO_2 after being inoculated at 2.67 x 10^5 cells/mL. A control batch (0 ng/mL) was used as a reference. Samples were taken every 24 hours; cell density and viability were estimated using a hemacytometer by the trypan blue dye exclusion method.

Results. The observed behavior of the cells in the 1.5 ng/mL flask was similar to those in the control batch (k_d= 0.05 h^{-1}) (Fig.1); cells viability decreased rapidly in both cultures (Fig.2), proving the cell line's dependency on M-CSF. On the other hand, both 5.4 and 11.6 ng/mL concentrations promoted good cell proliferation (μ_{max}=0.03 h^{-1}); with 11.6 ng/mL the cells continued their growth until they reached a 2.14 x 10^6 cells/mL density (Fig.1) with 92% viability after 72 hours (Fig.2). This suggests that it is possible to cultivate M-NFS-60 cells with a 11.6 ng/mL M-CSF concentration while performing the media exchange procedure after 72 hours instead of a 62 ng/mL concentration exchanged every 48 hours as suggested by the cell line provider.

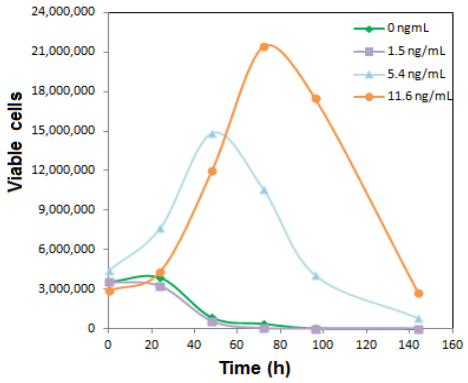

Fig.1 Viable cell density of the M-NFS-60 batch cultures at different M-CSF concentrations.

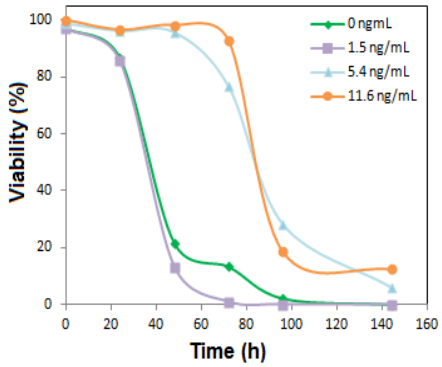

Fig.2 Cell viability of the M-NFS-60 batch cultures at different M-CSF concentrations

Conclusions. M-CSF at 11.6 ng/mL promotes and maintains the M-NFS-60 cell line proliferation until 72 hours, reducing by 5.4 times its expenditure as well as the usage of cell culture medium during the in vitro potency assay.

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

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