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IN VITRO EFFECTS OF *CHELIDONIUM MAJUS L.* ALKALOID THIOPHOSPHORIC ACID CONJUGATES (UKRAIN) ON THE PHENOTYPE OF NORMAL HUMAN LYMPHOCYTES

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Summary: *Several plant-derived drugs are used in medical oncology (1-3). The thiophosphoric acid-alkaloid derivative from the plant* Chelidonium majus L. *(Ukrain) has been produced since 1978 by Nowicky. (Austrian Patent No. 354644, Vienna, 25 January 1980). It has been reported that this drug may exert therapeutic effects in cancer patients by modulating their immune systems (4, 5-8). The aim of this study was to evaluate* in vitro *the possibility that Ukrain may influence the phenotype of lymphocyte subpopulations isolated from healthy donors.*

Introduction

It has been reported that the semisynthetic derivative of *Chelidonium majus L.* alkaloids conjugated to thiophosphoric acid (Ukrain) exert immunomodulatory effects in cancer patients. The aim of the present study was to evaluate the phenotype of lymphocyte subpopulations isolated from healthy donors after incubation with the semisynthetic drug Ukrain. Lymphocytes were isolated from peripheral blood of healthy donors and incubated *in vitro* for 30 min in the presence of the drug (final concentration 340μM). Lymphocyte subpopulations were quantitated by immunofluorescence using monoclonal antibodies against total T-cells, T-helper and T-suppressor cells before and after incubation with Ukrain. The T-helper/T-suppressor ratio was also calculated. Incubation of normal human lymphocytes with Ukrain resulted in:

a an increase of lymphocytes expressing T-helper phenotype.
b a decrease of lymphocytes with T-suppressor phenotype.
c an increase of T-helper/T-suppressor ratio.

These results indicate that Ukrain has a direct immunomodulatory effect on human lymphocytes. The mechanism of this phenomenon is unknown.

Materials and methods

The study was performed on lymphocytes isolated from peripheral blood of 12 healthy volunteers. The cells were isolated on Ficoll-Paque density gradient centrifugation (1.077 g/ml) (9). Viability of cells was determined by 0.1% trypan blue staining, and found to be ≥96%.

Lymphocyte subpopulations were quantitated by immunofluorescence using monoclonal antibodies against total T-cells (Leu 1), T-helper cells (Leu

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3a+3b) and T-suppressor cells (Leu 2a) (Becton-Dickinson, USA). Subsequently the cells were treated with FITC-conjugated rabbit F/ab/$_2$ fragments anti-mouse IgG, washed in PBS and mounted on slides using polyvinyl-alcohol (PVA) and glycerol (10). In control preparations, PBS or normal mouse serum was used instead of monoclonal antibodies. The lymphocytes at a concentration of 1.5 x 10^6 cells were incubated with Ukrain in a concentration of 340μM for 30 min at room temperature. Viability of the cells did not change and was estimated as ≥96%. The percentage of total T cells, T-helper and T-suppressor cells, as well as the TH/TS ratios, were compared before and after incubation with Ukrain. All lymphocyte preparations were evaluated in an Opton type III photomicroscope by means of incident light excitation and were statistically evaluated using Student's t-test.

Results

The effects of Ukrain on the total number of T-cells, T-helper and T-suppressor cells are presented in Figs. 1, 2, 3 respectively. There were no significant differences in the amount of total T-cells after incubation of lymphocytes in 340μM of Ukrain for 30 min at 22°C. A negligible increase in the percentage of fluorescent cells was, however, noted in six donors (Fig. 1).

When the percentages of T-helper lymphocytes, before and after *in vitro* Ukrain treatment, were determined, an increase of, on average, 32% was noted in the lymphocyte population in 7 of the 12 donors (Fig. 2).

The percentage of T-suppressor lymphocytes after incubation with Ukrain showed a clear decrease of these cells in 10 out of 12 donors. The decrease in TS cells was 26% (Fig. 3).

When the T-helper/T-suppressor cell ratio was calculated, before and after incubation with Ukrain, a clear increase was found in 6 lymphocyte populations, with a minor increase in 3 donors and a decrease in 3 out of the 12 donors (Fig. 4). The influence of Ukrain on the phenotype of lymphocyte subpopulations in individual donors is presented in Table I.

A comparison between all studied parameters of cellular immunity before and after incubation with Ukrain is summarized in Table II. An increase in the percentage of pan-T lymphocytes, as well as in the

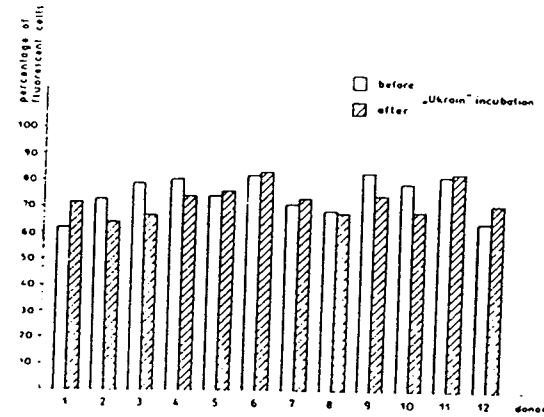

Fig. 1 Influence of "Ukrain" on total T cell population in healthy donors (*in vitro* studies).

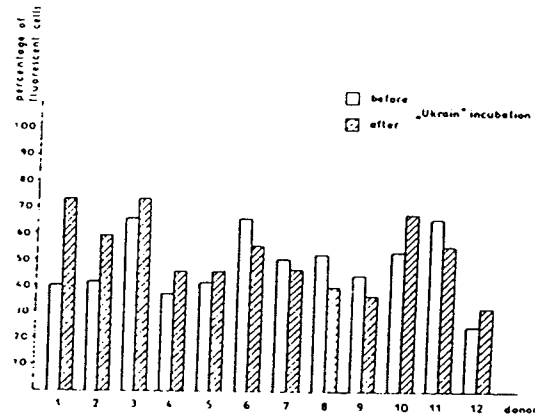

Fig. 2 Influence of "Ukrain" on T-helper cells in healthy donors (*in vitro* studies).

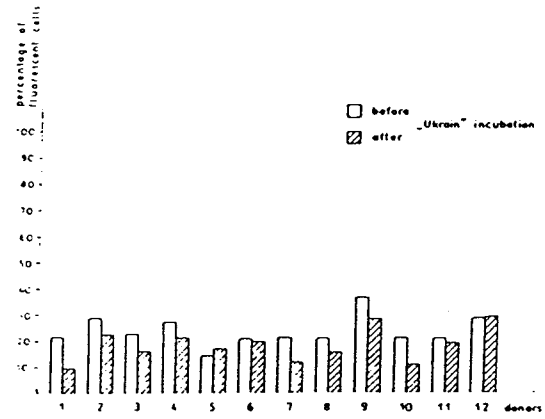

Fig. 3 Influence of "Ukrain" on T-suppressor cells in healthy donors (*in vitro* studies).

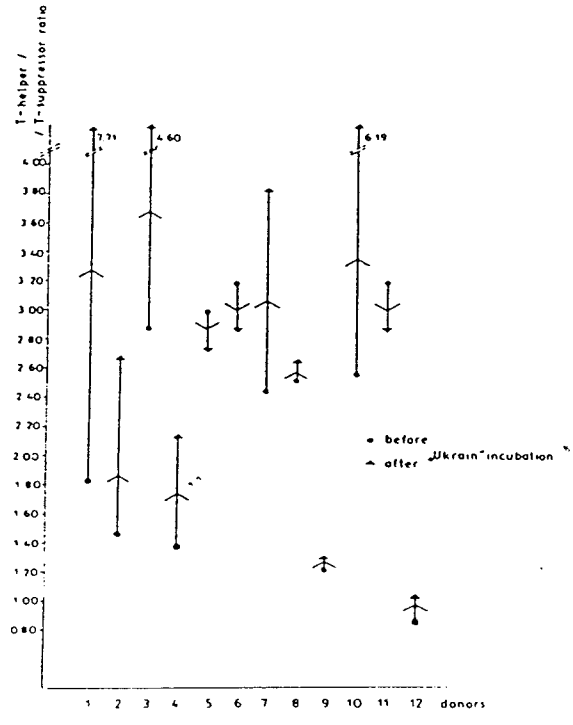

Fig. 4 Influence of "Ukrain" on T-helper/T-suppressor ratio in healthy donors (*in vitro* studies)

T-helper phenotype, was found in 50% and 58.3% of studied cases, respectively. Furthermore, a decrease was noted in the percentage of lymphocytes with T-suppressor phenotype in 10 out of the 12 donors (83.3%). These changes resulted in an increase of T-helper/T-suppressor cell ratios in 9 out of 12 lymphocyte donors (75%).

Discussion

The clinical need for agents to modify immune response in cancer patients has led to an increase in the search and development of biological substances with immunomodulatory activity (11–13). Preliminary clinical observations and the enhancement of cellular immunity in cancer patients treated with thiophosphoric acid alkaloid derivatives from *Chelidonium majus L.* (Ukrain) were indicative of an immunostimulatory effect (4, 5, 8, 15).

To evaluate the possible direct effect of Ukrain on cellular immune parameters the authors investigated the effects of Ukrain on lymphocyte phenotypes from 12 healthy volunteer donors. The dose of Ukrain for these studies was estimated in preliminary experiments according to the Liepins data (14). Liepins demonstrated that Ukrain enhances the cytolytic activity of primed spleen cells in a dose dependent manner and with a greater efficiency at a particular time (approximately 18 days) after alloimmunization in the mouse system (14).

In the present study, the prolongation of lymphocyte + Ukrain incubation time to 1 h did not cause significant changes in the percentage of positive reacting T-cell subpopulations compared to the 30 min incubation time. Incubation with Ukrain did not change the viability of studied cells. Prolongation of the incubation time for 24 h, as well as increase of Ukrain dose to 510μM, caused an increase in the percentage of dead cells to 20–25% (unpublished data).

The present findings (using 340μM of Ukrain concentration and 30 min preincubation time) indicated

Table I *The influence of Ukrain on behaviour of lymphocyte subpopulations in healthy donors* (in vitro *studies*)

No of donors	before Ukrain incubation				after Ukrain incubation			
	TH	TS	p-T	TH/TS	TH	TS	p-T	TH/TS
I	40.0*	21.5*	61.8*	1.81	73.2*	9.5*	71.7*	7.71
II	41.8	28.6	72.8	1.46	59.8	22.4	64.3	2.67
III	65.6	22.7	78.5	2.89	73.6	16.0	65.7	4.60
IV	37.1	22.9	80.1	1.38	45.7	21.1	73.7	2.17
V	41.6	13.9	74.5	2.99	45.6	16.8	75.4	2.71
VI	65.9	20.7	81.5	3.18	55.6	19.5	82.7	2.85
VII	50.7	20.8	71.2	2.44	46.5	12.2	72.9	3.81
VIII	51.9	20.7	68.1	2.51	39.9	15.2	67.6	2.63
IX	44.3	36.7	83.3	1.21	36.7	28.6	74.4	1.28
X	53.7	21.0	79.2	2.56	68.1	11.0	68.2	6.19
XI	65.9	20.7	81.5	3.18	55.6	19.5	82.7	2.85
XII	24.7	28.5	63.9	0.87	31.8	29.0	71.3	1.10

* percentage of positive cells (IF test)

Table II *The influence of Ukrain on behaviour of lymphocyte subpopulations in healthy donors* (in vitro *studies*)

		after Ukrain incubation			
	No of donors	Increase no/total	%	Decrease no/total	%
P-T CELLS	12	6/12 0.10>p>0.05	50.0	6/12 0.005>p>0.001*	50.0
T-HELPER CELLS	12	7/12 0.01>p>0.005*	58.3	5/12 0.005>p>0.001*	41/7
T-SUPPRESSOR CELLS	12	2/12 0.30>p>0.25	16.7	10/12 p<0.001*	83.3
T-HELPER/ T-SUPPRESSOR CELLS RATIO	12	9/12 0.05>p>0.025	75.0	3/12 p<0.001*	25.0

* statistically significant

Statistical analyses were based on the Student's t-test, where significance level was p<0.02

that the thiophosphoric acid derivatives from *Chelidonium majus L.* alkaloids produced direct immunomodulatory effects on T-lymphocyte subpopulations. The mechanism of this action remains unknown at this time.

The present study indicating the possibility of direct influence of Ukrain on T-cell subpopulations confirmed the earlier observations that *Chelidonium majus L* alkaloid-thiophosphoric acid derivatives (Ukrain) could be a good plant immunostimulator of cellular immunity in cancer patients (4–7, 15, 16).

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