

INFLUENCE OF UKRAIN ON PATIENTS WITH SURGICALLY TREATED BREAST CANCER. PART IV. ELECTROMICROSCOPIC AND CYTOCHEMICAL EVALUATION

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Summary: *The present studies were undertaken to evaluate by light and by electron microscopy the influence of Ukrain on the morphology of breast cancer. The studies were carried out on material obtained from ten patients with breast cancer, treated preoperatively with Ukrain. Control material for the studies was obtained from patients of similar age and advancement of the disease, who did not receive Ukrain. The data obtained in the present studies indicate that Ukrain is responsible for severe changes in morphology of tumour cells. Histological examination by both light and electron microscopy revealed cells characteristic of those undergoing apoptosis. The stromal changes of the tumour were characterized by intensive proliferation of the connective tissue accompanied by an immune reaction expressed by mononuclear infiltrates.*

Introduction

In recent decades breast cancer has been identified as one of the highest causes of death in women. Therapy for this disease is not at present definitively agreed upon or finally solved. It needs further perfecting, which should be sought at the very base of the process of neoplasm formation.

Ukrain is a new anticancer medicament which seems to be highly promising. It has cytotoxic properties based on alkaloids obtained from *Chelidonium majus* L. (1, 3-5).

The present study was undertaken to follow the

changes in the behaviour of breast cancer under the influence of Ukrain. These effects were evaluated by means of electron microscopy and cytochemical methods.

Patients and methods

The changes in breast cancer produced by Ukrain in ten patients were evaluated. Diagnosis of cancer was established clinically on the basis of ultrasonography (USG) and mammography as well as by histological study of material from the tumours taken by needle biopsy. The age of the patients varied within the limits of 38-65 years. Six

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patients were in the T2NOMO stage, two in T2N1MO, one in T3NOMO and one in T3N1MO.

Before surgical intervention the patients received intravenous Ukrain in a dose of 5 mg every second day for 20 days. Each patient received a total of 50 mg. The patients were operated 7 to 10 days after the last injection, by radical mastectomy according to the methods of Patey or Halsted.

The control group consisted of ten patients with similar diagnoses and of similar ages who did not receive Ukrain and were treated only by mastectomy.

Material for morphological examination was collected at operation. In each case the size of the primary tumour was evaluated; its situation in relation to the surrounding tissue and the cancerous tissue was evaluated on the cross-section. Tissue material was collected from the border and the centre of the tumour; the lymph nodes were also examined.

Material for histopathological inspection was fixed in 10% buffered formalin. For electronmicroscopic (EM) studies the material was fixed in cooled 2.5% glutaraldehyde. Material frozen in liquid nitrogen was used for histochemical analyses. Material for electron microscopic examination was routinely processed through 1% osmic acid and phosphate buffer, and after dehydration embedded in epon and araldite, according to Mollenhauer (2). Ultrathin sections were cut in a microtome LKB-3 (Sweden). Sections were stained with uranyl acetate after Reynolds (2) and inspected in the electron microscope PEM-100.

The sections for cytochemical investigation were cut in a cryostat at -20°C and stained for the presence of succinodehydrogenase, lactodehydrogenase and acid phosphatase. Staining was performed by the method of Pears (2). Quantitative evaluation was done with a cytospectrophotometer MFTX-2M (SNG).

Results

EM studies demonstrated that cancer cells in the examined patients with breast cancer were in

most cases poorly differentiated. The nuclei were big with coarse-grained chromatin translocated to the edge of the karyoplasm. One or several nucleoli observed in such nuclei were distinctly delineated, separate from the remaining nuclear structures. The pores in the nuclear membranes were not pronounced; however, in some places they were distinctly broadened. The cytoplasmic reticulum of the cancer cells was weakly developed with focal broadening of the cisternae. No secretion was found in them. Between the cytoplasmic reticulum numerous dispersed free ribosomes could be seen. A few mitochondria in the cytoplasm were swollen and the structure of the cristae was damaged. In some cells there were glycogen grains, a few liposomes not differing in size and myelin structures, many of them in the form of many layer rolls contrasted distinctly in the cytoplasm of the cancer cells. The Golgi complex was seldom found and usually compact.

Under the influence of Ukrain the cancer cell nuclei changed; they took polymorphic forms, became more dense and the chromatin shifted to the periphery of the nucleoplasm, sometimes even undergoing fragmentation. The structure of the nucleoli became condensed. In some cells their number reached 2-3. The pores in the nuclear membrane were markedly outlined and on the side facing them the cytoplasm was rarefied.

Under the influence of Ukrain the endoplasmic reticulum underwent fragmentation. Its lumen became wider, in some places forming cisternae or even vacuoles. Within their lumen their content of low electron density could be seen. Ribosomes on the membranes of the cisternae differed greatly in size, shape and electron density (Fig. 1). Within the cytoplasm, beside cisternae, rather numerous free ribosomes could be seen. The latter differed only slightly from the controls. The most pronounced changes due to Ukrain concerned the mitochondria, which became polymorphic, swollen, with the cristae damaged. The matrix exhibited lighter patches (Figs. 1, 2). Many of them were empty. In the cytoplasm, the number of lysosomes, myelin bodies and large phagolysosomes containing several mitochondria under-

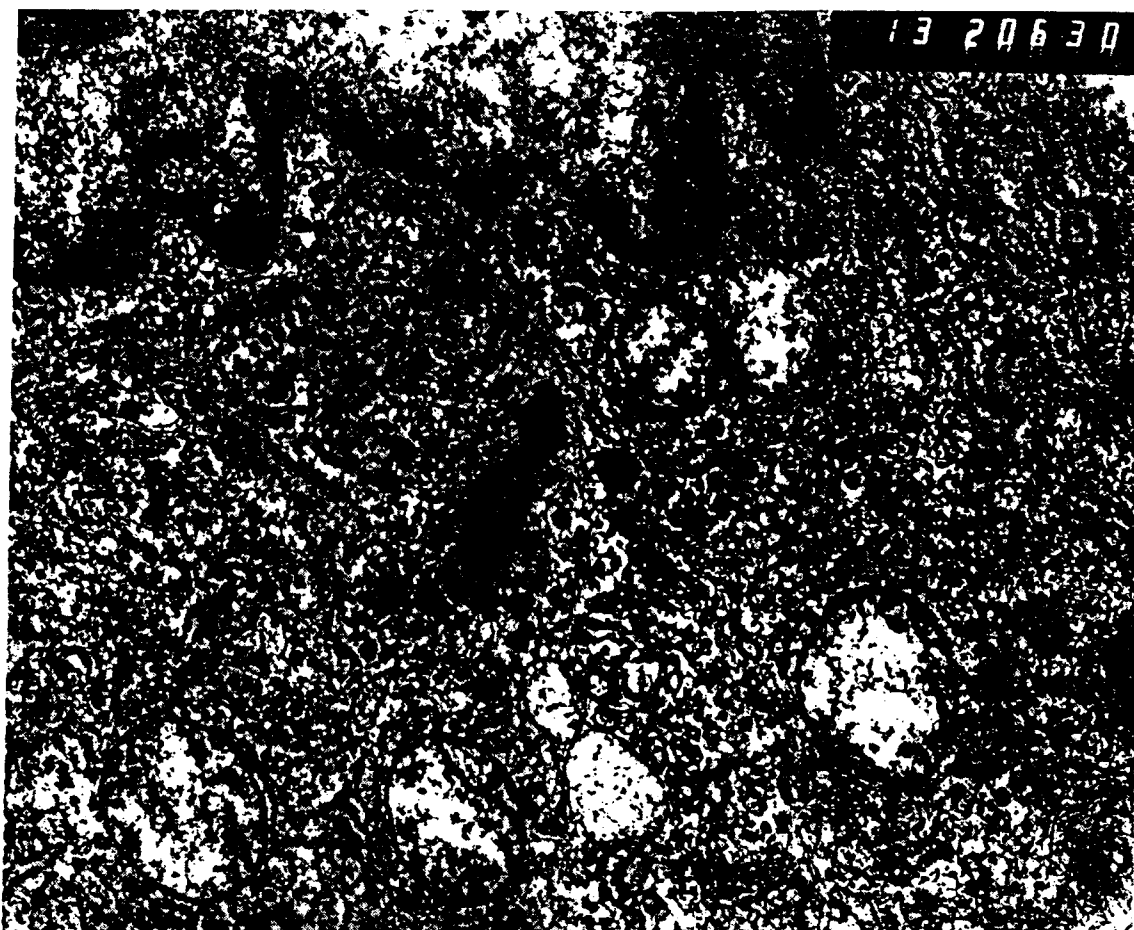


Fig. 1 Breast cancer cell under influence of Ukrain. Fragmentation of endoplasmic reticulum. Formation of vacuoles and cisternae. Swelling and destruction of mitochondria. (x 32,000).

going lysis and fragments of endoplasmic reticulum increased (Fig. 2). In such cells the number of equal-sized liposomes also increased. In many cancer cells the cytoplasm was swollen with deep changes in the cytoplasmic structures including even their destruction.

The influence of Ukrain on the vascular system was only slightly noticeable, characterized by swelling of endothelial cells with, however, their ultrastructure well preserved.

In tumour tissue from patients treated with Ukrain there was a markedly higher number of fibroblasts and extracellular connective fibres as compared with the controls (Figs. 3, 4). They had a normal ultrastructure with all organelles pronounced.

Histochemical examination of cells of breast cancer demonstrated quantitative differences with regard to enzyme content. These differences in the neoplastic tissue may best be explained by



Fig. 2 Breast cancer cell under influence of Ukrain. Disintegration of endoplasmic reticulum with formation of large phagolysosomes containing myelin bodies. (x 25,000).

the degree of differentiation of the particular neoplastic cells in the tumour tissue. In the analysed breast cancer cells enzyme activity was moderate. This concerned 75% of cells. To obtain results closer to the mean, the slides were subjected to cytospectrophotometric analysis.

The tissues of patients with breast cancer who were treated with Ukrain were subjected to the same study and demonstrated that in patients after Ukrain therapy, mitochondrial dehydrogena-

se activity responsible for respiration processes and being a component of the electron transport chain (6), succinodehydrogenase (SDH) and dehydrogenase (NADH), and in some smaller degree the cytoplasmic lactodehydrogenase (LDH) controlling neoglycogenesis, was reduced (7). The influence of Ukrain on the enzymes responsible for oxidating-reducing processes and oxygen production within the whole neoplastic cell indicates that Ukrain is active along the phe-

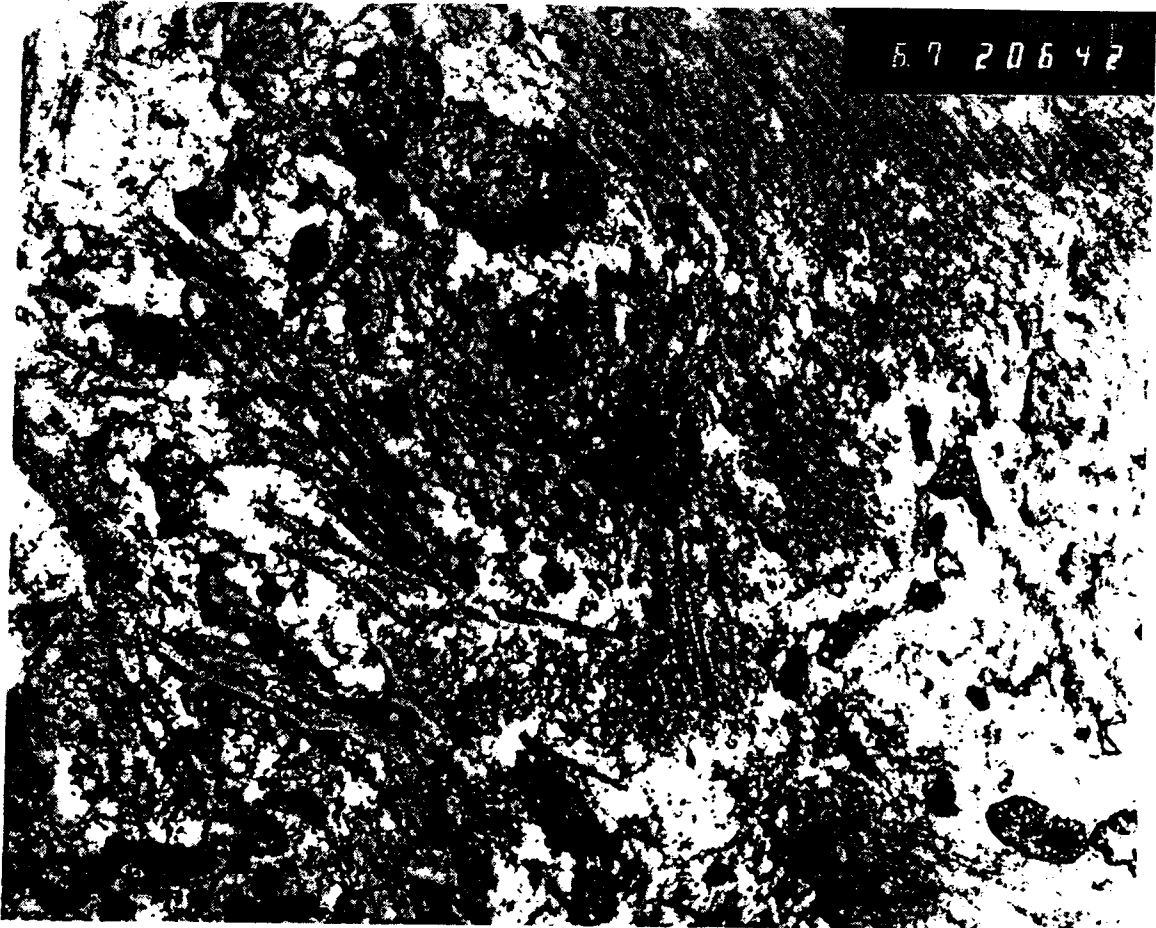


Fig. 3 Breast cancer cell under influence of Ukrain. Connective fibres surrounding remnants of disintegrated cancerous cells. (x 16,750).

nomena responsible for cell oxidation processes (3, 4). At the same time an enhanced activity of glyco-6-phosphatase dehydrogenase (G-6-P-DH) and a threefold increase of activity of the lysosomal enzyme, acid phosphatase (AP), was observed (Fig. 5). The latter observation indicates an intensification of processes leading to destruction of the neoplastic cells. These results are also shown in Table I.

Discussion

The main energetic substrate for the neoplastic tissue is glucose, and the intensive glycolysis serves to restore ATP in the cancer cells (8). A reduction of LDH, SDH and NADH-dehydrogenase (NADH-DH) activity by Ukrain may indicate a diminished ATP activity in the neoplastic tissue owing to the reduced glycolysis and anaerobic



Fig. 4 Breast cancer cell under influence of Ukrain and diffuse proliferation of connective tissue fibres responsible for characteristic hardness of the tumour after Ukrain treatment. (x 25,000).

decomposition of glucose (9). Neoplastic cells are known to have a tendency to accumulate lactic acid, since they utilise for oxidation of NADH pyruvate (10) instead of the mitochondrial mechanism. The increase in the reduction potential NAD/NADH, when Ukrain is applied can probably be obtained in neoglycogenesis by reducing LDH activity. This supposition is confirmed by the results of our biochemical investigations indicating in the tissue of patients treated with Ukrain an

increase of the major glycogenous amino acids, i.e., alanine, serine, glutamine, glycine, valine and histidine.

When enzyme activity is considerably lowered in the tumour tissue after Ukrain administration, the observed rise of G-6-P-DH activity is noteworthy. It is known that ATP is an inhibitor of this enzymatic system. Therefore, the increase in G-6-P-DH activity observed in the neoplastic tissue after Ukrain may indicate a lower ATP activity

Table 1 Activity of enzymes in cancer cells of patients with breast cancer treated with Ukrain

Groups	SDH	LDH	NADH-DH	G-6-P-DH	AP
Ukrain	0.038 ± 0.03	0.549 ± 0.041	0.483 ± 0.03	0.4822 ± 0.023	0.341 ± 0.46
Control	0.068 ± 0.003	0.791 ± 0.039	0.802 ± 0.057	0.3038 ± 0.032	0.108 ± 0.09
P	0.000002	0.0004	0.0002	0.0004	0.00002

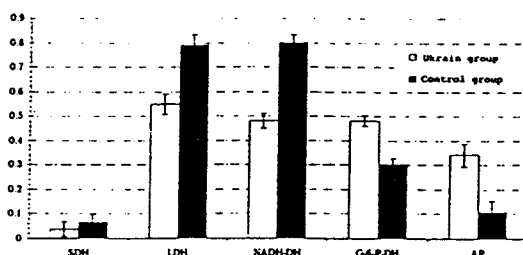


Fig. 5 Activity of enzymes in cancer cells of patients with breast cancer treated with Ukrain.

and thus a reduction of cellular ATP (11).

The threefold rise of acid phosphatase activity in the neoplastic tissue as the effect of Ukrain points to an intensification of the processes of tissue disintegration. This is confirmed in the electron microscopic pictures in the form of enhanced destruction of mitochondria and increase in the cells of the number of primary and secondary lysosomes. Additional confirmation of this was found in biochemical analyses which demonstrated normalisation of ethanolamines/phosphoethanolamines ratio under the influence of Ukrain.

Conclusion

In view of all the observations presented above, one may conclude that Ukrain is undoubtedly a cytotoxic substance to cancerous tissue,

leading to its disintegration, with subsequent proliferation of connective tissue. This conclusion is fully confirmed by our histological, histochemical and electron microscopic studies.

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