Report on the pathophysiological mechanisms of antiproliferative Flaraxin effect realization.

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Goal of experimental study

Flaraxin is an antitumoral remedy of vegetable origin, one of the few remedies with expressed cancerolitic effect under practical absence of toxicity (LD_{50} for rodents in the limits of 115-350 mg/kg of the body weight). The trials conducted in a number of leading scientific establishments of the Ukrainian Academy of sciences showed, that flaraxin has antioxidant properties, stimulates endogenic interferon output and tumor necrosis factor. Besides clinical application of flaraxin experience, namely the cases when the remedy gives complete clinical remission of tumoral process, accompanied by tumoral ganglions resolving, allow to doubt, that similar effect is stipulated by only mentioned above remedy properties. It's absolutely logical to suppose, that flaraxin is able to render effect on tumoral cells, as it is demonstrated for similar to it in non-toxicity antitumoral preparation "Ukrain". In this connection the phenomenon of chosen cytopathic action of flaraxin only on tumoral cells is of interest, besides in contrast to cytostatics the preparation acts on non-malignant tumors.

For elucidation of this universal mechanism of antiproliferative action of flaraxin we addressed to recently stated phenomenon of selective connection of cytostatics with serum-proteins in oncological patients. On the basis of the given phenomenon the diagnostic test was worked out and patented. However, to our mind, the phenomenon of selective connection of antitumoral preparations with onco-associated proteins opens new perspectives in the study of the mechanism of their effect on tumoral process.

Materials and methods.

The trial was carried out on 40 patients with various forms and stages of malignant oncological process, as well as 10 patients with non-tumoral pathology and non-malignant tumors. The object of the trial was blood serum of the patients in which the content to SH-group was defined before and after the contact with antitumoral preparations. Thus, the serum received was separated on several parts in accordance with the preparations used for testing. After incubation during 2 hours under t 37^0C in correlation 10:1 (serum: preparation) the number of free non-protein in mkm/l was defined with the help of amperometric titling. In control probe the serum was without preparations addition.

The following preparations: VINBLASTIN (the firm "Hedeon Richter"), CYCLOPHOSFAN (AS "Biochemist"), UKRAIN (Vena "Novitskapharm") and FLARAXIN (CCC Phoenix LLC, Kharkov) were used as reagents. The trial was fulfilled on the basis of immunology laboratory 411 of the Main Hospital of the South operative command (Odessa).

Results and their discussion.

In most of oncological patients under study the level of non-protein SH-groups in blood serum was rather high: 17,1±7,4 mkm/l. This is stipulated by the fact that most of the patients were with neglected stages of tumoral process, accompanied by, as it is known, violations of hemorheological homeostasis.

Presence of free non-protein SH groups in this situation reflects conformational non-stability of serum proteins on the background of catabolic processes. In connection with the presence of starting

level of non-protein SH-groups in native serum, absolute indices of selective connection for VINBLASTIN and CYCLOPHOSFAN were higher, then in the trial, conducted earlier (table. 1).

Table. 1. The content of non-protein SH group in reaction mixtures of examined oncological patients (BS – blood serum)

Reaction mixture	Level of non-protein SH groups mkm/l M±m
BS + Vinblastin	18,4 ± 8,3
BS + Cyclophosfan	20,7 ± 7,1
BS + Ukrain	23,6 ± 5,7
BS + Flaraxin	32,2 ± 9,9

From the table mentioned above it is seen, that Flaraxin gives the highest indices of selective connection with proteins of blood serum in patients. The interpretation of this phenomenon, to our mind, is directly connected with the analysis of pathophysiological mechanisms of antitumoral activity of the preparation. Speaking about cytostatics, it is necessary to mention, that their ability to selective connection with onco- associated proteins has no direct relation to antitumoral activity. Cytostatics have also other reactive-able groupings, causing denaturizing changes not only tumoral specific, but also physiologically necessary proteins of healthy organism. In the test presented only narrow circle of proteins is mentioned, with their own distinctive features to tumoral process and having labialized tyolodysulfer connections. As Flaraxin has no harming action on proteins of normal tissues, it is quite logical to suppose, that its antitumoral effect is realized mostly through denaturizing injures of labialized according to SH-SS groups of onco-associated proteins. The same can be said in relation with other non-toxic preparation UKRAIN.

Mentioned above is related only to the patients with malignant forms of tumoral process. During the examination of blood serum in patients with non-malignant tumors an unexpected picture was found. Thus, blood serum in patients with fibrocystic mastopathy (4 cases), uterus fibromyoma (2 cases), prostate adenoma (1 case), thyroid ganglion adenoma (1 case) was examined. In non cases VINBLASTIN and CYCLOPHOSFAN gave selective connection with proteins of blood serum, FLARAXIN gave the connection in 7 from 8 cases, UKRAIN – in 6 cases. Medium indices for FLARAXIN comprised: 8,5 ± 3,2 mkm/l.

The ability of FLARAXIN to connect with proteins of blood serum of the patients with non-malignant tumors, exposed by us, i.e. the ability to denaturizing injures of these proteins, quite correlates with the propositions mentioned above as for the fact, that antitumoral effect of the preparation is realized through this mechanism. As it is known, cytostatics do not act on non-malignant tumors, they also do not give denaturizing injures (speaking about only injures, realized through tyolodysulfer connections) of the proteins, taking place under non-malignant tumoral processes.

At last, in casual trials of blood serum in pregnant (2 cases) we found quite high selective connection relation to FLARAXIN M±m = 25,6 ± 0,4 mkm/l. Neither cytostatics nor UKRAIN in these cases showed no selective connection. Thus, we have every reason to consider, that FLARAXIN realizes its antiproliferative effect through denaturizing injures of oncofetal proteins. These injures are realized thanks to conformational vulnerability (labilisation) of tyolodysulfer connections in this group of proteins. Probably such conformational vulnerability of onco- associated proteins of embryonal period is stipulated by the execution by them exclusively proliferative function, demanding other level of tertiary protein organization.

Main conclusions.

1. FLARAXIN side by side with cytostatics, has the ability to denaturizing injures of onco- associated proteins, realized through labilized tyolodysulfer connections.
2. Different from cytostatics FLARAXIN renders denaturizing effect of the mentioned above type on proteins of blood serum in patients with non-malignant tumors and pregnant.
3. Antitumoral effect of FLARAXIN is due to its universal antiproliferative properties, realized through mechanism of denaturizing injure of labialized through tyolodysulfer connections of proteins, taking part in proliferative process.

EXECUTION OF THE TEST ON SELECTIVE CONNECTION

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1. Taking not less than 10 ml of blood from vein.
2. Receiving of serum, division of it into equal parts according to the number of tested preparations, one part – control.
3. Into all parts, except control, reagent in correlation 10:1 is added. The reagent solution is necessary to select empirically.
4. Mixture of serum with reagent is incubated during 2 hours under t 37^0C.
5. After incubation deproteinization of serum is done.
6. In deproteinized serum free non-protein SH-groups.

   For spectrophotometric definition of SH-groups it is necessary to select the level of reagents solution (cytostatics, flaraxin or others).

Selection of preparations solution.

1. First therapeutic solution is taken.
2. Then definition of non-protein SH-groups in control probe and after preparation addition is done. Correctly selected solution must not cause addition of SH-groups in comparison with control. If it is absent, therapeutic solution must be more durable.