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BIOPHYSICS OF COMPLEX SYSTEMS

Cellular Effects of the Antitumor Drug Aurumacryl

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Abstract—The cellular effects of aurumacryl (a drug based on gold polyacrylate, which previously showed significant antitumor activity in vivo against solid tumors in mice) were studied in the model of the MCF7 stable cell line of human breast carcinoma. It was found that aurumacryl possesses cytotoxic and cytostatic effects on tumor cells. The dose-dependent cytotoxic effect of aurumacryl is expressed as the death of 60% of tumor cells after incubation with aurumacryl at a dose of 1 mg/mL for 24 h. The proliferation kinetics of the surviving fraction of tumor cells also undergoes significant changes, which is expressed in the predominant accumulation of the cells (93%) in the G_0 phase of proliferative rest and in a significant decrease in the number of proliferating cells to 7%. These data could be interpreted as evidence of the loss of the reproductive ability of the surviving cells after treatment with aurumacryl.

Keywords: gold polyacrylate, MCF7 human tumor cell culture, cytotoxic and cytostatic effects
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INTRODUCTION

The study of organometallic compounds as potential antitumor drugs has been recognized as one of the most promising areas of research in the field of biomedical chemistry, as well as experimental and clinical oncology [1, 2].

The interest in organometallic compounds, especially in structures containing noble metals, is largely determined by the discovery of the high level of antitumor activity in a series of platinum complex compounds (cisplatin, carboplatin, oxaliplatin, etc.), which are widely used in modern chemotherapy of tumors [1, 3].

Recent studies have revealed significant antitumor activity of organometallic compounds containing gold, which is another platinum group metal. Certain differences found in the spectrum of antitumor activity, as well as in the mechanism of action of compounds containing gold and platinum, along with the lack of tumor cross-resistance towards some of these drugs, give particular relevance to this area of research.

In the last 2 decades, the study of gold-containing compounds as potential antitumor drugs has been widely developed. A large number of varying substances that contain gold, including in the form of complex compounds and nanostructures, have been synthesized; many of them show significant antitumor activity in vitro against human tumor cell lines, including cell lines that are resistant to platinum drugs [4–6].

We first found significant anticancer activity of polyacrylates containing noble metals, in particular gold (aurumacryl), on models of a solid tumor in animals during the investigation of new compounds for oncology belonging to the class of metal polyacrylates. It was established that aurumacryl effectively inhibits the development of solid tumors in mice (Lewis lung carcinoma, Acatol adenocarcinoma, and Ca755 adenocarcinoma), causing suppression of their growth by 70–90% compared to the control [7–10].

It should be noted that metal polyacrylates developed under the leadership of Academician M.G. Voronkov have diverse biological activity; in particular, they constitute a group of local hemostatics of a new generation, which combine a hemostatic effect with a high level of bactericidal activity against 27 major strains of microbial pathogens [11].

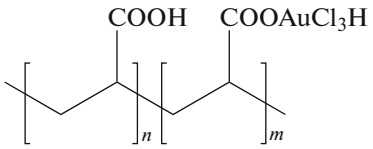
The objective of this work was to study the cellular effects of aurumacryl in vitro on the MCF7 model of human breast carcinoma.

MATERIALS AND METHODS

Drug. The studied drug with the conventional name aurumacryl is an incomplete gold salt of polyacrylic acid containing 8.03 wt % Au and corresponds to the general formula:

$(-CH_2-CHCOOH-)_n(-CH_2CHCOOAuCl_3H-)_m$,

where $n = 1263$; $m = 124$ (see Scheme 1):



In this paper, the effect of aurumacryl applied as an aqueous solution was studied at the concentrations of 0.001, 0.01, 0.1 and 1.0 mg/mL.

Cell culture. The experiments were carried out in vitro using a cell culture of human breast carcinoma of the MCF7 line. The cells were cultured in the standard culture medium DMEM/F12 (Life Science Thermo Fisher Scientific, United States) supplemented with 2 mM L-glutamine (PanEco, Russia), 100 U/mL penicillin, 100 mg/mL streptomycin (PanEco, Russia) and 10% FBS (Life Science Thermo Fisher Scientific, United States) at 37°C in 5% CO_2 (MCO-18A, Sanyo, Japan). The cells were then removed from the surface using a 0.25% trypsin–EDTA solution (PanEco, Russia) and passaged at the density of 200 cells/cm^2 in the same culture medium. Passaging of the cells was carried out until the culture reached an 80–90% monolayer. The medium was replaced every 4 days.

The experimental study of aurumacryl was carried out on cells in the phase of exponential growth (cell population density ~80%). Cells were removed from the plastic with trypsin–EDTA solution, followed by inactivation of trypsin and washing the cells in the complete medium. The cells were passaged in 35-mm Petri dishes containing coverslips (SPL Life science, South Korea). The effect of aurumacryl at different concentrations was studied under incubation of the cells with the drug at 37°C for 1, 6 and 24 h, with parallel incubation of intact cells as a control.

Cell viability. The cell viability was determined from the susceptibility to staining with a 0.4% solution of trypan blue. The cells were washed twice from the medium with phosphate-buffered saline (pH 7.4), removed from the dishes with trypsin-EDTA solution, and transferred to a suspension. The solution of 0.4% trypan blue was then mixed with the cell suspension in equal proportions, the mixture was resuspended, and cell death was immediately evaluated by counting the stained (dead) cells in a hemocytometer.

Cell proliferation. To evaluate the effect of aurumacryl on cell proliferation, immunocytochemical analysis of a marker of cell division, the Ki-67 protein, was used. This protein is present in the nuclei of dividing cells, but is absent in resting cells [12]. Analysis of the Ki-67 expression is widely used in clinical oncology to evaluate the effectiveness of treatment and the prognosis of relapses [12, 13].

The cells on the coverslips were washed twice from the medium with phosphate-buffered saline (pH 7.4) and fixed with paraformaldehyde (4% solution in phosphate-buffered saline, pH 7.4) for 15 min at room temperature and then washed twice with phosphate buffered saline (pH 7.4). The cells were permeabilized with 0.3% Triton X-100 in phosphate-buffered saline (pH 7.4) containing 2% bovine serum albumin in order to block nonspecific binding.

The slides were incubated with murine monoclonal antibodies to the Ki-67 protein (Anti-Ki-67, Merck–Millipore, United States) diluted at the ratio of 1 : 200 with phosphate-buffered saline (pH 7.4) containing 1% bovine serum albumin for 1 h at room temperature.

After washing two times with phosphate-buffered saline (pH 7.4), the slides were incubated at room temperature for 1 h with secondary goat polyclonal antibodies against murine IgG conjugated with the Alexa 488 fluorochrome (Merck–Millipore, United States) diluted at the ratio of 1 : 400 with phosphate-buffered saline (pH 7.4) containing 1% bovine serum albumin.

ProLong Gold mounting medium (Life Technologies, United States) containing DAPI was used for DNA staining and the prevention of photobleaching.

Visualization, documentation and processing of immunocytochemical microimages were performed on an Eclipse Ni-U luminescent microscope (Nikon, Japan) equipped with a ProgRes CFcool high-resolution video camera (Jenoptik AG, Germany) using DAPI (excitation at 340–380 nm and emission at 435–485 nm) and B-2E/C (excitation at 465–495 nm and emission at 515–555 nm) filter sets. At least 200–500 cells per point were analyzed with subsequent counting of the proportion of Ki-67-positive/Ki-67-negative cells.

Double-strand DNA breaks. The evaluation of double-strand breaks of DNA was carried out immunocytochemically using antibodies specific to a marker protein of double-strand breaks of DNA: phosphorylated histone H2AX (γH2AX).

The complex dynamic microstructures formed during the repair of double-strand DNA breaks, consisting of thousands of γH2AX copies, are visualized after immunocytochemical staining as bright dots/foci [14]. Quantitative evaluation of γH2AX foci is currently considered to be the most sensitive method for the analysis of double DNA breaks [15].

We used the technique described in detail earlier in [16] to analyze γH2AX foci. At least 200 cells per point were analyzed. The Focicounter program (http: //focicounter.sourseforge.net/) was used to calculate the number of γH2AX foci.

Statistics. Statistical processing of the obtained results was performed using the Statistica 8.0 program. The results are presented as the mean of three independent results ± standard error.

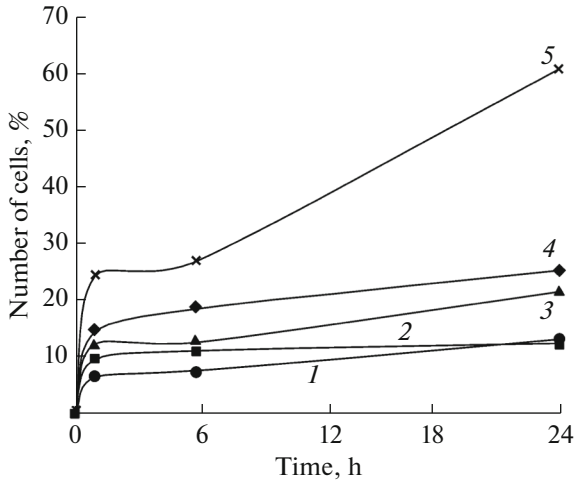


Fig. 1. The effect of the drug aurumacryl at a series of concentrations on kinetics of MCF7 cell death. The exposure time is along the abscissa, the number of dead cells is along the ordinate. Concentrations of the drug (mg/mL): *1*, control; *2*, 0.001; *3*, 0.01; *4*, 0.1; *5*, 1.0.

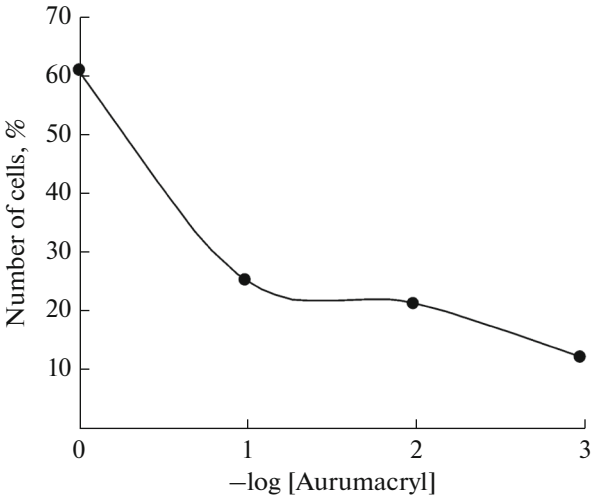


Fig. 2. The change in the fraction of dead cells of the MCF7 culture depending on the dose of aurumacryl (24-h exposure). The logarithm of the drug concentration is along the abscissa, the number of dead cells is along the ordinate.

RESULTS

Investigation of the effects of aurumacryl in vitro included the evaluation of cell survival after the exposure to the drug, the study of its effect on cell proliferation kinetics, and the determination of the ability to induce double-strand DNA breaks for this agent.

It was established that aurumacryl has a cytotoxic effect on tumor cells, causing their death, the degree of which depends on the time of drug exposure and its concentration (Fig. 1). After 1 h of incubation, cell death reached 10–25%, varying within this range depending on the dose. The observed effect increases with time and cell death reaches 60% after 24 h of incubation under the drug treatment at the concentration of 1 mg/mL (7 μM/L) (Fig. 1).

The cytotoxic effect of aurumacryl has a pronounced dose-dependent character. The maximum effect, the death of 60% of the tumor cells, was observed when the drug is applied at the dose of 1 mg/mL, the largest of the studied ones (Fig. 2).

Note that for aurumacryl the calculated dose that is lethal for 50% of the tumor cells (ED_{50}), is 0.5 mg/mL (Fig. 2).

The proliferation intensity of the surviving fraction of tumor cells underwent significant changes under the influence of aurumacryl compared with the control, which are the most pronounced when the drug is applied at the highest of the studied doses, 1 mg/mL (Figs. 3 and 4).

It was found that the drug causes the accumulation of cells in the G_0 phase of proliferative rest. As can be seen from Fig. 3, the proportion of resting cells after 24 h of incubation increased from the 40% observed in the control to 93% recorded for this period when exposed to the drug at the dose of 1 mg/mL (Fig. 3).

In addition, a decrease in the fraction of dividing cells was observed among the surviving cells under the influence of aurumacryl. Thus, after the incubation of tumor cells with the drug at the dose of 1 mg/mL for 24 h, the fraction of dividing cells decreased from 60% as found at this time in the control, to 7% (Fig. 4).

These data indicate that aurumacryl has a pronounced cytotoxic and cytostatic effect, causing both cell death (60%) and significant changes in the cell-

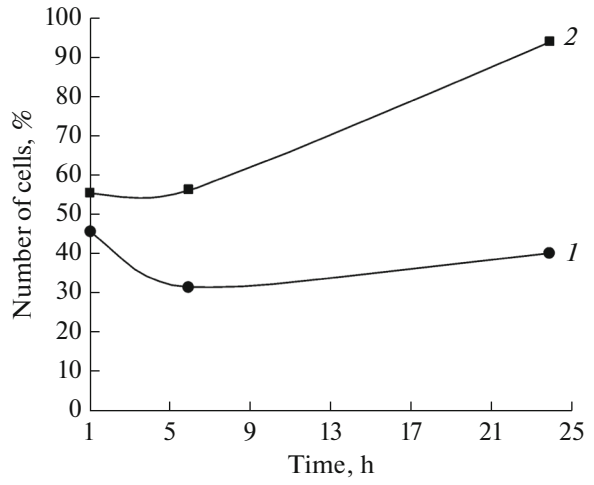


Fig. 3. The effect of aurumacryl on kinetics of the change in the fraction of MCF7 resting cells: *1*, control; *2*, aurumacryl, 1 mg/mL. The exposure time is along the abscissa, the number of cells in the G_0 phase is along the ordinate (%).

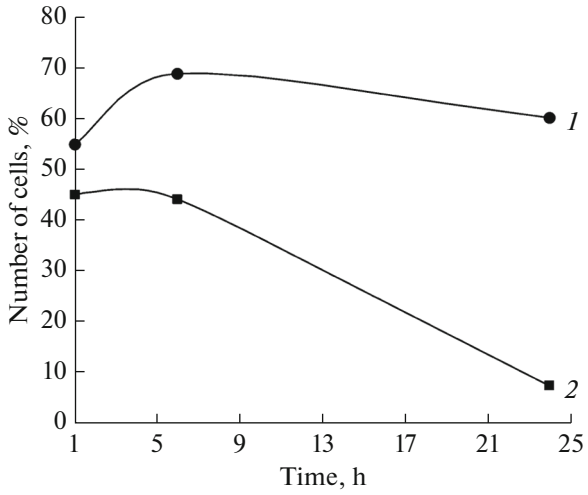


Fig. 4. The effect of aurumacryl on the kinetics of the change in the fraction of dividing MCF7 cells: *1*, control; *2*, aurumacryl, 1 mg/mL. Exposure time is along the abscissa, the number of dividing cells is along the ordinate.

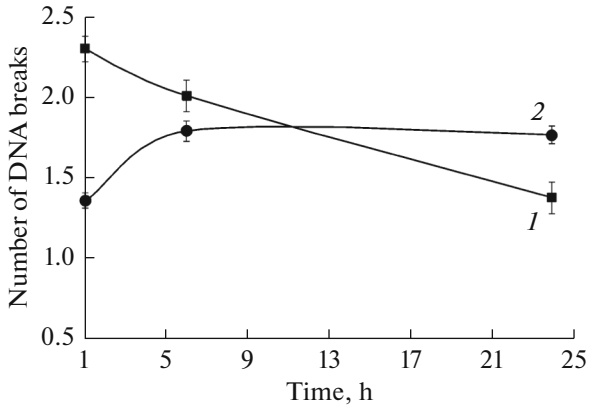


Fig. 5. The effect of aurumacryl on kinetics of the change in the fraction of double-strand DNA breaks per cell (MCF7 cell culture): *1*, control; *2*, aurumacryl, 1 mg/mL. Exposure time is along the abscissa, the number of DNA breaks per cell is along the ordinate.

proliferation intensity of the surviving fraction of tumor cells, as shown by a decrease in the fraction of dividing cells.

When studying the effect of aurumacryl on the DNA of tumor cells, it was found that short term (for 1 hour) incubation of cells with the drug leads to an almost twofold increase in the number of breaks (from 1.3 in the control to 2.3 with the drug). However, further exposure to aurumacryl does not increase this effect; moreover, after incubation for 24 h the number of double breaks in the control and experiment was practically the same and had values equal to 1.8 and 1.4, respectively (Fig. 5).

Apparently, this is a reflection of the process of reducing the proportion of cells in the phase of DNA synthesis (S phase). It is known that in S phase the largest number of spontaneous double breaks occurs from the collapse of replicative forks [17]. The data presented here indicate that there is no significant effect of aurumacryl on the index characterizing the number of double DNA breaks per cell.

DISCUSSION

The cellular effects of aurumacryl in vitro found in the study on the MCF7 model of human breast carcinoma are in good agreement with the data on the antitumor activity of the drug against solid tumors in animals in vivo obtained earlier [8–10].

These results not only indicate the significant cytotoxic effect of aurumacryl, but also show the effect of the drug on the proliferative activity of tumor cells, which indicates a pronounced cytotoxic effect of the drug.

It should be noted that these effects in experiments in vitro were achieved at very low nanomolar amounts of the substance, which are significantly lower than the doses at which the antitumor effect was detected in experiments in vivo.

Our data on the antitumor activity and cellular effects of aurumacryl together indicates the prospects of further study of this compound as a potential antitumor agent.

The possible molecular targets and mechanisms of the antitumor action of aurumacryl, which are necessary for the use of the drug in accordance with modern approaches to antitumor chemotherapy, remain unclear.

According to the existing ideas, enzymes involved in the regulation of tumor-cell proliferation in the development of apoptosis and angiogenesis can serve as biotargets for gold-containing drugs.

The highly specific inhibition of an enzyme of the pyridine nucleotide oxidoreductase family, mitochondrial thioredoxin reductase, which has selenium in the active center and is highly sensitive to the action of heavy metals, has been shown under the influence of gold drugs, in particular, in a number of models of stable human tumor cell lines. The result of this interaction is the damage to the mitochondrial membrane, the release of cytochrome *c* into the cytosol, and the induction of apoptosis. The modulation of the proteasome activity by gold drugs and a decrease in the content of antiapoptotic proteins are also found, which leads to the stimulation of apoptosis of the cells. There is a high probability of an influence of gold on proteins involved in the signaling cascade of mitogen signal transduction, which may lead to the suppression of cell proliferation and cell death [4–6, 18–20].

Considering that aurumacryl is the first, and thus far the only, polymeric compound among the studied

gold-containing substances, and based on the known data on some preferential predisposition of tumor cells to interaction with polyanions, it can be assumed that gold polyacrylate, as a pronounced polyanion, is likely to interact selectively with tumor cells. It is also known that polyanions interact intensively with positively charged proteins, in particular histones, which may lead to chromatin disruption and subsequent disturbance of DNA functions [21].

The investigation of the detailed mechanism and targets of the antitumor effect of the drug are goals for further studies of this compound.

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