IMMUNOPHARMACOLOGY OF PIDOTIMOD: EFFECT ON NATURAL KILLER CELL ACTIVITY AND THYMOCYTE CELL DEATH.

G. Migliorati, G. Coppi*, F. D'Adamio, C. Pagliacci^, I. Nicoletti^ and C. Riccardi.
Institutes of Farmacologia Medica and ^Clinica Medica I, Perugia University Medical School, 06100 Perugia, and *Poli Research Center, 20089 Rozzano, Italy.
Key words: Pidotimod, Natural Killer, Apoptosis.

We have studied the posssible effect of the immunomodulating agent Pidotimod (pyroglutamoil-tiazolidin-carboxylic acid) on mouse Natural Killer (NK) cell activity and glucocorticoid(GC)-induced thymocyte apoptosis.

NK cell activity is an important immune function involved in the control of tumor cell growth and of infectious diseases, as well as in the regulation of growth and differentiation of normal non neoplastic tissues (1). To analyze the possible effect of Pidotimod on NK activity, 20-week-old C3H/HeN mice were treated with different dose of the drug (ranging from 200 to 50 mg/Kg ip) for 5 days. After tratment, animals were sacrified and the NK activity was measured with the classical *in vitro* cytotoxicity assay against the NK-sensitive YAC-1 target (data are expressed as % specific cytotoxicity). Data shown in Table I clearly indicate that *in vivo* treatment with Pidotimod (200 mg/Kg ip for 5 days) results in a significant augmentation of NK activity.

TABLE I

Treatment	% CYTOTOXICITY		
	100:1	50:1	25:1[a]
Medium	12.5	9.2	7.0
Pidotimod (200 mg/Kg ip)	18.8	15.3	11.5

[a] Effector to target ratio.

Apoptosis is an important physiological mechanism involved in tissue remodelling during embryogenesis and in tumor regression in adults. It is characterized by extensive DNA fragmentation, followed by a decrease of nuclear content of DNA and cell death (2). Apoptotic cell death is also responsible of the elimination of self-reactive T-lymphocytes in the thymus and both Ag-TCR-interaction as well as interaction with GC induce apoptotic cell death suggesting that both are physiological events involved in the control of T-cell development.

We performed *in vitro* experiments to analyze the possible protective effect of Pidotimod on Dexamethasone (DEX)-induced thymocyte apoptosis. For that purpose thymocytes (10^6

1043–6618/92/26II0154–02/$03.00/0

cells/ml) of 4-week-old C3H/HeN mice were cultured *in vitro* for 24h with medium alone, medium plus DEX (10^{-7} M) or medium plus DEX plus Pidotimod at different concentrations (ranging from 400 to 10 μg/ml). After incubation cells were treated with propidium iodide and the levels of apoptosis evaluated by flow cytometry as previously described (3). Results of a representative experiment are shown in Table II.

TABLE II

Treatment	% APOPTOSIS[a]
Medium	9.5
DEX	88.7
DEX + Pidotimod (200 μg/ml)	38.2

[a] As evaluated by flow cytometry as previously described (3).

As shown in Table II DEX induced a high percentage of apoptosis and Pidotimod was able to inhibit such a phenomenon. Moreover, this inhibition appeared to be dose-dependent and was also exerted against TPA or Ca^{++}ionophore-induced apoptosis (data not shown).

Taken together the above data suggest that Pidotimod is able to stimulate the NK activity and also to counteract the lymphocyte apoptotic cell death.

REFERENCES.

1) Riccardi C., Puccetti P., Santoni A. and Herberman R.B. Rapid in vivo assay of mouse natural killer activity. JNCI 1979; 53:1041-1045.

2) Willie A.H., Kerr J.F.R., Currie A.R. Cell death the significance of apoptosis. Int. Rev. Cytol. 1980; 68:251-265.

3) Nicoletti I., Migliorati G., Pagliacci M.C., Grignani F., Riccardi C. A rapid and simple method for measuring thymocytes apoptosis by propidium iodide staining and flow cytometry. J. Immunol. Methods. 1991; 139:271-279.