

PRELIMINARY RESULTS ON ANTIPROLIFERATIVE EFFECT OF NEW DEACETYLASE INHIBITORS ON HUMAN LEUKEMIA CELL LINES

Vitali Federica^a, Turriziani Mario^b, D'Alfonso Rossella^b, Lombardo Marco^b, Valenti Sergio^c, Mai Antonello^c, Tita Beatrice^a

^aDepartment of Human Physiology and Pharmacology, University of Rome "Sapienza"

^bDepartment of Internal Medicine, University of Rome "Tor Vergata"

^cDepartment of Pharmaceutical Studies, University of Rome "Sapienza"

The acetylation and deacetylation of histones mediated by histone acetylases and deacetylases influence DNA accessibility to factors regulating replication, repair, and transcription. Histone deacetylase inhibitors (HDACi) have been shown to be potent inducers of growth arrest, differentiation, and/or apoptotic death of transformed cells by altering the transcription of a small number of genes (1). The HDACi, suberoylanilide hydroxamic acid (SAHA), has multiple antitumor effects against a variety of human cancers. SAHA can cause growth arrest and death of a broad variety of transformed cells both in vitro and in tumor-bearing animals at concentrations not toxic to normal cells. SAHA is in clinical trials as monotherapy and in combination with various anticancer drugs. In phase 1/2 clinical trials, SAHA has shown significant anticancer activity in both hematologic and solid tumors at doses well tolerated by patients (2). In our laboratory, a series of SAHA analogues have been synthesized. The essential chemical characteristic of SAHA-analogues is the presence of a substituted *meta*- or *para*-acylamido cinnamyl hydroxamide moiety. The antiproliferative, cytotoxic and apoptogenic activities of SAHA-analogues on human leukemia cell lines has been investigated. Yurkat (lymphoblastic leukemia T cells; p53 null) and HL-60 (acute promyelocytic leukemia cells; p53 deficient) were maintained in RPMI 1640, 10% FBS. Cells (5×10^5 cells/ml) were treated with SAHA (0.5-5-50 μ M) or equal concentrations of new HDACis or DMSO vehicle (0.02%) for 6-15-24-48 hours. The growth inhibitory effects were evaluated by tetrazolium-dye and trypan blue exclusion assays (3, 4). Cell cycle progression and apoptosis were measured by flow cytometry using propidium iodide and fluorescein isothiocyanate-labeled Annexin V (5, 6). Experimental data represent the results obtained from three independent experiments and expressed as mean \pm SEM. Statistical analysis was performed utilizing the ANOVA, followed by Bonferroni *t*-test. The exposure suppressed the proliferation of leukemia cells in a time- and dose-dependent manner. In particular, the inhibitory effect did not appear to correlate on a direct cytotoxic action of the drugs. In fact, the viability index was not significantly modified. The antiproliferative effects were due to apoptotic cell death caused by G2/M-phase arrest and to a lesser extent to G1 arrest. The findings from the present study suggest that the new HDACis might possess potent antineoplastic activity having antiproliferative, cytotoxic and apoptogenic activity against leukemic cells. At present, considerable attention focuses on the role of epigenetic modifications in carcinogenesis and the development of drug resistance. SAHA analogues are promising new anti-leukemia agents.

(1) La Thangue N.B. (2004) *J. Chemother.* Nov;16 Suppl 4: 64-67.

(2) O'Connor O.A. *et al.* (2005) *J. Clin. Oncol.* 24: 166-173.

(3) Hansen M.B. *et al.* (1989) *J. Immunol. Methods* 12; 119 (2): 203-210.

(4) Patterson M.K. (1979) *Methods in enzymology* (eds Jacoby & Pastan) vol. LVIII: 141-152.

(5) Shirin H. *et al.* (1999) *Cancer Research* 59: 2277-2281.

(6) Ji J. *et al.* (2006) *Leuk. Lymphoma.* 47 (12): 2617-2624.