INFLUENCE OF AMITOZYN AND SOME CELANDINE ALKALOIDS ON TRANSCRIPTION *IN VITRO*

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Effective antitumor preparation amitozyn is produced by modification of great celandine (Chelidonium majus L.) alkaloids with thiophosphoramide (thiotepa). Amitozyn reveals antimitotic activity like its initial compounds. But its antitumor effect is much stronger than additive effect of celandine alkaloids and thiotepa. Moreover, amitozyn shows higher specificity and lower toxicity [1, 2]. In therapeutic doses amitozyn is selectively accumulated in tumor cells and causes their death. Nevertheless, both the structure of amitozyn and mechanisms of its action remain unknown.

Previous study of experimental tumor models of mouse and rat showed the decreased amount of nucleic acids after amitozyn treatment [1]. In order to clarify the reason of such declination we studied the influence of amitozyn and some celandine alkaloids on transcription *in vitro*.

DNA-dependent RNA-polymerase (RNAP) of bacteriofag T7 was used as a test-system *in vitro*. It is the one-subunit ferment and is able to accomplish a complete cycle of transcription without protein factors in contrast to the most of other RNAPs [3]. These properties of the ferment made it to be quite convenient model for investigation of both transcription and various factors influence including the influence of bioactive compounds on RNA biosynthesis [3, 4]. It is worthy to note, that the three-dimensional structure of RNAP T7 is essentially similar to the broad range of DNA-, RNA-polymerases and reverse transcriptases, including evolutionary distant ones [3, 5]. Their spatial structures of polymerazing domains have significant similarities. Moreover, all DNA- and RNA-polymerases without exceptions have two conservative motifs in the absence of evident homologies in the primary structures [3].

Therefore the data on the influence of bioactive compounds on transcription *in vitro* in cell-free systems with DNA-dependent RNA-polymerase of bacteriofag T7 might be extrapolated to a certain extent on the ferments catalyzing similar reactions, i.e. the formation of diether bonds between r-NTP- or dNTP-5'-phosphate and 3'-hydroxyl of RNA or DNA end nucleotide [3, 5].

Studied substances were tested in the concentrations of 100 μg/ml.

Electrophoresis data (Fig 1.) showing synthesized RNA products, demonstrates the influence of celandine alkaloids and amitozyn on transcription *in vitro* with DNA-dependent RNA-polymerase of bacteriofag T7. As it is seen on the figure, sanguinarine hydrochloride (1) completely inhibits the total synthesis of RNA.

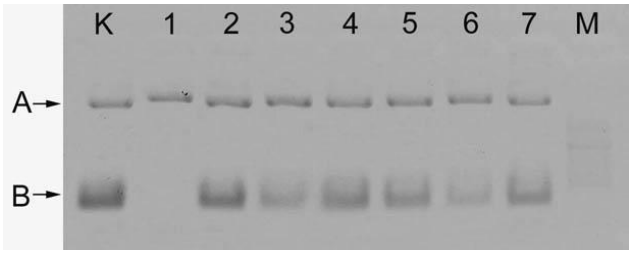

Figure 1. *In vitro* inhibition of RNA synthesis by celandine alkaloids and amitozyn.

A – DNA matrix; B – synthesized RNA. Alkaloids and amitozyn were used in concentration 100 μg/ml. K - control; 1 –sanguinarine chloride; 2 – chelidonine hydrochloride; 3 – berberine chloride; 4 – tribetamide; 5 – amitozyn I; 6 – amitozyn II; 7 – thiotepa; M – markers.

Amitozyn I, amitozyn II, thiotepa and berberine chloride significantly, but not completely inhibit T7 RNAP – synthesis. The insignificant inhibition was observed in case of chelidonine hydrochloride and thribetamide.

As is known, the celandine alkaloids berberine and sanguinarine have planar multinuclear angular structure and are able to intercalate into DNAs and RNAs [6, 7]. Sanguinarine intercalates into DNA completely and strongly binds to the B-form DNA [7], while both ligand-DNA and ligand-enzyme interactions are important for the poisoning by berberine [6, 8]. Inhibition of transcription by sanguinarine can be caused by its intercalation into DNA. The interaction of sanguinarine and DNA-matrix makes formation of productive complex between DNA and the ferment impossible. Berberine chloride inhibits RNA polymerase much worse than sanguinarine (Fig 1.). Comparing to sanguinarine it binds DNA weaker and just partially intercalates into DNA [7]. Hence we have observed some correlation between the transcription inhibition level by the alkaloids and their DNA interaction ability.

Amitozyn, a product of great celandine alkaloid mix and thiotepa interaction, inhibits RNA synthesis weaker than the minor alkaloid sanguinarine, but much stronger than one of the major celandine alkaloids chelidonine. Therefore, the contribution to RNA polymerase inhibitory activity of amitozyn can made not only the alkaloid relative contents but also their interaction with thiotepa which can change the character of their interaction with nucleic acids.

The further investigation of amitozyn mechanisms of action will concern the elucidation of the interaction of constitutive compounds of amitozyn with both nucleic acids and RNA polymerase.

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