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Effect of Ukrain preparation on immune response in mice affected by influenza virus

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INTRODUCTION

The preparation Ukrain is a semi-synthetic substance containing alkaloids of *Chelidonium majus* [1-3], which has been used in the therapy of neoplastic diseases [3-6]. It has been shown that the preparation acts in a selective way on neoplastic cells without influencing normal cells [3,5,7]. Ukrain has not been demonstrated to be toxic and is well tolerated by patients [7-10].

Experimental and clinical studies have indicated that one of the mechanisms of antineoplastic action of the above preparation may be connected with the stimulation or modulation of the immune system [8-11]. Trials aiming to assess the effectiveness of this preparation in virus infections have been undertaken in infections caused by influenza as well as HIV viruses [7,12,13].

We evaluated the effectiveness of Ukrain on prevention and healing of infections caused by influenza viruses in mice. Because of our encouraging results as measured by survival rate, we also evaluated some parameters of the immune response.

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MATERIALS AND METHODS

The studies were carried out on Balb/c mice, 10-12 weeks old, of both sexes, weighing 24-26 g. For the experiments, the Ukrain preparation, batch No. 290614, produced by the J.W. Nowicky firm (Vienna), was used. Ukrain was administered subcutaneously. Mice were divided into three groups. The first group of mice was then divided into three subgroups of 10 mice each to which the preparation was administered at the following doses, respectively: 0.04 mg, 0.4 mg and 4.0 mg/kg body weight at 48, 24 and 2 h before infecting the animals, and at 2, 24 and 48 h after infecting them. The second group was also divided into three subgroups of 10 mice each, receiving the preparation at the same doses as the previous group but every other day for 20 days, for a total of 10 doses. Afterwards, the mice were subjected to a short ether narcosis and infected nasally with the influenza virus of the group APR8/HON1/13 at the dose 2 x LD_{50}. The infectious dose was determined experimentally. Mouse survival was observed within 10 days. The mice infected with the same doses of viruses and healthy or uninfected mice were examined as the control groups. The third group of 40 mice were administered Ukrain at the dose of 0.4 mg/kg body weight every other day for 20 days. These animals were infected on day 20 with influenza viruses using 1/5 x LD_{50} dose. Examinations were carried out each time on 10 mice, on days 3, 6, 9 and 14 after infection. One day before examination the mice were given 1 ml of 0.1% glycogen solution. On the day of examination, they were bled and the blood was tested for antibody titer. Next mice were killed and after disinfection of skin integument with 70% ethanol, 0.9% sterile NaCl solution at a dose of 5-7 ml was administered intraperitoneally. After massaging abdominal integuments, the peritoneum was dissected and incised, collecting the peritoneal fluid into a sterile vessel containing 5 IU of heparin. After centrifugation, granulocytes were suspended in 1 ml of Hanks solution, counted and suspended again in Hanks solution to reach a suspension containing 5 x 10^6 cells in 1 ml. These cells were then used for determination of phagocytic reaction according to Trippestad method (modified) in Tchorzewski et al [14]. Afterwards the

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spleen was dissected free and homogenized in a glass homogenizer with 5 ml of Hanks solution. After filtering the cell serum, cells were centrigued and washed 3 times with Hanks solution. After final centrifugation, the cells were suspended in Hanks solution containing 10% serum and antibiotic (120 U penicillin and 100 µg streptomycin per milliliter), and calculated in a hemocytometer. Cell viability was then estimated and the suspension containing 5 x 10^6 cells/ml was prepared. The spleen cells were used for carrying out the test of inhibition of leukocyte migration (LIF) according to Sanberg [15]. Mouse blood was evaluated for clotting; after removing the clot, inhibition of hemagglutination in serum was measured according to Heirholzer [16].

RESULTS

The survival rate of uninfected mice was 100%. The survival rate of mice infected by influenza viruses on day 10 was 10%. The mice which had been receiving Ukrain for 20 days at the dose of 0.4 mg/kg body weight showed the longest survival time, 70%. A lower survival index was observed for mice receiving 0.04 mg/kg for 20 days, being 60%. Within 10 days of observation, no mouse survived in the group receiving 0.04 mg of Ukrain at 48, 24 and 2 h before being infected and at 2, 24 and 48 h after infection (0% survival). The remaining mice survived similarly to the group of infected mice, i.e., about 10% (*Figures 1* and *2*).

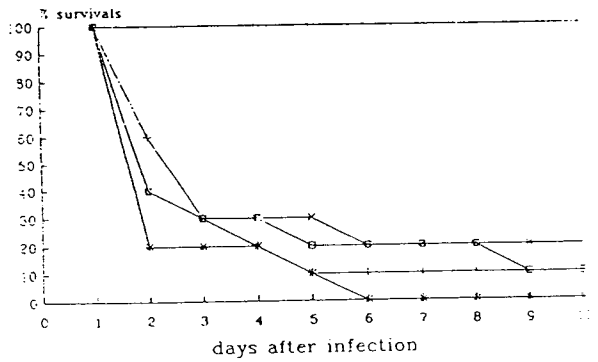

FIGURE 1 - Survival of influenza-infected mice and those treated with Ukrain 48, 24, and 2 h before infection and 2, 24 and 48 h after infection.
—•— uninfected mice; —|— infected mice; —*— infected mice + Ukrain 0.04 mg; —□— infected mice + Ukrain 0.40 mg; —×— infected mice + Ukrain 4.00 mg

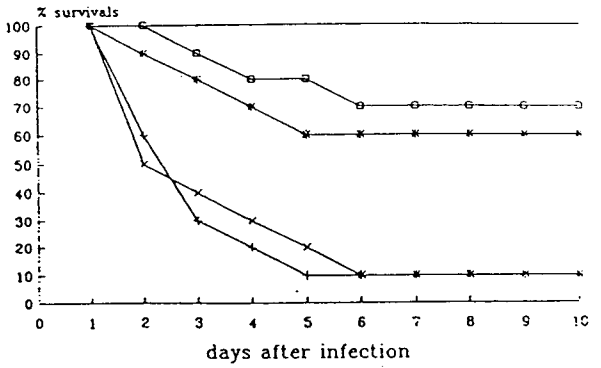

FIGURE 2 - Survival of influenza-infected mice and those treated with Ukrain every second day for 20 days.
—•— uninfected mice; —|— infected mice; —*— infected mice + Ukrain 0.04 mg; —□— infected mice + Ukrain 0.40 mg; —×— infected mice + Ukrain 4.00 mg

The inhibition of leukocyte migration (LIF) increased as the infection took place and was highest 14 days after infection. No significant difference between the LIF values for mice being infected and those for mice infected and receiving at the same time the preparation was found. However, it should be stressed that in this second group the LIF values were a little higher than those of the first group (*Figure 3*).

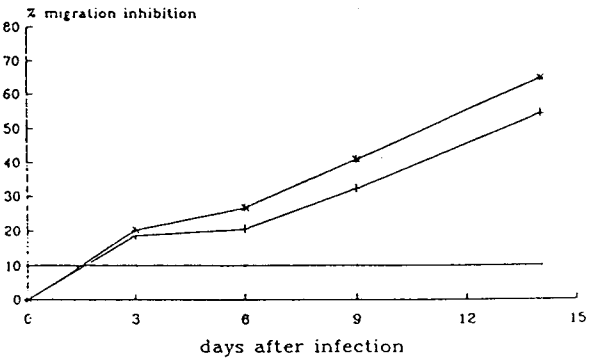

FIGURE 3 - Migration inhibition of spleen cells (LIF) in the presence of influenza antigen in mice treated with Ukrain.
—•— uninfected mice; —|— infected mice; —*— infected mice + Ukrain

The phagocyte activity of granulocytes of peritoneal exudate was decreased in both groups studied. Its value was within the range of 0.5 to 0.7. The indexes had similar behavior as in the case of LIF with the exception of day 9 after infection, when a lower phagocyte index of granulocytes was observed for mice

receiving the preparation *(Figure 4)*. In our studies this preparation did not show any effect on the antibody titer against viral hemagglutinin *(Figure 5)*.

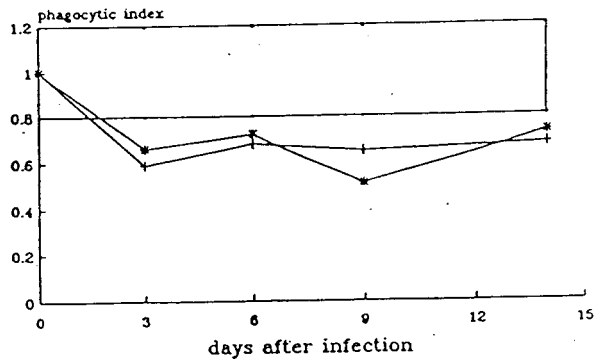

FIGURE 4 - Phagocytic activity of peritoneal exudate granulocytes in mice treated with Ukrain.
—•— uninfected mice; —|— infected mice; —*— infected mice + Ukrain

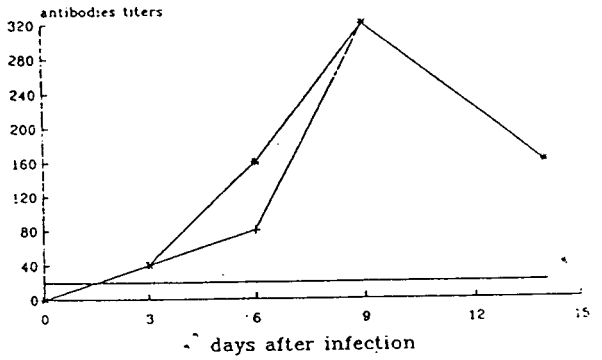

FIGURE 5 - Anti-HA antibody titers in sera from mice treated with Ukrain.
—•— uninfected mice; —|— infected mice; —*— infected mice + Ukrain

DISCUSSION

Experimental and clinical studies show that the preparation Ukrain possesses antineoplastic activity. This activity depends on the dose administered, time of administration [1,4,9] and kind of neoplasm [3,4,7]. Higher effectiveness is achieved with long and multiple administrations of the preparation [5,6,17].

In our investigations we observed a protective effect in mice infected by influenza viruses if Ukrain is administered every other day for 20 days. The action of Ukrain does not seem connected with stimulation of the immune system. Our results do not show any stimulation effect on immune response parameters as is observed in neoplastic diseases, where an increased number of T, NK and K lymphocytes, stimulation of phagocytic activity of granulocytes, normalization of the proportion of T helper/T suppressor lymphocytes, increase in complement components as well as in serum immunoglobulin levels was observed [4,9,10,11,17,18].

Ukrain selectively affects neoplastic cells without destroying normal cells [3,5,7]. The selective effect of this preparation is probably connected with the expression of neoplastic antigens on the cell surface and a secondary change in the permeability of the cell membrane. It is probable that viral antigens such as neoplastic antigens are able to change the cell membrane permeability for the preparation being administered. The presence of this preparation inside the infected cell and its combining with cell DNA [2,7] may have an inhibitory effect on some phases of influenza virus replication cycle taking place in the cell nucleus.

Together with this mechanism increased exhaustion of energetic and oxygenic resources of the cell may occur due to the presence of Ukrain [1,2,12]. These disturbances negatively affect the phases of the cell development cycle and its metabolic process, which may limit or prevent organization of virions or its release from the cell.

Our present study does not allow us to say whether the increased survival rate of mice infected by influenza viruses but treated with Ukrain may be correlated with the activating effect of the preparation on the immune system. We studied only two parameters of cell response and one humoral one. In viral infections an important role is played by immunity which is dependent on cytotoxic cells and interferon. The stimulation of cytotoxic cells under the influence of Ukrain in neoplastic diseases has already been described [3,7,8,13]. It is possible that in viral infections Ukrain also activates cytotoxic cells and increases the production of interferon. The explanation of this question will contribute to a better evaluation of the effectiveness of Ukrain in chemotherapy of viral infections. Thus Kamyshentsev's [12] theory of

chemotherapy of viral infections based on blocking of metabolic processes in the cell essential for viral replication cycle could be supported by interferon and cytotoxic defense.

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