Influence of chelidonin, cheliritrin, and sanguinarin on the cellular cycle of lymphoblastic human leukemia MT-4 line: comparative analysis of their DNA-recognizing capability

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Celandine alkaloids and their derivatives can decelerate proliferation and cause malignant cells death that is the reason why the preparations, made on their bases, are offered as antitumor therapy means [1, 2]. The mechanisms of action of such preparations on target cells are not adequately explored, but, in particular, there are still controversial questions concerning direct relation of antiproliferative and cytotoxic alkaloid effects, and their capability to interact with cells DNA.

The purpose of the work – is to study the action of particular celandine alkaloids: chelidonin, cheliritrin, and sanguinarin on apoptosis induction and allocation for the cells cycle of lymphoblastic human leukemia MT-4 in comparison with DNA-intercalary characteristics of these substances.

The researches were conducted on subinoculative cells line of acute lymphoblastic human leukemia MT-4. The cells were cultivated according to established procedure. Chelidonin, cheliritrin, and sanguinarin alkaloids were obtained from celandine (*Chelidonium majus L.*) rootstocks according to the method described in [3]. Mother alcoholic 1 % solutions were prepared using these preparations; to obtain specified resulting concentration aliquot was introduced into the cellular suspension. Cells were cultivated during 24 hours at the presence of mentioned above alkaloids.

The apoptotic cells were revealed using cytological method after Giemsa staining, and also using flow cytometry of cells filled by ionic propidium.

Cells distribution on cycling states was analyzed in cytofluorimeter using "ModFit" mathematical program. To give quantitative assessment of intercalating possibility we determined DNA termodenaturation dynamics of salmon sperm (Sigma Chem. Co.) at the increasing alkaloids concentrations presence. Relation degree of specified alkaloids against DNA was estimated by determining alkaloid concentrations that leads to half-effect of DNA melting temperature increasing [4].

All three investigated alkaloids revealed toxic effect to the cells line of acute lymphoid leukemia MT-4 approximately in the same range of concentrations. At 10 mkg/ml concentration practically full cells death in 24 hours was registered. To reveal cells apoptosis and their cycling state distribution, alkaloids preparations were used at 1-2 mkg/ml concentration, at that alkaloids toxicity during 24 hours was moderately expressed.

The results of determining the apoptotic cells percentage and the highest DNA melting temperature increasing at the celandine alkaloids presence are shown in the table. In the accompanying figure cells cycling state distribution under the mentioned above alkaloids influence is shown.

Table. Apoptosis induction by specified alkaloids in MT-4 cells and intercalating possibility of these alkaloids relatively DNA standard preparation

Alkaloids	**The apoptotic cells percentage**	**DNA melting temperature increasing, °C**
Control (without alkaloids)	5,8	–
Chelidonin	33,7	0,5
Cheliritrin	8,7	16,1
Sanguinarin	19,1	15,0

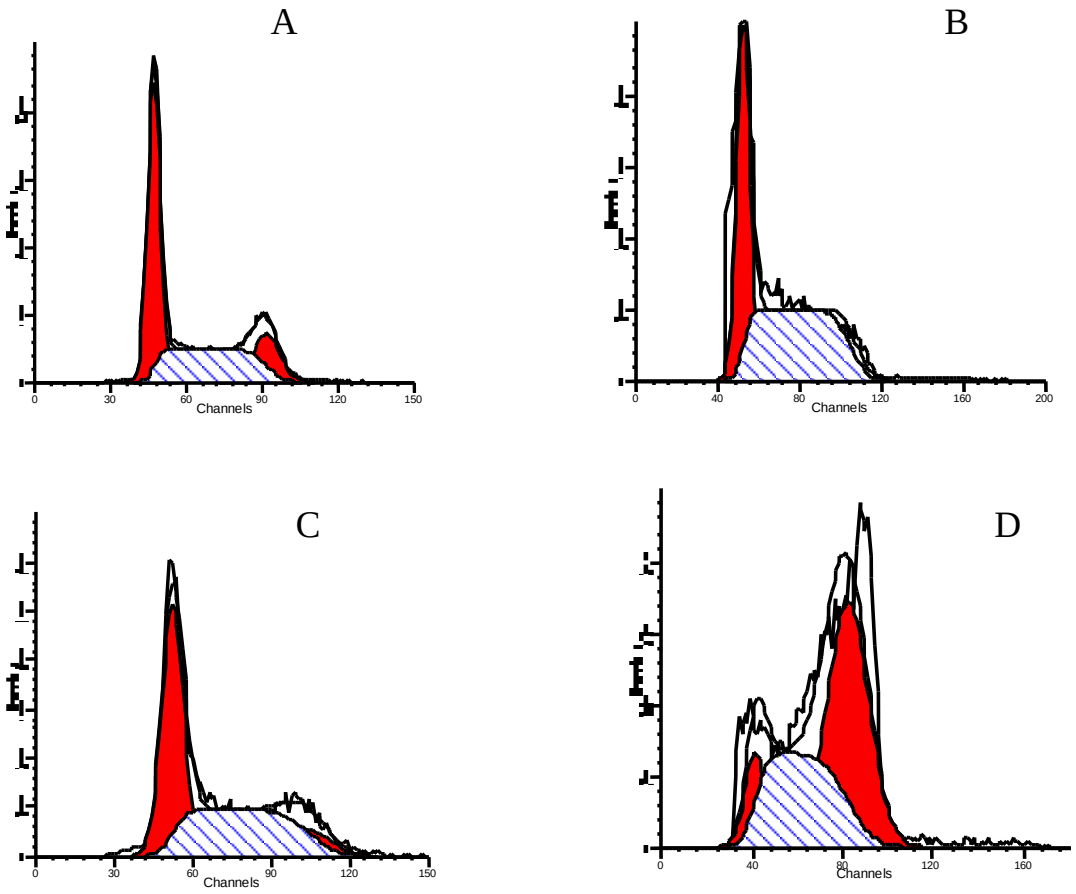

Figure. MT-4 cells cycling state distribution at the celandine alkaloids action: A – intact cells; B –cheliritrin; C – sanguinarin; D – chelidonin.

As may be seen from data above, chelidonin has the highest apoptosis-inducting possibility among investigated alkaloids. At that, in contrast to cheliritrin and sanguinarin, this alkaloid leads to significant increasing of cells part in G_2/M phase up to 53±3% comparing to 15±4% in control. This happens at the expense of cells decreasing in G_0/G_1 phase (10±4% comparing to 48±2% in control). This effect is similar to the action of amitozyn preparation and cumulative celandine alkaloids extract [5]. Cheliritrin and sanguinarin in the investigated concentrations range cause certain decreasing of the cells part in G_2/M phase with cells part corresponding increasing in S phase (46±4% comparing to 33±3% in control). These alkaloids have similar structure, strong intercalary properties and bind to DNA with high affinity that is specified by coplanar conformation of the alkaloids molecules and their favorable energy location between nitrogen bases pairs in

DNA helix. This brings to the helix stabilization (melting temperature increasing) and, correspondingly, to the replication process inhibition. In such a way, cells accumulation in S phase at sanguinarin and cheliritrin presence can be explained. Low content of apoptotic cells in the population at these alkaloids presence may be conditioned by their high toxicity, rapid death, and cells disintegration without their accumulation.

Chelidonin differs from the other alkaloids with higher hydrogenation of cycles and cycle area deviation from coplanarity that hinders from entering of chelidonin molecule between DNA bases pairs and leads to the DNA-intercalary capability loss by alkaloid. From the literature data is known that chelidonin has tubulin-binding property and is antimitotic factor [6]. It is also relatively less cytotoxic factor comparing to the other alkaloids. Using these properties, a significant cells number increasing in G_2/M phase with apoptosis characteristics can be explained.

References

1. **A.I. Potopalsky** Chelidonium majus L. preparations in biology and medicine. Kiev, "Naukova dumka", 1992, p. 200.
2. **Taborska E., Bochorakova H., Dostal J., Paulova H.** The greater celandine (*Chelidonium majus L.*) – review of present knowledge. Ceska Slov. Farm. 1995; **44**: 71-75.
3. **Slavik J., Slavikova L.** Minor alkaloids from *Chelidonium majus L*. Coll. Czechoslov. Chem. Commun. 1977; **42**: 2686-2693.
4. **Schmeller T., Latz-Bruning B., Wink M.** Biochemical activities of berberine, palmatine and sanguinarine mediating chemical defense against microorganisms and herbivores. Phytochem. 1997; **44**: 257-266.
5. **M.P. Zavelevich, N.N. Hranovskaya, L.A. Zaika, Yu.A. Potopalskaya, Yu.A. Grinevich, A.A. Filchenkov** Cell cycle standstill in G_2/M point and apoptosis induction in malignant lymphoid human cells under amitozyn action *in vitro*. Materials of the Ist All-Ukrainian scientifically-practical conference “Domestic antitumor preparations: analyses of the present and future”. Kyiv, the 14-15th of October 2004. The appendix to “Oncology” journal 2004; **6**: 10.
6. **Colombo M.L., Bosisio E.** Pharmacological activities of *Chelidonium majus L*. Pharmacol. Res. 1996; **33**: 127-134.