Activation of kaspaze-3 under amitozyn effect on the cells of lymphatic human leukemia MT-4 line

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The large group of natural preparations and compounds, obtained using their various chemical modifications, were revealed among substances with antitumor activity. Alkaloid-ethyleneimines, recently being under study, are among these substances. Amitozyn antitumor preparation was obtained in Ukraine using celandine (*Chelidonium majus L.*) alkaloids modification by thiophosphamide in accordance with original elaboration of A.I. Potopalsky together with co-authors [1]. Wide range of its antitumor action against different solid malignant neoplasms was shown. Meanwhile, the action of the preparation on leucosis and lymphoma cells almost has not been studied, and mechanisms that mediate amitozyn antitumor activity are still undiscovered.

Apoptosis induction and activation of kaspaze cascade are of a great importance in realization of the most chemical preparation cytotoxic action [2]. Meanwhile, these mechanisms contribution to amitozyn antitumor activity almost have not been studied to present day.

The purpose of the given investigation was to study phase-specificity of amitozyn action *in vitro* on model of subinoculative cells line of acute lymphoblastic human leucosis MT-4, and also analysis of activation of kaspaze-3 during apoptosis process, activated by amitozyn.

Material and methods: The researches were conducted on subinoculative cells line of acute lymphoblastic human leucosis MT-4. Amitozyn preparation was used in 25-250 mkg/ml concentration. Apoptotic cells content in the samples was

determined using light-optical microscopy (May-Grunwald–Giemsa blood staining) and flow cytoflourimetry (propidium iodide staining). Flow cytoflourimetry was used for analysis of the cells mitotic cycling state distribution. Kaspaze-3 active form in the cells was revealed using set of "mAb Apoptosis Kit FITC" (BD Bioscience Pharmingen, USA).

Results: MT-4 cells treatment with amitozyn at concentrations higher than 100 mkg/ml resulted in significant cells growth inhibition, but was not accompanied by mass cells death. Hypodiploidic (apoptotic) cells content in 24 hours after preparation adding was 24.2% (table 1). At that, evident accumulation of cells, cultivated at amitozyn presence in G_2/M point of the cell cycle, was observed (figure). The similar researches, conducted with etoposide (DNA-topoisomerase II inhibitor), showed that apoptosis induction was accompanied by the delay of cells passing S-phase (see table 1).

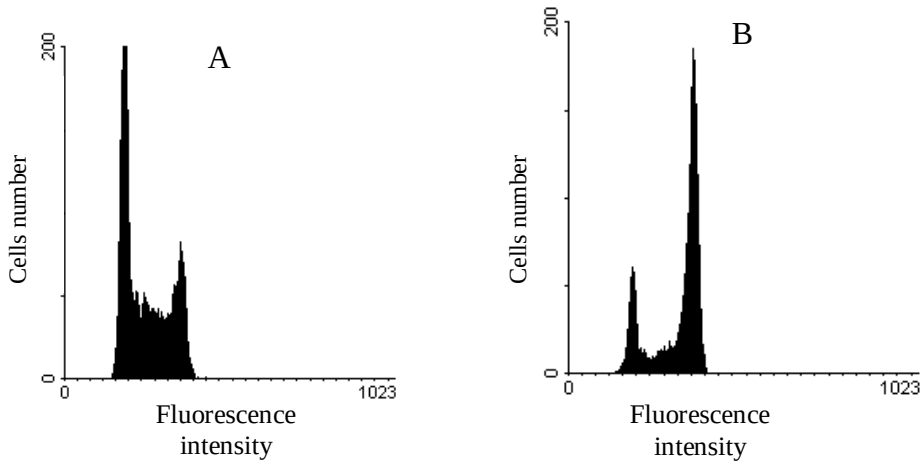

Figure. MT-4 cells cycling state distribution at amitozyn action: A – control; B – amitozyn, 100 mkg/ml, 24 h

Table 1. Data of flow cytoflourimetry of MT-4 cells, stained with propidium iodide

Preparation	Cells cycling state distribution, %			Apoptotic cells content, %
	G_0/G_1	S	G_2/M	
Amitozyn 25 mkg/ml	38.96	46.75	14.25	7.1
Amitozyn 100 mkg/ml	13.01	20.03	66.96	24.2
Control	41.25	46.26	12.48	5.4
Preparation for comparison – etoposide, 40 mkg/ml	79.43	18.12	2.45	54.1

Using monoclonal antibodies C92-605 against kaspaze-3 active form, its activation in 24 hours after cells cultivation with amitozyn different dosages at comparatively low apoptotic index value was shown (table 2). At that, amitozyn concentration increasing does not lead to significant increasing of the cells percentage content with activated kaspaze-3 (see tables 1, 2).

Table 2. Content of active kaspaze-3 form in MT-4 cells, treated with amitozyn or etopisodine during 24 hours

Preparation, dosage mkg/ml	Percentage of cells, containing kaspaze-3 active form
0	6.67
Amitozyn 25	10.93
Amitozyn 125	12.84
Amitozyn 250	17.71
Etoposide 40	51.20

Conclusions: It was shown that amitozyn caused inhibition of the malignant human lymphoid cells proliferation, at that inducting the arrest in G_2/M point of the cell cycle. Such a mechanism of action of amitozyn and other widely used

antitumor preparations (e.g. doxorubicine or vincryctine [3, 4]) is a subject of interest, because amitozyn usage in combination with preparations that act on the other phases of cell cycle can lead to additive or synergic effects. Besides, amitozyn, comparatively nontoxic preparation, causes apoptosis of the malignant human lymphoid cells, accompanied by effector kaspaze-3 activation.

References

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