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INFLUENCE OF UKRAIN ON BREAST CANCER

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Summary: *The influence of Ukrain on breast cancer was studied in ten women and monitored in each patient by clinical examination, mammography, ultrasonography, histopathology and immunofluorescence in the tissue material obtained after radical mastectomy performed ten days after discontinuation of the treatment. Observations accumulated during the study and analytical data support the view that Ukrain, by changing the antigenic expression of malignant cells, make them recognizable for the immune system which rejects them in a host-versus graft manner.*

Introduction

In our previous studies done in patients with ovarian and colorectal cancers it was demonstrated that Ukrain may suppress tumour growth by a direct cytostatic effect as well as by an increase of anti-tumour immunity, mediated by cytotoxic T lymphocytes and NK cells (1). In the present studies Ukrain anti-tumour potency has been tested in women with breast cancer.

Patients and methods

The studies were carried out in 10 women from 38 to 65 years old (mean 56) in whom stage II and stage III of breast cancer on the basis of clinical evaluation, mammography (MG), ultrasonography (USG), and histopathology (HP) of the material obtained by needle biopsy were diagnosed.

Prior to radical mastectomy performed according to Patey and Halsted-Merier from eight to ten days after discontinuation of the treatment with Ukrain, each patient received intravenously (i.v.) ten injections of the drug in a dose of 5 mg in 5 ml of distilled water per injection every second day. Thus each patient received finally 50 mg of Ukrain.

One week after discontinuation of treatment with Ukrain and before mastectomy, the influence of the drug on the cancer was analysed clinically by mammography (MG) and ultrasonography (USG), and the tissue material after surgery by histopathology (HP) and immunomorphology (IM). For HP, tissue material was taken from the primary tumour and lymph nodes, fixed in formalin, embedded in paraffin and stained with eosin and

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haematoxylin. For immunomorphology, the tissue material obtained from the primary tumour and lymph nodes was snap-frozen in liquid nitrogen, cut in a cryostat, fixed in acetone and stained for localization of IgG, IgM, IgA, CD3, CD4, CD8, CD16, CD20, CD45, HLA-DR and Il-2. Localization of Ig was performed by direct staining with rabbit anti-IgG, IgM and IgA reagents labelled with FITC. The remaining reactions were performed indirectly with monoclonal antibodies with second-layer anti-mouse reagent labelled with FITC. The slides were analysed in a Reichert microscope, and for photography Agfa and Kodak film were used.

Results and discussion

After Ukrain treatment, no clinical side-effects were noted in our patients, except two who complained of pains of the breast invaded by the tumour. Influence of the drug on tumours was characterized by tumour hardening and its sharp contrast with the surrounding tissue. By MG the tumour hardness was demonstrated by its higher density. By USG, it was found that the tumours, besides their higher contrast, increased in size (mean 5-6 percent) to the size before Ukrain treatment.

During surgery it was also found that parallel to the changes induced by Ukrain in tumours, the lymph nodes increased in size and hardness. They were easier to locate and evaluate.

By HP, tumours were found to be surrounded by connective tissue heavily infiltrated by numerous mononuclears composed mostly of lymphocytes and plasma cells (Fig. 1). The tumours were also found heavily infiltrated by mononuclears similar to those found in a marginal zone (Fig. 2). In some places around the foci of neoplastic tissue, the mononuclear infiltrates formed structures similar to lymph nodules (Figs. 3,

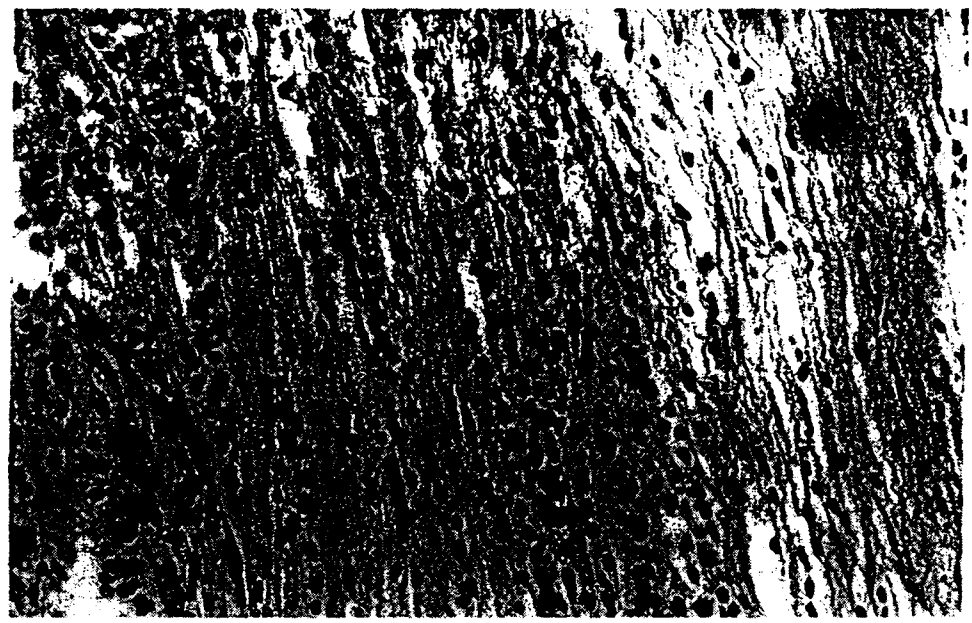


Fig. 1 Connective tissue surrounding the tumour infiltrated by lymphocytes and plasma cells. Staining H+E, mag. 400 x.

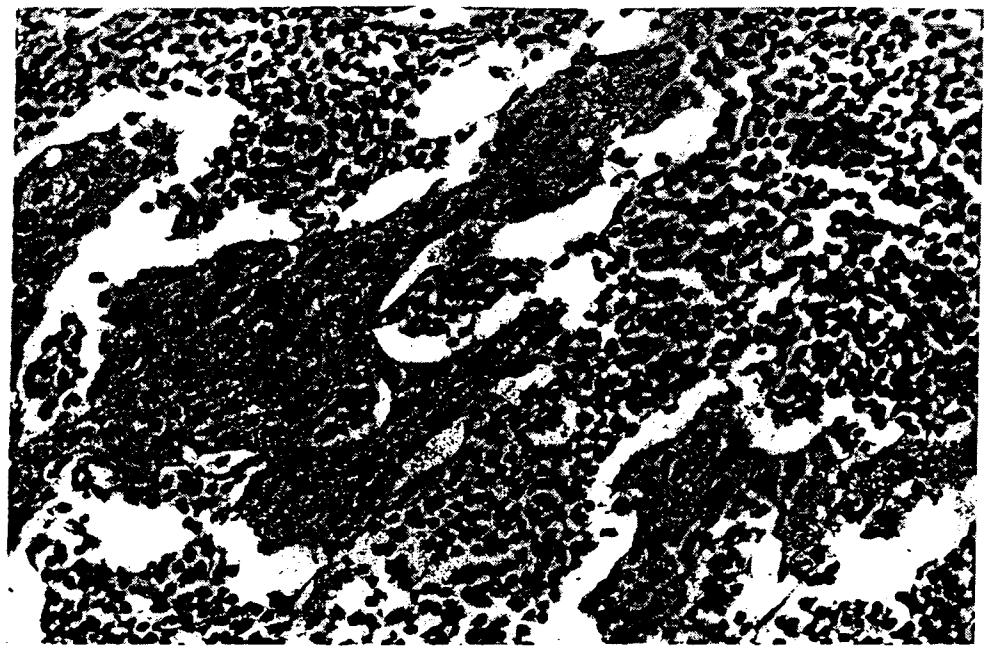
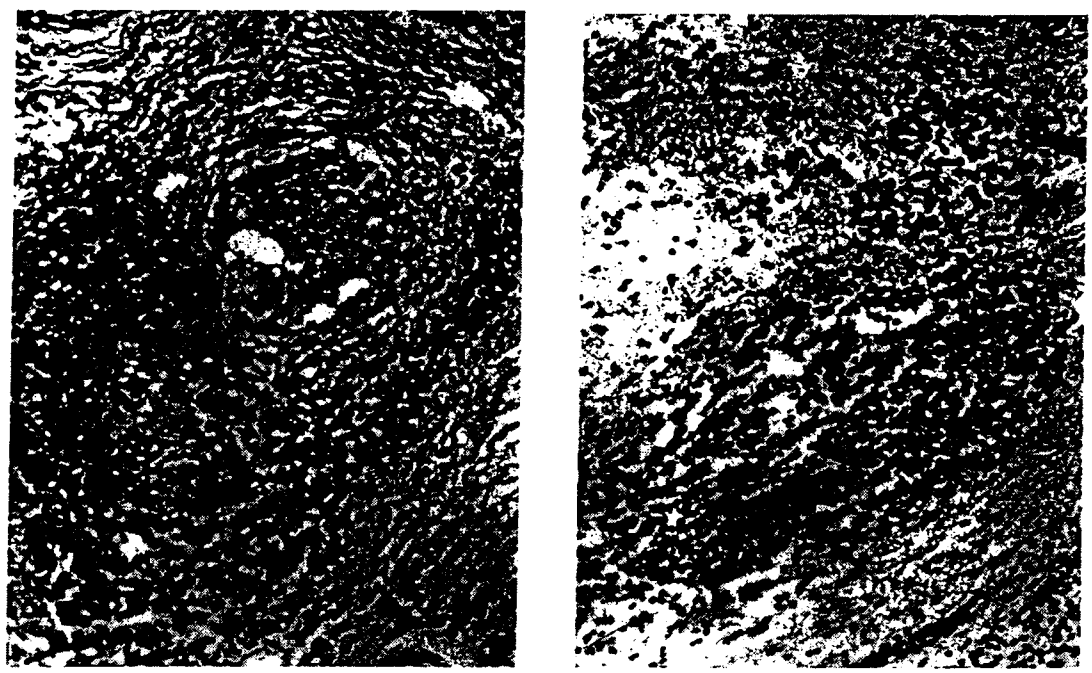


Fig. 2 Inflammatory infiltrates of the tumour. Staining H+E, mag. 400 x.



Figs. 3-4 Inflammatory infiltrates forming the germinal centre around the tumour cells. Staining H+E, mag. 160 x.

4). Many neoplastic cells surrounded by inflammatory infiltrates were found degenerated, enlarged with vacuolated cytoplasm, undergoing necrosis or already necrotic (Fig. 5).

By immunofluorescence (IF), the connective tissue located in tumours was found heavily embedded in IgG, as well as some necrotic foci or groups of carcinomatous cells undergoing necrosis (Fig. 6). IgG-positive cells were sparsely spread throughout the neoplastic tissue.

IgM-positive cells were predominant and IgA-positive cells not very numerous. Among the IgM-positive cells were found lymphocytes infiltrating bundles of connective tissue, plasma cells in different stages of differentiation (Fig. 7), and carcinomatous cells either scattered along the connective tissue (Fig. 8) or located in groups forming a mesh (Fig. 9). IgM located in neoplastic cells was found either in the cytoplasm and nucleus or in the form of droplets on the surface of the cell membrane. In necrotic foci, IgM covered all disrupted fragments of cells (Fig. 10).

Mononuclears infiltrating tumours were found to be B lymphocytes (CD20) and T lymphocytes almost exclusively CD8-positive (Fig. 11). They were located along the connective tissue surrounding the mesh of carcinomatous cells. Mononuclears with CD16 marker were almost absent. Single positive HLA-DR cells were noted among carcinomatous cells. The neoplastic cells positive to Ukrain, expressing its own autofluorescence, were only single, but focally correlated with the intensity of mononuclear infiltrates.

The above-presented results confirm previous observations indicating an anti-tumour potency of Ukrain (2-5). However, its mode of action is mostly operational not by direct cytotoxicity of the drug, but through the influence of

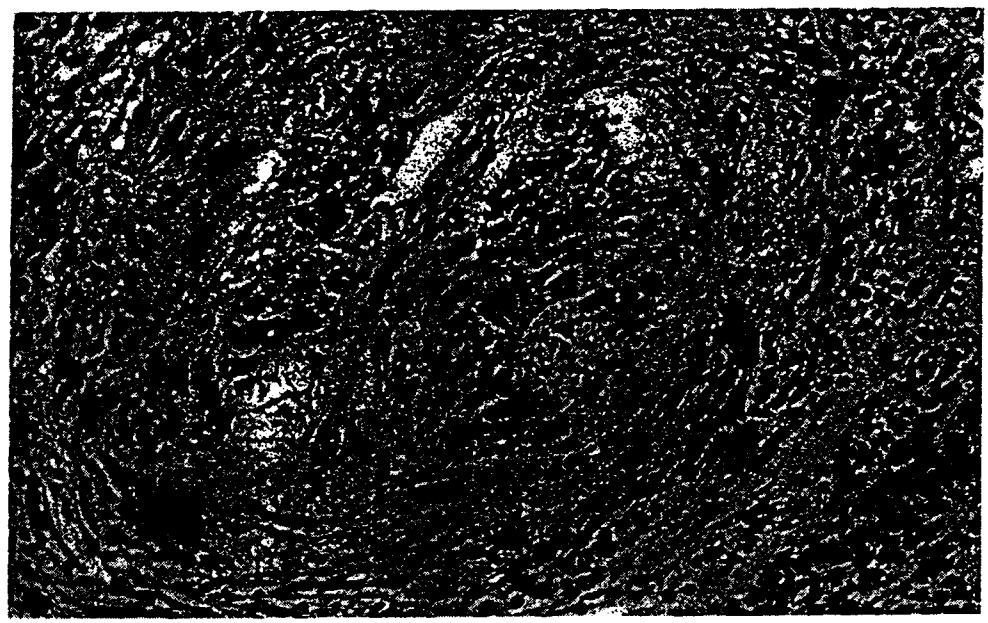


Fig. 5 Group of carcinomatous cells undergoing degenerative changes surrounded by inflammatory infiltrates composed of lymphocytes and plasma cells. Staining H+E, mag. 400 x.

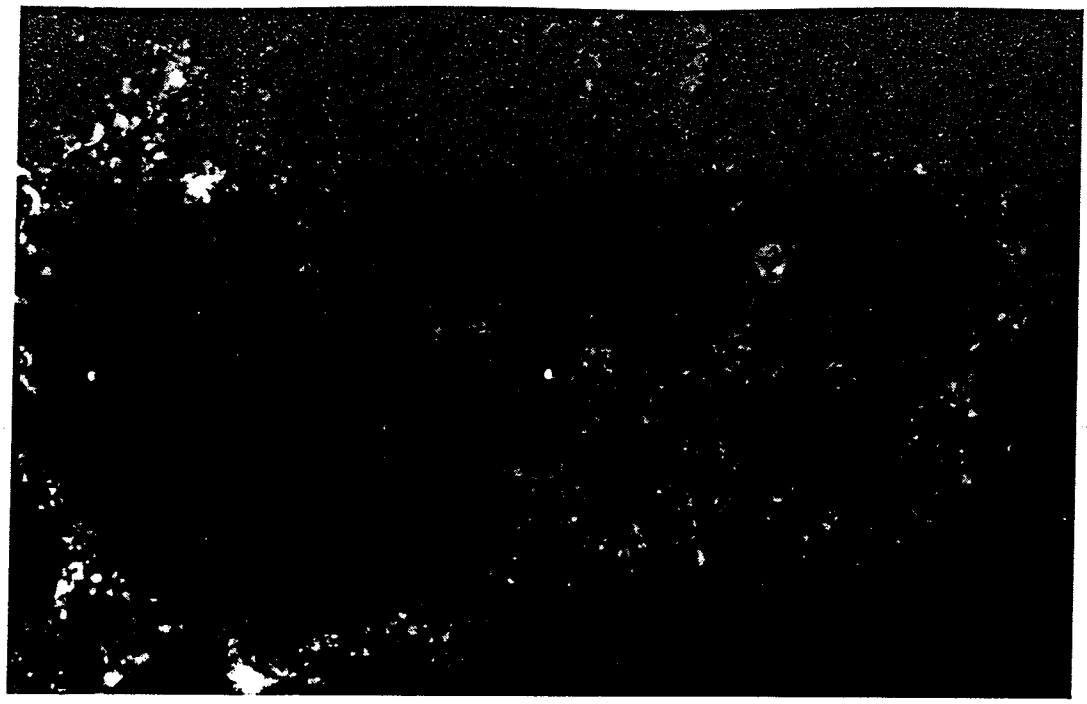


Fig. 6 IgG localization in the connective tissue and vascular bed of the tumour. Staining, Rab. anti-Human IgG FITC, mag. 400 x.

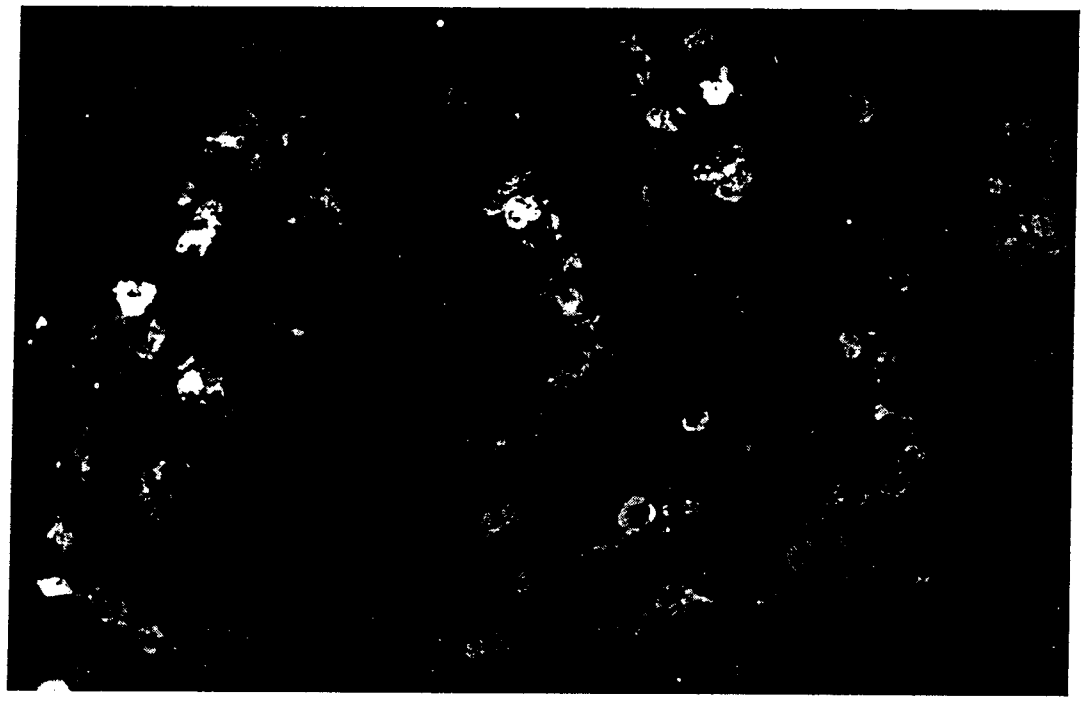


Fig. 7 IgG-positive plasma cells infiltrating the tumour tissue. Staining, Rab. anti-Human IgG FITC, mag. 400 x.

Fig. 8 Group of carcinomatous cells infiltrated by IgM and surrounded by IgM positive plasma cells. Staining, Rab. anti-Human IgM FITC, mag. 400 x.

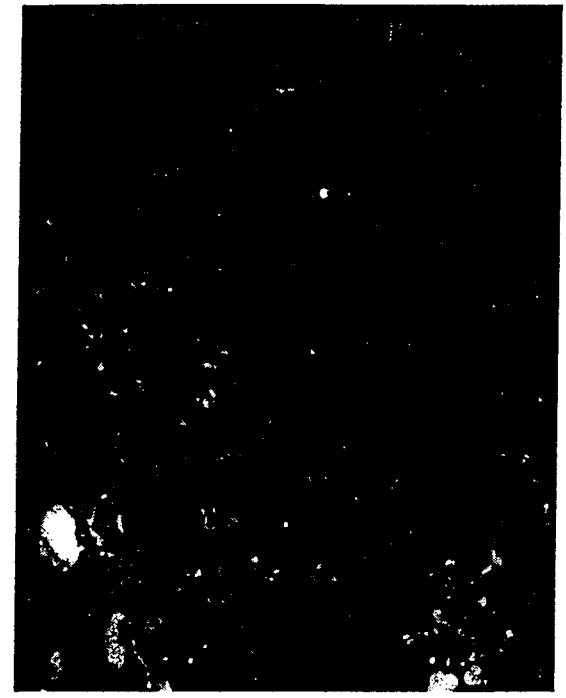


Fig. 9 Foci of carcinomatous cells undergoing necrosis with IgM present both on the surface and/or inside the cytoplasm of cells. Staining, Rab. anti-Human IgM FITC, mag. 400 x.

the drug on neoplastic tissue and changes in its antigenic expression which make it recognizable for the immune system. This viewpoint is fully supported both clinically by MG, USG and HP observations and by IF analysis of the tumours.

The increase in tumour size and its hardness may correspond to the diffuse and intensive inflammatory reaction observed in the tumours as well as inflammatory-mediated increase of the connective tissue. The local immune reaction observed in the tumours (breast carcinomas) which was detected in all our patients resembles a rejection reaction exerted by the host versus graft.

From the clinical viewpoint, pretreatment of patients with breast carcinoma with 50 mg (10 injections) of Ukrain is highly recommended. The drug with no side-effects helps the surgeon to locate the primary tumour as well as the metastatic lymph nodes.

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References

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Fig. 10 Necrotic foci of the tumour covered by IgM. Staining, Rab. anti-Human IgM FITC, mag. 400 x.

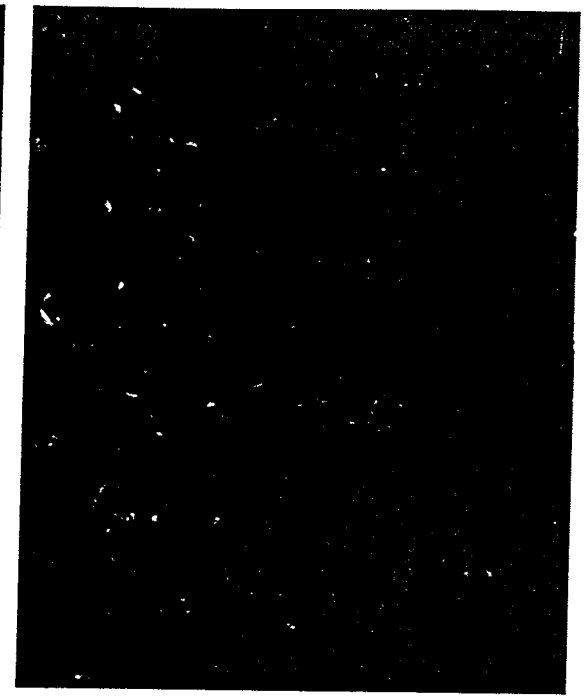

Fig. 11 CD8 T lymphocytes of mononuclear infiltrates in the tumour tissue. Staining, Mouse anti-T CD8/Rab. anti-Mouse FITC, mag. 400 x.

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