MITOGENIC PROPERTIES OF UKRAIN (NSC-6315170) ON HUMAN PERIPHERAL BLOOD MONOCYTES: CLINICAL IMPLICATIONS

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Summary: *Using a cell-proliferation assay, the authors examined and compared the mitogenic effects of Ukrain and phytohaemagglutinin (PHA) on human peripheral blood mononuclear cells (PBMCs), as well as their synergic effects. It was found that even a short period of pretreatment of the cell with Ukrain had a potent synergic effect on PHA mitogenesis resulting in significantly higher cell stimulation indices than those of PHA alone. Moreover, it was found that a short period of PHA treatment of the cells is almost imperative for Ukrain to exert its mitogenic effects. The mitogenic effect of Ukrain on human PBMCs is consistent with a previous clinical report which found that circulating lymphocytes were significantly increased in cancer patients treated with Ukrain. Thus the* in vitro *assay used in these studies may serve as a prognostic assay for potential patient responsiveness to Ukrain treatment, as well as a clinical parameter during Ukrain therapy.*

Introduction

Mitogens are compounds that induce polyclonal proliferation of T and B lymphocytes. Common mitogens are proteins, for example lectins, the majority of which are derived from plants and bind to carbohydrates on lymphocyte cell surface membranes. Some lectins stimulate preferentially proliferation of T lymphocytes, B lymphocytes or both. Phytohaemagglutinin (PHA) and Concanvalin A are mitogenic to T lymphocytes, whereas the lectins derived from the crab *Homarus americanus* and from the slime mould *Dictyostelium purpureum* are selectively mitogenic to B lymphocytes only; hence they are referred to as T-cell and B-cell mitogens, respectively. On the other hand, pokeweed mitogen is able to stimulate both T and B lymphocytes (1).

The lipopolysaccharide (LPS) component of the Gram-negative bacteria cell wall mainly functions as a B-cell mitogen. The mitogenic activity is due to its lipid A moiety which binds to a 73 kDa protein (2, 3) on lymphoreticular cells (4, 5).

Materials and methods

Peripheral blood mononuclear cells. Human PBMCs were isolated from heparinized venous

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blood obtained from healthy donors. The blood was diluted with an equal volume of PBS containing 1mM EDTA, pH 7.4, and was layered over Histopaque 1077 (Sigma Chemicals, St. Louis, MO). The tubes were centrifuged at 2,000 rpm for 30 min. The interface layers containing lymphocytes were collected and washed three times with RPMI tissue culture medium.

Cell-proliferation assay. The purified PBMCs were seeded in 96-well microtitre plates at a cell density of $1x10^5$ cells/well in RPMI medium containing 10% heat inactivated FCS, 2mM L-glutamine, 50 µm 2-mercaptoethanol, 100 U/ml penicillin and 100 µm/ml streptomycin for 72 h followed by a 3-h incubation with 1 µCi of ^{3}H-thymidine. The ^{3}H-thymidine incorporated by the cells in the culture medium alone and in the presence of various concentrations of PHA and/or Ukrain was measured and compared in terms of stimulation of thymidine uptake. The stimulation index was calculated by dividing the ^{3}H-cpms of lymphocytes cultured in the presence of PHA or Ukrain by that in tissue culture medium alone. Cell proliferation was also examined for PBMCs which were preincubated with an agent of a defined concentration followed by incubation with a second agent at various concentrations.

Results and discussion

In view of the clinical evidence obtained from cancer patients treated with Ukrain, which showed a significant increase in the CD-2, CD-4 and CD-8 lymphocyte populations (6), *in vitro* evaluation of the mitogenic effects of Ukrain is warranted. The thirty-six cancer patients in the clinical study cited were in stage III of malignancy. Seven of them had ovarian cancer, thirteen had rectal cancer, eight had breast cancer, four had skin cancer and four had liver cancer. The clinical study raised the possibility of developing an *in vitro* assay that may serve as a prognostic indicator for the outcome of patients with advanced cancers treated with Ukrain. For this purpose, human PBMCs were used to evaluate possible *in vitro* mitogenic effect of Ukrain.

Figure 1 shows the stimulation indices obtained by preincubating PBMCs in 5.0 µg/ml of Ukrain for 1 h followed by 72 h incubation with various concentrations of PHA. The maximum stimulation index was obtained when PBMCs were preincubated with Ukrain followed by incubation with 0.1 µg/ml of PHA for 72 h. It is noteworthy that preincubation of PBMCs with Ukrain induced higher stimulation indices than those of PBMCs incubated with PHA alone, i.e., without preincubation with Ukrain. These results demonstrated that even a short period of preincubation of the cells with Ukrain (1 h) resulted in a higher stimulation index than that of cells incubated with the T-cell mitogen PHA alone for a much longer time period (72 h).

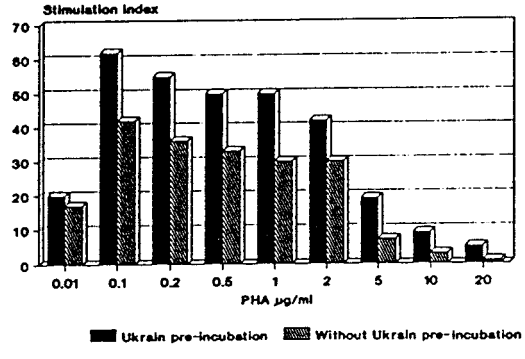

Fig. 1 Human peripheral blood mononuclear cells were isolated from blood of healthy donors as described in Materials and methods. The cells were either incubated in RPMI tissue culture medium containing 5.0 µg/ml of Ukrain or the medium alone without Ukrain for 1 h. Subsequently, the cells were incubated in the media containing indicated concentration of PHA for 72 h. The ^{3}H-thymidine incorporation of the cells was measured and their stimulation indices calculated as described in Materials and methods. The data are presented as the mean of nine individual blood donors.

Since the highest stimulation index was obtained by preincubation of PBMCs with 5.0 µg/ml of Ukrain followed by the incubation with 0.1 µg/ml of PHA for 72 h, this optimal concentration of PHA (0.1 µg/ml) was used in subsequent experiments. Figure 2 shows the stimulation indices obtained by preincubation of the cells with the optimal con-

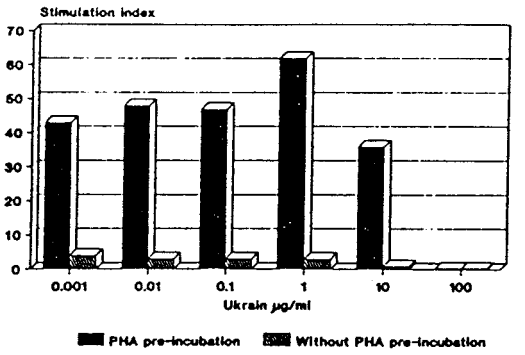

Fig. 2 Human peripheral blood mononuclear cells were isolated from blood of healthy donors as described in Materials and methods. The cells were either incubated in RPMI tissue culture medium containing 0.1 µg/ml of PHA or the medium containing indicated concentrations of Ukrain for 72 h. The ^{3}H-thymidine incorporation of the cells was measured and their simulation indices were calculated as described in Materials and methods. The data are presented as the mean of nine individual blood donors.

centration of PHA (0.1 µg/ml) for 1 h, followed by incubation of the cells with indicated concentrations of Ukrain for 72 h. As shown in Fig. 2, the highest stimulation index was obtained at 1.0 µg/ml of Ukrain. It is of interest that the highest stimulation index of 62 was equal to that obtained when the cells were preincubated with 5.0 µg/ml of Ukrain followed by the incubation of 0.1 µg/ml PHA for 72 h (Fig. 1). Preincubation of PBMCs with PHA (0.1 µg/ml/1h) followed by incubation with Ukrain, gave relatively uniform stimulation indices at Ukrain concentrations of 0.001, 0.01 and 0.1 µg/mL with the highest stimulation index of 62 at 1 µg/ml, followed by an index of 36 at 10 µg/ml of Ukrain (Fig. 2). Moreover, we found that PBMCs cultured in the various concentrations of Ukrain without preincubation in PHA, i.e., preincubation in medium only, gave negligible stimulation indices of 4.0 or less (Fig. 2). Thus, a short period (1h) of preincubation of the cells in PHA was imperative in order to obtain high stimulation indices from the cell subsequently incubated with Ukrain. Similarly, short 1 h preincubation of PBMCs in 5.0 µg/ml of Ukrain followed by 72 h incubation of culture with various concentrations of PHA, also produced higher stimulation indices than PHA alone (Fig. 1).

The broad range of Ukrain concentrations, 0.001-10.0 µg/ml, which induced high stimulation indices for PBMCs pretreated with PHA for 1 h, may be of clinical relevance in that this compound induces an increase in tumour antigen primed lymphocytes. This interpretation is in agreement with the clinical finding previously reported (6) which showed a significant increase in circulating lymphocytes in patients in advanced stages of malignancies treated with Ukrain.

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