INT. J. IMMUNOTHERAPY **XIX**(2-4) 47-53 (2003)

# UKRAIN, A THIOPHOSPHORIC ACID DERIVATIVE OF ALKALOIDS OF *CHELIDONIUM MAJUS L.,* IS EFFECTIVE IN THE TREATMENT OF RECURRENT BRONCHOPULMONARY PATHOLOGY IN CHILDREN FROM AREAS CONTAMINATED AFTER THE CHERNOBYL ACCIDENT

**ZAHRIYCHUK O.**

Ukrainian Anti-Cancer Institute, Vienna, Austria.

**Summary:** *A total of 38 children, drawn from areas contaminated after the Chernobyl accident and suffering from recurrent bronchopulmonary pathology, were included in the study. To ascertain the effects of Ukrain, an anticancer and immunomodulating drug, it was administrated intravenously at a dose of 5 mg twice a week, up to a total dose of 35 mg. The control group included 10 children with the same pathology who received standard anti-inflammatory therapy. Compared with the control group, the group treated with Ukrain showed marked anti-inflammatory activity: rapid decrease in white blood cell count and blood sedimentation rate. The strong immunomodulatory effect of Ukrain was indicated through the improvement in specific humoral and cellular immunity: increases in the immunoglobulin G (IgG) level, the phagocytic activity of neutrophils, the number of total lymphocytes, T-lymphocytes and T-helpers, and the T-helpers/suppressors ratio. In view of the positive results of this pilot study and the great importance of preventive and clinical investigation of this problem given the widespread distribution of nuclear power plants and of nuclear military equipment, further studies devoted to the impact of Ukrain on children with immune disorders from contaminated areas would be interesting and could lead to positive results.*

## Introduction

The health of a child can be affected by many environmental factors. Nowadays, one of the most serious and sometimes fatal influences is ionizing radiation, which can lead to the development of cancer and other diseases, mental retardation and, in conjunction with other concomitant circumstances, psychic and social disadaptation. Ionizing radiation is one of the main factors defining the health status of adolescents in Ukraine as one of the countries most profoundly affected by the Chernobyl disaster.

*Address for correspondence:* Oleh Zahriychuk, Ukranian Anti-Cancer Institute, Margaretenstr. 7, 1040 Vienna, Austria. E-mail: zahriychuk@yahoo.com

0255-9625/2003/2-4 00047+6 $02.50/0

On April 26, 1986, the Chernobyl nuclear power station suffered an accident that led to the prolonged release of large amounts of radioactive substances into the atmosphere. Specific features of the incident favored a widespread distribution of radioactivity throughout the northern hemisphere, especially across Europe. Contributing factors were varying meteorological conditions and wind regimes during the period of release. Radioactivity transported by multiple plumes from Chernobyl was measured not only in northern and southern Europe, but also in Canada, Japan and the United States. Only the southern hemisphere remained free of contamination. Released radioactive isotopes ($^{33}$Xe, $^{131}$I, $^{134}$Cs, $^{137}$Cs, $^{132}$Te, $^{89}$Sr, $^{90}$Sr, $^{140}$Ba, $^{95}$Zr, *etc.*) in the form of gases, aerosols and finely fragmented nuclear fuel particles had an extremely detrimental agricultural and environmental impact. Currently, the frequency of late health stochastic effects of the radiation is the subject of numerous studies (1).

A large number of reviews and clinical and epidemiological investigations have been devoted to defining the precise impact of the Chernobyl accident on the health of children from contaminated territories. Special attention has been paid to possible increases in cancer diseases, in accordance with the data that children are much more radiosensitive than adults: a 1-year-old infant has a 10- to 15-fold greater risk than a 50-year-old adult of developing a malignancy from the same dose of radiation (2).

One type of radiation-induced malignant disease in children, with a minimum latency period of less than 10 years, is thyroid cancer (3). Because of relatively high thyroid doses (up to a few grays) resulting from inhaled and ingested radioiodine, including $^{131}$I and a few short-lived iodine-isotopes, it was not really surprising that childhood thyroid cancer was the first tumor type to show signs of marked increase in many areas in the vicinity of Chernobyl. This increase began in 1990, and was first observed in Belarus (4-6). In relative terms, the increase was pronounced and has been over 100-fold in some areas. The initial reports met with considerable skepticism in some scientific quarters (7), and it was pointed out that many types of thyroid carcinoma were clinically indolent, and that any active search for such tumors might greatly influence the number found. Further criticism was based on the apparent early finding that an increase was observed only in Belarus and not in other areas which had experienced relatively high fallout. Later, however, significant increases were also detected in northern districts of Ukraine and in parts of the Bryansk and Kaluga regions of Russia, where widespread radioactive pollution had been observed (8). To date, nearly 700 extra cases of childhood thyroid cancer have been detected in a population of about 3 million children at risk, and estimates of the future rate of childhood thyroid cancers vary from 0.5 to 3 per 1 million children (3). Published rates of childhood thyroid cancer are shown in Table I.

A notable factor in the observed increase in thyroid cancer incidence is distance from the accident site. For instance, in Finland, which is about 1,000 km from the release site, no apparent increase in childhood thyroid cancer seems to have occurred by the end of 1993 (3). However, it should be underlined that in Finland all children at risk of radioactive $^{131}$I exposure received iodine treatment, whereas children from radioactivity-contaminated territories in the Ukraine, Belarus and Russia in the former Soviet Union did not have this opportunity at the time.

Evidence as to the frequency of childhood leukemia in areas near Chernobyl has shown no distinct association with radioactive fallout (9). However, the rate of infant leukemia as a result of *in utero* exposure, a distinct form associated with specific genetic abnormality, was increased in Greece 2.6-fold compared to unexposed children, and in Germany, after the Chernobyl accident (10, 11). In Belarus, increased incidence of autoimmune thyroiditis, and endocrine,

**Table I** *Rates of childhood thyroid cancer in those territories of Belarus, Ukraine and Russia contaminated in the Chernobyl accident (7)*

| | 1981-1985 | | 1986-1990 | | 1991-1994 | |
|---|---|---|---|---|---|---|
| Area | No. | Rate (per million) | No. | Rate (per million) | No. | Rate (per million) |
| Belarus (all) | 3 | 0.3 | 47 | 4 | 286 | 30.6 |
| Gomel region | 1 | 0.5 | 21 | 10.5 | 143 | 96.4 |
| Ukraine: five northern regions | 1 | 0.1 | 21 | 2 | 97 | 11.5 |
| Russia: Bryansk and Kaluga regions | 0 | 0 | 3 | 1.2 | 20 | 10 |

digestive, dermatologic, hematopoietic and mental disorders have been reported (6).

It is worth noting that children's exposure to ionizing radiation is not limited to the environment. Medical radiation exposure occurs during diagnosis, therapy and dental radiography. Epidemiologic studies have shown that people exposed to high levels of ionizing radiation have an increased risk of cancer, particularly leukemia and, later in life, breast and thyroid cancer (12). In addition, some epidemiologic studies have found that radiation exposure during childhood carries a higher risk of cancer than exposure at other ages (12, 13). Thus, the problem of childhood protection from the harmful action of radiation is of widespread significance.

The issue of the treatment of children living in contaminated areas is particularly pressing. Almost all of them suffer from various chronic health disorders, including anemia and a decrease in both specific and nonspecific immunity. Therefore, drugs used in the therapy of these children must have the minimum of side effects and, in the optimal scenario, improve the status of their immune system. In accordance with these requirements, Ukrain attracted our attention as a drug with proven immunomodulating properties (14). Of special significance is the fact that Ukrain has unique radioprotective effects: It protects normal human fibroblasts from radiation toxicity, while enhancing radiation toxicity in colorectal and brain tumor cells (15). The pronounced radioprotective effect of this drug was also described previously (16).

The absence of serious side effects, and our experience of Ukrain administration in children, prompted us to use this drug in the treatment of recurrent bronchopulmonary pathology in children from areas contaminated after the Chernobyl accident. In all these children, various abnormalities in immune status were found, and so administration of Ukrain as an immunomodulatory drug was directly indicated.

## Patients and methods

Ukrain (Nowicky Pharma, Vienna, Austria) was clinically administered at three pediatric centers in the Kiev region. The study included 38 children aged 3-14 years with chronic bronchopulmonary diseases, who were from areas contaminated after the Chernobyl accident. The Pharmacological Committee of the Ministry of Health of Ukraine gave permission for clinical studies with Ukrain to be conducted, and the study design was approved by the local ethics committee. Before the treatment was started, a written informed consent had been obtained from parents of all children involved in the study.

Ukrain was administrated intravenously at a dose of 5 mg (5 ml) twice a week, up to a total dosage of 35 mg (35 ml). The control group included 10 children with the same diagnosis who were treated with standard nonspecific anti-inflammatory therapy. A further healthy group consisted of 20 children of the same age without health disorders, who received no treatment.

All children were given a detailed clinical examination before treatment, and those in the two treatment groups were found to have considerable immune status abnormalities.

The influence of Ukrain on hematological (*i.e.,* blood count) and immunological parameters was evaluated. The influence of Ukrain on cellular immunity was monitored by monoclonal antibodies to CD4

**Table II** *Blood count parameters of patients treated with Ukrain (Ukrain group), patients treated traditionally (control group), and healthy children (healthy group)*

| Parameters | Group | On admission | After the course of treatment |
|---|---|---|---|
| RBC ($10^{12}$/l) | Healthy | 4.8 ± 0.2 | – |
| | Control | 4.7 ± 0.2 | 4.6 ± 0.2 |
| | Ukrain | 4.7 ± 0.2 | 4.7 ± 0.2 |
| Hemoglobin (g/l) | Healthy | 130 ± 5 | – |
| | Control | 119 ± 3 | 126 ± 7 |
| | Ukrain | 123 ± 5 | 122 ± 5 |
| Platelets ($10^9$/l) | Healthy | 266 ± 16 | – |
| | Control | 235 ± 23 | 240 ± 26 |
| | Ukrain | 246 ± 31 | 252 ± 21 |
| WBC ($10^9$/l) | Healthy | 5.0 ± 0.6 | – |
| | Control | 13.9 ± 2.7 | 7.3 ± 3.8* |
| | Ukrain | 14.3 ± 2.2 | 5.9 ± 1.5* |
| Eosinophils (%) | Healthy | 1.8 ± 0.4 | – |
| | Control | 1.2 ± 0.6 | 1.4 ± 1.0 |
| | Ukrain | 1.3 ± 0.2 | 1.9 ± 0.8 |
| Stabs (%) | Healthy | 1.7 ± 0.2 | – |
| | Control | 2.2 ± 0.6 | 1.9 ± 0.2 |
| | Ukrain | 2.0 ± 0.5 | 2.0 ± 0.4 |
| Filamented (%) | Healthy | 56.8 ± 6.9 | – |
| | Control | 60.2 ± 8.1 | 55.6 ± 4.4 |
| | Ukrain | 58.9 ± 9.0 | 59.9 ± 6.7 |
| Basophils (%) | Healthy | 0.2 ± 0.1 | – |
| | Control | 0.3 ± 0.2 | 0.2 ± 0.1 |
| | Ukrain | 0.2 ± 0.1 | 0.2 ± 0.1 |
| Lymphocytes (%) | Healthy | 34.1 ± 2.8 | – |
| | Control | 30.9 ± 5.4 | 31.2 ± 6.1 |
| | Ukrain | 32.7 ± 6.6 | 33.1 ± 4.2 |
| Monocytes (%) | Healthy | 6.7 ± 2.8 | – |
| | Control | 7.9 ± 3.3 | 6.5 ± 0.2 |
| | Ukrain | 8.4 ± 5.5 | 4.4 ± 1.2 |
| BSR (mm/h) | Healthy | 6.1 ± 2.4 | – |
| | Control | 14.7 ± 2.9 | 8.3 ± 2.7* |
| | Ukrain | 15.3 ± 3.3 | 5.3 ± 0.9* |

*$p < 0.05$ in comparison with control group. RBC = red blood cells; WBC = white blood cells; BSR = blood sedimentation rate.

(helpers) and CD8 (suppressors-killers); the number of T-cells in the peripheral blood was determined by their capacity to form rosettes with three or more sheep erythrocytes. Serum immunoglobulins were measured by the radial immune-diffusion method (Fisher Scientific GmbH, Schwerte, Germany).

Statistical analysis of the data was carried out using Student's *t*-test; $p < 0.05$ was considered statistically significant.

## Results

Observation of the impact of Ukrain was carried out over a 5-week period. The resulting data were compared with the results of standard treatment in children with a similar pathology in the control group and also with the data for the 20 children without any health disorders.

It was clinically established that after two to three injections of Ukrain, a decrease in the clinical symptoms of disease was observable: general debility lessened, sense of well-being improved, and in nine children high body temperatures normalized. In all children in the Ukrain-treated group there was also increased release of bronchial secretion and easier separation, the purulent mucus acquiring a mucilaginous character. After three to four injections of Ukrain, coughing became more productive in character, then gradually decreased, and after five to six injections it disappeared completely. During the course of treatment, the results of percussion and auscultation objective examinations returned to normal in all these children.

Laboratory findings of the blood count parameters and the parameters of the immune status before and after treatment are presented in Tables II, III and IV, respectively.

Compared with the group treated traditionally, the Ukrain group exhibited more pronounced anti-inflammatory activity: this was indicated by greater decreases in both white blood cell count and blood

**Table III** *Serological parameters of the immune status of patients treated with Ukrain (Ukrain group), patients treated traditionally (control group) and healthy children (healthy group)*

| Parameters | Group | On admission | After the course of treatment |
|---|---|---|---|
| IgG (g/l) | Healthy | 10.05 ± 1.12 | – |
| | Control | 8.42 ± 1.43 | 8.61 ± 1.07 |
| | Ukrain | 7.80 ± 0.56 | 9.01 ± 1.22* |
| IgA (g/l) | Healthy | 1.91 ± 0.22 | – |
| | Control | 1.46 ± 0.17 | 1.43 ± 0.56 |
| | Ukrain | 1.51 ± 0.46 | 1.57 ± 0.71 |
| IgM (g/l) | Healthy | 1.23 ± 0.17 | – |
| | Control | 1.10 ± 0.09 | 1.20 ± 0.14 |
| | Ukrain | 0.96 ± 0.11 | 1.10 ± 0.10 |
| Complement titre (%) | Healthy | 58 ± 7 | – |
| | Control | 56 ± 9 | 58 ± 10 |
| | Ukrain | 57 ± 11 | 55 ± 6 |
| Phagocytic activity of neutrophils (%) | Healthy | 63 ± 4 | – |
| | Control | 52 ± 5 | 55 ± 2 |
| | Ukrain | 52 ± 3 | 59 ± 1* |

*$p < 0.05$ in comparison with control group. Ig = immunoglobulin.

**Table IV** *Cellular parameters of the immune status of patients treated with Ukrain (Ukrain group), patients treated traditionally (control group), and healthy children (healthy group)*

| Parameters | Group | On admission | After the course of treatment |
|---|---|---|---|
| Total lymphocytes (%) | Healthy | 34.09 ± 2.06 | – |
| | Control | 35.09 ± 1.98 | 34.99 ± 1.48 |
| | Ukrain | 34.75 ± 1.32 | 37.18 ± 1.23* |
| T-lymphocytes (%) | Healthy | 42.30 ± 1.12 | – |
| | Control | 42.27 ± 0.87 | 42.01 ± 1.01 |
| | Ukrain | 42.50 ± 0.24 | 45.00 ± 0.24* |
| T-helpers (CD4) (%) | Healthy | 57.44 ± 2.01 | – |
| | Control | 56.89 ± 2.44 | 57.05 ± 1.83 |
| | Ukrain | 57.03 ± 1.08 | 62.45 ± 1.12* |
| T-suppressors (CD8) (%) | Healthy | 9.02 ± 0.18 | – |
| | Control | 9.79 ± 0.24 | 9.55 ± 0.37 |
| | Ukrain | 9.91 ± 0.31 | 8.59 ± 0.55 |
| T-helper/T-suppressor ratio | Healthy | 6.01 ± 0.09 | – |
| | Control | 5.82 ± 0.17 | 5.97 ± 0.10 |
| | Ukrain | 5.76 ± 0.08 | 7.22 ± 0.13* |

* $p < 0.05$ in comparison with control group.

sedimentation rate. The strong immunomodulatory effect of Ukrain was indicated in the stimulation of specific humoral and cellular immunity: increases in the immunoglobulin G (IgG) level; the phagocytic activity of neutrophils; the number of total lymphocytes, T-lymphocytes and T-helpers; and the T-helper/suppressor ratio.

## Discussion

The differences in the results of therapy between the two treatment groups (with and without Ukrain) are remarkable. In the Ukrain group, the improvement of immune status, decrease in inflammation and improvement in clinical situation were really surprising, especially in comparison with the results from the group treated traditionally.

The study was performed in children with deep and long-lasting changes in immune status provoked by external ionizing radiation. Diseases and health abnormalities in this group of young patients are hard to prevent and hard to cure. Thus, the observed results are of great significance.

In the 15 years after the Chernobyl accident, there was an increase in the incidence of endocrinal and dermatological disorders, disorders of the digestive organs, chronic tonsillitis and adenoiditis, and autoimmune thyroiditis (6).

We suggest that this entire complex of health disorders in children, being the most sensitive section of the human population, can be labelled Chernobyl syndrome: a decrease in the immune resistance of the organism to infection and cancer as a result of the effects of exposure to radiation from Chernobyl. In view of the results of this pilot study and the importance of preventive and clinical investigation of this problem given the widespread use of nuclear power stations and the risk of radiation from military sources, further studies devoted to the effect of Ukrain on children with immune disorders from radiation-contaminated areas would be of great significance and great potential benefit.

## Acknowledgments

This study was performed thanks to the support of Dr. Oleh Tyahnybok from Kiev, Ukraine, a member of the Ukrainian Parliament, and of Dr. Wassil Nowicky from Vienna, Austria.

## References

(1) Fry R.J. *Deterministic effects.* Health Phys., **80**(4), 338, 2001.

(2) *1990 Recommendations of the International Commission on Radiologic Protection.* Ann. ICRP, **21**, 1, 1991.

(3) Rytomaa T. *Ten years after Chernobyl.* Ann. Med., **28**(2), 83, 1996.

(4) Lindholm C., Salomaa S., Tekkel M., et al. *Biodosimetry after accidental radiation exposure by conventional chromosome analysis and FISH.* Int. J. Radiat. Biol., **70**(6), 647, 1996.

(5) Likhtarev I.A., Sobolev B.G., Kairo I.A., et al. *Thyroid cancer in Ukraine.* Nature, **375**(6530), 365, 1995.

(6) Lomat L., Galburt G., Quastel M.R., Polyakov S., Okeanov A., Rozin S. *Incidence of childhood disease in Belarus associated with the Chernobyl accident.* Environ. Health Perspect., **105** (Suppl. 6), 1529, 1997.

(7) Stsjazhko V.A., Tsyb A.F., Tronko N.D., Souchkevitch G., Baverstock K.F. *Childhood thyroid cancer since the accident at Chernobyl.* Br. Med. J., **310**(6982), 801,1995.

(8) Parkin D.M., Cardis E., Masuyer E., et al. *Childhood leukaemia following the Chernobyl accident: the European Childhood Leukaemia-Lymphoma Incidence Study (ECLIS).* Eur. J. Cancer, **29A**(1), 87, 1992.

(9) Kuchuk A.A. *Health problems of the population in different regions of the Ukraine.* Toxicol. Lett., **72**(1-3), 213, 1994.

(10) Sali D., Cardis E., Sztanyik L., et al. *Cancer consequences of the Chernobyl accident in Europe outside the former USSR: A review.* Int. J. Cancer., **67**(3), 343, 1996.

(11) Steiner M., Burkart W., Grosche B., Kaletsch U., Michaelis J. *Trends in infant leukaemia in West Germany in relation to in utero exposure due to the Chernobyl accident.* Radiat. Environ. Biophys., **37**(2), 87, 1998.

(12) Miller R.W. *Delayed effects of external radiation exposure: A brief history.* Radiat. Res., **144**(2), 160, 1995.

(13) Yoshimoto Y., Delongchamp R., Mabuchi K. *In-utero exposed atomic bomb survivors: Cancer risk update.* Lancet, **344**(8918), 345, 1994.

(14) Uglyanitsa K.N., Nefyodov L.I., Doroshenko Y.M., et al. *Ukrain: A novel antitumor drug.* Drugs Exptl. Clin. Res., **26**(5/6), 341, 2000.

(15) Cordes N., Plasswilm L., Bamberg M., Rodemann H.P. *Ukrain®, an alkaloid thiophosphoric acid derivative of Chelidonium majus L. protects human fibroblasts but not human tumour cells in vitro against ionizing radiation.* Int. J. Radiat. Biol., **78**(1), 17, 2002.

(16) Boyko W.N., Zholus R.B. *A comparative evaluation of the influence of the complex drug Ukrain and its components on the effects of radiation.* Drugs Exptl. Clin. Res., **24**(5/6), 13, 1998.