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INFLUENCE OF UKRAIN ON IMMUNOLOGICAL BLOOD PARAMETERS *IN VITRO* AND *IN VIVO*

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Summary: *Immunological changes are often seen in subjects suffering from oncological disease. Decreased NK activity, alterations of the T4/T8 quotient, decreased phagocytic activity, low reactivity and decreased recognition of the "foreign" are common features seen in these patients. Cytostatic therapy (chemotherapy and radiation therapy) very often enhance these negative properties, thus limiting therapeutic possibilities by highly toxic sequels. Ukrain, being cytostatic and immune-stimulating and -modulating, has no adverse effects on the organism.*

Introduction

Ukrain, (Tris[2-[5bS-(5ba, 6b, 12ba)-5b,6,7, 12b,13,14-Hexahydro-13-methyl[1,3]benzodioxolo[5,6-c]-1,3-dioxolo[4,5-i]phenanthridinium-6o-l]-Ethaneaminyl]Phosphinesulfide6HCL)**, a semisynthetic compound from *Chelidonium majus L.* alkaloids and thiophosphoric acid triaziridide, has cytotoxic (1), mediated cytotoxic (2), immunostimulating and -modulating properties (3). In the clinic it can induce partial and complete remissions in oncological patients (4). The average direct cytotoxicity lies within 10^{-4} µg/ml expressed in 100% growth inhibition. This concentration has no inhibiting influence on normal cells tested to date (5). ThioTepa is a well known cytostatic used in polychemotherapy which may induce remissions in some kinds of human tumours. Whereas Ukrain highly significantly increases in a dose-dependent manner the monocytic leukocyte compartment in Wistar rats of both sexes, the control substance ThioTepa had not shown such properties. The stimulating activity of Ukrain and phytohaemagglutinin (PHA) in combination was evaluated in one assay showing a synergistic effect of both substances.

Material and methods

93 Wistar rats (SPF) were used for this study. The initial age was 16 weeks for both male and female rats. The initial mean body weight was 290g and 260g, respectively. The experiments were carried out according to the guidelines of Good Laboratory Practice (GLP).

The animals were housed individually in

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Makrolon cages (type III) at a room temperature of 21 ± 1°C. Haematological investigations were carried out in all dose groups (controls with NaCl, 90, 100, 150, 200 and 300 mg/kg/bw) including haemoglobin according to Rick, erythrocytes and leukocytes with the Coulter Counter, differential blood cell count with Giemsa staining, reticulocytes with brilliant cresyl blue staining and monocytes/large granular lymphocytes (LGL) cells with ANAE/PAS according to Romeins in "Colorimetric Methods in Histopathology". The examinations were performed on days 0, 3 and 14.

Ukrain and PHA were tested in a ^3H thymidine test on T lymphocytes to evaluate the stimulation index in doses from 0.01 to 20 µg/ml. Ukrain alone could not stimulate the lymphocytes of a healthy donor but PHA combined with Ukrain enhanced the stimulating capability.

Results

At 90 to 300 mg/kg/bw there was a significant reticulocytosis. A slight hypochromic anaemia was noticed at 200 mg. The Re increase was dose-dependent: 70% at 90 and 260% at 150 mg of Ukrain. In the same range a relative granulocytosis was registered; this did not seem to be dose-related: 60% PMN increase at 150 mg. However, there was a marked, highly significant and clearly dose-related increase in the monocytic leukocyte compartment in the order of 75% at 90 and of 475% ($p \subseteq 0.01$) at 150 mg. Most of the cells of this lympho-monocytic fraction showed a strong α-naphtylacetate esterase (ANAE) reaction. They also stained weakly with PAS. In the Giemsa colouration they contained azurophilic granules, classifying them morphologically as LGL cells which are known to comprise the cells with NK activity (Table I and Fig. 1). By day 14 all values had returned to normal with the exception of the LGL cells, which dropped from +475% on day 3 to +100% on day 14 after a single dosage of 150 mg Ukrain kg/bw. The increase in LGL cells was accompained by a dose-related relative lymphocytopaenia. Whatever the dose of Ukrain, no changes were registered in absolute leukocyte counts. 1/10 and 1/100 of the LD_{17} of ThioTepa chosen for the haematological studies did not induce any changes in blood cell counts on day 3. Ukrain does not stimulate healthy T lymphocytes alone (Table II and Fig. 2), but has strong "cooperative" action when given together with the stimulating compound PHA.

Table I *Values of haematological changes on day 3 after application in percent increase to day 0*

Ukrain	Reticulocytosis	Granulocytosis	LGL* cells
0 mg	0	0	0
90 mg	70	50	75
100 mg	120	59	82
150 mg	260	60	475
200 mg	114	65	400
300 mg	40	59	365

* large granular lymphocyte

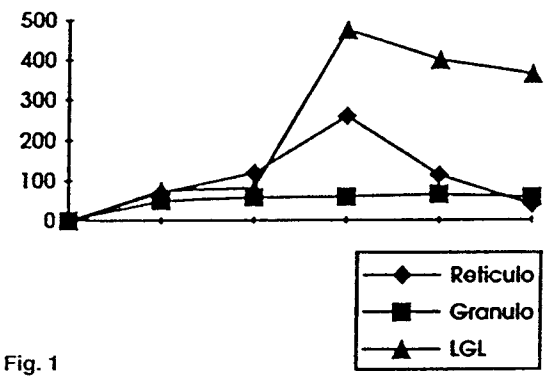

Fig. 1

Discussion

Ukrain stimulates different subsets of the haematopoietic and immunological systems (6). In this experiment reticulocytosis is induced as a possible sign of stimulation of certain stem cells or of general activation of the erythropoietic system. As no changes in the absolute leukocyte counts could be demonstrated, it may be postulated that by the

Table II - *T-lymphocyte-stimulation of Ukrain, phytohaemagglutinin (PHA) and synergy of both.*

	PHA-Ukrain	PHA	Ukrain-PHA	PHA
20/100	5	1	0.3	0.3
	9	3	0.9	36
	19	7	3	62
2/0.1	42	30	3	47
1/0.01	50	30	3	48
0.5/0.001	50	33	4	43
0.2	55	36		
0.1	62	42		
0.001	20	17		

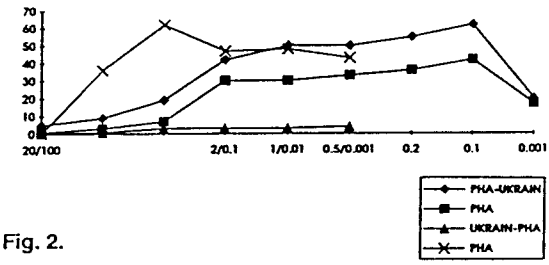

Fig. 2.

action of Ukrain only strong modulating properties, e.g., a dislocation of the different subsets, happened in this experiment. Compared to a study of healthy volunteers, Ukrain was unable to change the leukocyte subsets significantly in healthy subjects with normal values (7). It is therefore astonishing that the stimulation in healthy laboratory rats may be achieved up to 475% of the normal values. Stimulation comparable to that gained in these experiments was seen *in vitro* (6), inducing apoptosis in cancer cells (8) and clinically in immune compromised cancer patients (3, 4). In one experiment with T-lymphocytes from a healthy donor, Ukrain was unable to stimulate the ^{3}H thymidine incorporation alone, if normal values are non existent, being a specific and not an unspecific stimulator. In any case, in combination it enhances the action of PHA.

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