Study of anticancer and modificating activity of amitozyn complex application

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Experiments aimed at in-depth study of amitozyn have been performed with classical experimental tumors on rats originated from the vivarium of Institute of experimental pathology, oncology and radio-biology of NAS of Ukraine. Altogether 118 rats were used in the experiments. The following subcutaneous transplantable tumors have been investigated: Gu-rin's carcinoma, sarcoma 45. Tumor transplantation and treatment were conducted according to commonly-accepted methods.

To study the mechanism of action of the preparation, the experiments were conducted to determine the concentration of malonic dialdehyde (final product of lipid peroxidation as markers of free radical particles formation) in both tumor and normal tissues, the concentration of reduced glutathione that characterizes the ability of the tissue to react upon deleterious for the organism factors (for instance, free radical particles) and neutralize them, it can also be used as an indicator of a tumor reaction to antitumor influence; the activity of glutathione-S- transferase that is involved in the neutralization of hazardous substances and metabolism products. Also, a tumor reaction to the preparation has been defined through the determination of a tissue bioenergetics level using ^{31}P NMR spectroscopy. The given above biochemical indicators of a tissue metabolism are rather characteristic for determining any antitumor agents both in experiment and in clinic. It was strongly shown that antitumor preparations or substances, which are studied as promising antitumor agents, lead to the reduction of concentration of reduced glutathione in tumor and the activity of glutathione-S- transferase. They also cause a dramatic decrease in the level of a tumor bioenergetics potential. The outlined above indicators are being used in clinic for the determination of typical signs of a positive tumor response to chemo- and radio-therapy, treatment effectiveness and its prognosis. The concentrations of malonic dialdehyde, reduced glutathione, the activity of glutathione-S- transferase were determined according to conventional biochemical methods. The signals ^{31}P were registered by NMR spectrometer 300 (Varian Mercury 300, USA). The data obtained were statistically processed.

The influence of the preparation used independently as well as combined with hyperglycemia, local and general hyperthermia and in a complex with chemotherapeutical preparation platydiam has been studied. The studies on a combined application of amitozyn and platydiam have proved to offer greatest promise (Tab.1).

We have established that the concentration of malonic dialdehyde (MDA) in sarcoma 45 showed no increase after amitozyn administration. This indicates the absence of lipid peroxidation activation which can evidence the absence of free radicals formation in tumor tissue under the administration of amitozyn into a tumor bearing organism. These conclusions are valid for normal tissues as well.

The content of reduced glutathione was somewhat increased. This indicates the involvement of glutathione system in the neutralization of some metabolites but not the products of free radical oxidation. Special attention should be given to an increase in reduced glutathione content in kidneys after amitozyn introduction. This may be an indication of some toxic effect on kidneys.

Table 1

Results of the treatment of rats with Gu-rin's carcinoma with amitozyn (20mg/kg.) and platydiam

Treatment	Tumor size before treatment (cm^3)	Tumor growth inhibition, (%)	Animals cured (%)	Average life (days)
Control (3)	1.0	--	--	28 (16-38)
Amitozyn + platydiam (8)	1.0	100 complete tumor regression – 75%	***	***
Platydiam (6)	0.7	90 complete tumor regression – 0	0	21 (16-44)

Notes

***- given mean values, deviation of parameters is given in brackets; TGT – tumor growth time (time during which tumor reaches 25 cm^3), TGIT – tumor growth inhibition time (difference in time when tumor reaches 25 cm^3 in control and experiment), AC- animals cured, AL – average life of rats starting from day of tumor inoculation.

Amitozyn was administered at 20 mg/kg of mass weight 60 min before platydiam every day, total injections – 10, platydiam was administered 3 times in both groups; platydiam with amitozyn administered on day 1,4,7 of treatment; total number of animals is indicated in brackets.

On the whole the data arrived at allow to conclude that the preparation does not activate lipid oxidation and it does not induce free radicals formation, while it can induce a certain toxic effect on kidneys. Therefore, based on the data on the content of MDA and reduced glutathione in tumor, the conclusion about a direct cytostatic action of amitozyn at given dosage cannot be made.

The administration of amitozyn was found to result in an increase in the content of MDA in Gu-rin's tumor that indicates lipid peroxidation activation. Of special interest is a similar effect of amitozyn observed in kidneys.

The content of reduced glutathione has been found to reduce in tumor located in the liver as well (the latter is especially important for explanation). It can be an indication to the activation of the system of glutathione, which serves as a cell protection system against various deleterious metabolites, including the products of lipid peroxidation. The validity of our assumption is supported by the absence of changes in the content of reduced glutathione in kidneys on the background of an increase in MDA concentration. On the whole these results suggest a direct action of amitozyn on Gu-rin's carcinoma, which manifests itself in a certain cytostatic effect, about which one can judge on the basis of changes in MDA and reduced glutathione content. We should seek for the proofs in direct experiments on the determination of a cytostatic effect of amitozyn on tumor cells. Also, it is noteworthy that in contrast to sarcoma 45, Gu-rin's carcinoma is epithelial neoplasm, which may suggest different mechanism of amitozyn action on cancerous neoplasms and sarcomas.

It has also been found that amitozyn has no inhibitory action on the bioenergetics of muscular tissue. At the same time certain changes of bioenergetics level take place in tumor after the introduction of amitozyn. On the whole they can be characterised as a moderate reduction in bioenergetics level, which at the same time cannot be unambiguously attributable to an inhibitory effect of amitozyn on the energy maintenance of a tumor cell functioning under the administration of amitozyn at given dosage and regimens.

Conclusions

1. Amitozyn exerts a certain anticancer action under a rather prolonged administration. With regard to quantitative characteristics this effect can be considered moderate, though in some cases a complete tumor regression was observed. It is of great importance that this effect had a more pronounced character on connective tissue tumors.
2. Artificial hyperglycemia does not level the anticancer effect of amitozyn, at the same time we cannot state that when combined with artificial hyperglycemia the effect of the preparation was enhanced though in some cases we observed a strong enhancement of amitozyn action on epithelial neoplasms.
3. The action of amitozyn has been observed to greatly increase when it was combined with local microwave hyperthermia in the treatment of rats with sarcoma 45.
4. When combined with amitozyn, platydiam has been found to increase its action on the background of almost absolute absence of the toxicity of platydiam action in rats with epithelial neoplasms. At this dosage change factor was 2.0.
5. We suggest applying amitozyn in an experiment with animals at dose 20.0 mg/kg mass, administration intravenously every day or every other day.
6. The data obtained allow us to make an assumption that amitozyn antitumor effect is based first of all on immune modulatory effects which may turn out to be non-classical. We can also assume that amitozyn influences vitally important systems of tumor, inhibiting their functioning, which leads to a considerable decrease in tumor vital activity, delaying its growth and removing a tumor toxic effect on the organism. On the basis of the experiments with animals in which the retardation of tumor development was observed after amitozyn treatment was initiated, but tumor did not disappear completely, whereas the general condition was rather satisfactory and so they lived much longer than the control ones, we can make a suggestion that amitozyn exerts a certain biological effect both on tumor and a tumor bearing organism, which leads to a long coexistence of tumor and the organism or to the organism living with tumor. The possibility of this phenomenon and its therapeutic significance were pointed out by M. fon Ardene (1980). The mechanism of this state still needs understanding but its therapeutic value could be of great importance for the treatment of patients with extensive tumors and metastases with amitozyn.

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