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CLINICAL STUDIES OF UKRAIN IN HEALTHY VOLUNTEERS (PHASE 1)

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Summary: *Phase I of a clinical study of Ukrain was performed in 19 healthy outpatient volunteers. Their general clinical conditions were evaluated, as well as the following parameters: biochemical, haematological, immunological, electrolyte and trace elements, neopterin, immune complexes and non specific blocking factors. Ukrain was administered intramuscularly (i.m.) or intravenously (i.v.) every one, two or three days in doses of 5 to 50 mg for 7 to 40 days. In one case the drug was administered for three years in the dose of 5 to 50 mg/injection in repeated courses. During the investigation no significant changes were found in clinical states. During the intramuscular injections the volunteers felt only localized pain; some reported drowsiness, increased thirst and polyurea. There was a slight, insignificant increase in body temperature and negligible decrease of blood pressure in some cases. In conclusion, it can be said that Ukrain is well tolerated in healthy volunteers in the doses of 5, 10, 20, and 50 mg/injection, even during prolonged (up to three years) administration.*

Introduction

Ukrain is a semisynthetic derivative of *Chelidonium majus L.* alkaloid Tris {2-[5bS-(5bα,6β, 12bα)]-5b,6,7,12b,13,14-Hexahydro-13-methyl[1,3] benzodioxolo[5,6-c]-1,3-dioxolo[4,5-i] phenanthridinium-6-ol]-ethaneaminyl}Phosphinesulfide 6HCl (USA Patent No. 2,670,347). Preclinical investigations performed both *in vitro* (1, 2, 3, 4) and *in vivo* (5) on laboratory animals demonstrated that Ukrain has malignocytolytic and immunomodulating properties and is non toxic (6, 7, 8) even in doses many times greater than biologically and therapeutically active doses. Taking into account the results of all studies performed in various laboratories and in various countries, it can be concluded that Ukrain is safe and well tolerated when administered in relatively high doses over many weeks. Based on this, as well as on the results of an earlier trial performed on one volunteer, it was decided to undertake the first clinical studies on healthy volunteers.

Subjects and methods

The investigations were performed on 19 volunteers, (10 females and 9 males) aged 25–65 years. Their states of health were established on the basis

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of their medical histories, physical examinations and subsequent laboratory investigations: (ESR, morphology, blood smears, urinalysis, bilirubin, GOT, GPT, BUN, creatinine, uric acid, cholesterol, chest X-ray examinations, ECG, ultrasonography and internal and gynaecological examinations. Fasting blood samples were taken and the parameters were analysed within 8 h. Lymphocyte subpopulations were quantified by immunofluorescence using monoclonal antibodies against: total T-cells (Leu 1), T-helper (Leu 3a & 3b) and T-suppressor (Leu 2a) cells (Becton-Dickinson, USA). The percentage of NK cells (VEP-133) (Boehring, West Germany) and the T-helper/T-suppressor ratio were also evaluated. The number of B cells was determined by surface immunoglobulin immunofluorescence.

All examinations were carried out before, and 7 day after the end of Ukrain administration.

The 19 volunteers were divided into five subgroups according to the method of drug administration and dosage.

Subgroup 1 consisted of seven volunteers. They received Ukrain intravenously (i.v.) in a dose of 10 mg every day for 7 to 17 days.

Subgroup 2 consisted of six volunteers. Three of them were injected intramuscularly (i.m.) and three intravenously. All of them received Ukrain in a dose of 5 mg every day for 20 days.

Subgroup 3 consisted of four volunteers. The drug was given intravenously in dose of 20 mg per injection every two days. Each volunteer received 20 injections.

Subgroup 4 consisted of one volunteer and was a special study. A 38 year old male received Ukrain intravenously in the dose of 10 mg/injection, for a total of 20 injections (daily for first five injections, then every second day for next 15 injections). Every morning and evening the temperature, pulse and blood pressure were checked. Haematological and immunological examinations were performed before and after the course of Ukrain.

Subgroup 5 consisted of one volunteer and was also a special study. 5–50 mg Ukrain per injection was administered i.m. or i.v. every one, two or three days. One course of treatment lasted from 20 to 40 days. Intervals between courses were from 10 days to 3 months. Over a period of three years the volunteer took a total of 3500 mg Ukrain. He felt that the drug was well tolerated. This volunteer was hospitalized during the first course of Ukrain administration in the dose of 50 mg/injection. The following examinations were performed every year (twice in 1985, once in 1986 and once in 1987): haematological and biochemical parameters of the blood together with electrolytes, GOT, GPT, some immunological parameters, a neopterin test and others.

In all the above volunteers the following examinations were performed before and after Ukrain administration.

1. Clinical condition (including X-ray of the chest, ECG.) (Table I)
2. Blood morphology (haemoglobin, RBC, WBC, haematocrit, white bood count erythrocyte sedimentation rate); biochemistry (BUN, creatinine, uric acid, cholesterol, bilirubin, GOT, GPT, electrolytes and trace elements)
3. Urinalysis (pH, specific weight, protein, glucose, urobilinogen, sediment).
4. Immunological parameters (pan-T, T-helper, T-suppressor, B-cells, NK-cells, T-helper/T-suppressor ratio, rosetting lymphocytes, immunoglobulins immune complex). (Tables II, III, IV)
5. Miscellaneous (neopterin, non-specific blocking factors)

Results

In all cases Ukrain was generally well tolerated. The volunteers reported only a localized pain with burning sensation during i.m. injection. The pain disappeared spontaneously after two minutes. The addition of 0.5 ml of 1% Xylocain completely eliminated this pain. Drowsiness during the day was also reported by some volunteers. No adverse reactions were noted. There were no notable changes in the

Table I *Results of initial and final clinical, morphological and biochemical examinations of healthy volunteers*

Parameter	Before Ukrain (mean value ± s.d.)	After Ukrain (mean value ± s.d.)
ESR	12.25/26.08 ± 4.53	13.28/25.89 ± 4.56
Hb	13.65 ± 1.6	14.23 ± 1.5
RBC	4,470,000 ± 49.23	4,710,000 ± 49.54
WBC	5958 ± 16.8	6171 ± 16.1
HI	42.91 ± 5.02	44.43 ± 5.01
Segmented cells	54.54 ± 5.7	51.13 ± 5.4
Eosinophils	3 ± 1.6	3.5 ± 1.5
Lymphocytes	39 ± 6.7	41. ± 6.6
Macrophages	3.8 ± 1.9	3.4 ± 1.7
Urine pH	acid (in all volunteers)	acid
Specific gravity	1022 ± 5.4	1018 ± 6.1
Protein in urine	absent (in all volunteers)	absent
Sugar in urine	absent (in all volunteers)	absent
Urobilinogen in urine	normal (in all volunteers)	normal
E. in urine sed.	negligible number in all cases	negligible number in all cases
L. in urine sed.	negligible number in all cases	negligible number in all cases
BUN	24.76 ± 4.9	27.98 ± 4.7
Creatinine	1 ± 0.3	1.1 ± 0.3
Uric acid	4.5 ± 1.1	4.4 ± 1.1
Cholesterol	216 ± 5.6	195 ± 5.4
Bilirubin	0.5 ± 0.1	0.6 ± 0.1
GOT	19.8 ± 3.25	20.9 ± 324
GPT	24.4 ± 2.8	26.9 ± 2.9
X-ray examin	normal – in all volunteers	normal – in all volunteers
ECG	normal – in all volunteers	normal – in all volunteers

(The results of the second examination after Ukrain administration did not differ from the initial values obtained.)

Table II *Influence of Ukrain administration on the immunological parameters*

Parameter: Monoclonal antibodies	Before Ukrain	After Ukrain
OKT_2	73.50 ± 6.80	76.00 ± 9.32
BI	22.30 ± 8.06	21.80 ± 8.10
OKT_4	38.50 ± 9.50	41.25 ± 11.68
OKT_8	34.00 ± 4.72	32.25 ± 6.69
NK-cells	8.30 ± 4.90	11.60 ± 4.40
OKT_4/OKT_8	1.15 ± 0.64	1.26 ± 0.19

Table III *Influence of Ukrain administration on the immunoglobulin status*

Parameter: IM status	Before Ukrain	After Ukrain
A	166.37 ± 40.64	171.12 ± 82.64
G	131.75 ± 49.89	156.37 ± 34.88
M	216.00 ± 80.07	256.25 ± 74.04
CIC	2.28 ± 0.74	2.25 ± 1.24
IK	1.35 ± 0.19	1.37 ± 0.22

No statistical differences were found between the mean values of all immunological parameters before and after Ukrain administration.

Table IV *Influence of Ukrain administration on electrolytes and trace elements*

Parameter: Electrolytes	Before Ukrain	After Ukrain
Na	138.60 ± 4.00	137.60 ± 5.02
K	4.34 ± 0.34	4.33 ± 0.29
Ca	90.50 ± 7.14	92.75 ± 3.20
P	38.75 ± 6.55	35.00 ± 3.36
Mg	21.33 ± 2.07	21.33 ± 1.14
Fe	95.40 ± 17.28	97.80 ± 14.73
Cu	93.75 ± 10.37	92.50 ± 8.66
Zn	0.93 ± 0.12	0.90 ± 0.17

clinical conditions. All parameters studied revealed only minimal fluctuations within the norm. Spasmotic and cholagogic actions of the preparation were reported by two volunteers who had mild dyspepsia. During treatment their symptoms disappeared.

It is worth emphasizing that during the period of Ukrain administration, numerous catarrhal and parainfluenzal infections were prevalent in some countries. However, no such infections were observed in any of the volunteers taking Ukrain. The tendency to increase the T4/T8 ratio was noted.

The results of this study showed no evidence that Ukrain had any harmful side effects. After drug administration all volunteers were in good or even better general states of health than before initiation of therapy.

One volunteer (Subgroup 4) who received 20 injections of Ukrain in doses of 10 mg/injection showed no adverse changes in the clinical state (pulse, blood pressure, body temperature, urine volume). The general comfort and psychic condition were unchanged. No pain at the site of injection was noted.

One volunteer (Subgroup 5) who received i.v. and i.m. injections of 3500 mg of Ukrain in single doses of 5 to 50 mg during 3 years showed that Ukrain was well tolerated during the whole time of administration regardless of the administered dose. No subjective or objective changes were found.

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Discussion and conclusion

Ukrain was administered to 19 healthy volunteers in repeated doses of 5 to 50 mg/injection for 20 to 40 days (in one case even for three years). Investigations were performed according to the principles of open trial in various clinics.

It can be concluded that repeated administration of Ukrain following schemes similar to those recommended for therapy showed no significant changes in the haematological, biochemical and immunological parameters as well as in the clinical conditions of healthy volunteers. This was also the case with doses which were far above the normal therapeutic dose. No allergic reactions were observed. At the beginning of Ukrain administration some volunteers felt slight fatigue, a slight increase of body temperature and increased thirst and polyurea. Slight, short-lasting pain at the site of injection was also reported.

On the basis of these observations, the investigators concluded that Ukrain is well tolerated and non-toxic, at least according to the criteria studied.

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