ONCOLOGY

Olivomycin Induces Tumor Cell Apoptosis and Suppresses p53-Induced Transcription

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Olivomycin (DNA-binding antibiotic) in nanomolar concentrations induces apoptosis of human tumor cells and inhibits p53-dependent transcription of the reporter gene (basal and induced by antitumor drugs). Olivomycin aglycon induces no cytotoxicity and does not block transcription.

Key Words: *olivomycin; apoptosis; transcription; cancer*

The structure of olivomycin (OM), aurelic acid group antibiotic produced by *Streptomyces olivoreticuli*, is presented by aglycon with a disaccharide branch and a trisaccharide branch (Fig. 1, *a*) [5,10]. An important characteristic of OM and chromomycin and mitramycin, possessing similar structure, is their interaction with GC pairs in DNA minor groove [2]. Since DNA is one of the main targets of antitumor therapy, OM was used as an antineoplastic drug in the treatment of testicular tumors (teratoblastoma, fetal cancer), tonsils, uterine chorionepithelioma, and soft tissue sarcoma [3]. The molecular mechanisms underlying the effect of OM deserve detailed studies. We studied the effects of OM essential for its antitumor activity: induction of human tumor cell death and regulation of gene transcription.

MATERIALS AND METHODS

The study was carried out on human transformed cells: K562 (leukemia), HepG2 (hepatoma), A549 (lung cancer), MCF-7 (breast cancer), BE(2)C (neuroblastoma), A2780 (ovarian cancer) and cisplatin-resistant A2780/DDP4 variant obtained by long-term culturing of A2780 cells with cisplatin in increasing concentrations (0.25-8.00 μg/ml); HCT116 (colonic cancer) and HCT116WafConALacZ variant with a construction including *p21* gene promoter site with p53-dependent regulatory elements and reporter gene (β-galactosidase, GS). The cells were cultured in RPMI-1640 (K562, BE(2)C), DMEM (MCF-7, HCT116, HCT116WafConALacZ), or in Dulbecco-modified Iscove medium (A2780, A2780/DDP4) with 5% FCS (BioWhittaker), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C and 5% CO_2 in a humid atmosphere. The cells in the logarithmic growth phase were used in the experiments. All reagents (except otherwise specified) were from Sigma. OM was obtained on an experimental device at G. F. Gauze Institute of New Antibiotics. Aglycon (olivin) was obtained by acid hydrolysis of OM [1]. Olivin structure (Fig. 1, *a*) was confirmed by the data of ^{1}H- and ^{13}C-NMR spectroscopy. OM and olivin were dissolved in DMSO to 10 mM and stored at 4°C.

The range of cytotoxic concentrations for OM and olivin was evaluated by cell capacity to reduce 3-(4,5-dimethylthiasol-2-yl)-5-(3-carboxymethoxyphenyl)-2-

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(4-sulfophenyl)-2P-tetrazolium salt to formazan (MTT test) [8]. The cells were incubated with OM or olivin in 96-well plates (5×10^4 cells/well) for 72 h at 37°C and 5% CO_2. Preliminary experiments showed that DMSO (up to 0.1% in culture medium; control) did not change cell viability and proliferation. After the end of incubation, 100 μg MTT was added to wells for 2 h, formazan was dissolved in DMSO, and optical density was measured at λ=540 nm. The cytotoxicity was expressed in percent of optical density of formazan formed by cells incubated with OM or olivine. Optical density of formazan in control wells was taken as 100%.

For morphological studies of cell death, HCT116 were inoculated onto slides, treated with OM, and fixed with glutaraldehyde. Transmission electron microscopy was carried out by the standard method. The preparations were examined under a JEM-1200 EX-II microscope.

For evaluation of internucleosomal DNA degradation, the cells (2×10^6) incubated with 100 nM OM for 24-72 h were precipitated at 1000*g*, the supernatant was centrifuged at 12 000*g*, the precipitate was pooled and lyzed in buffer containing 20 mM Tris HCl (pH 7.4), 0.35 M NaCl, 0.5% NP-40, 2 mM $MgCl_2$, and 1 mM dithiotreitol. DNA was extracted with phenol-chloroform mixture (1:1) and precipitated with ethanol in the presence of 0.3 M sodium acetate at -20°C. The precipitate was treated with RNase A for 20 min at 65°C and analyzed by electrophoresis in 1.5% agarose gel.

For studies of p53-dependent transactivation of GS reporter gene, HCT116WafConALacZ cells were

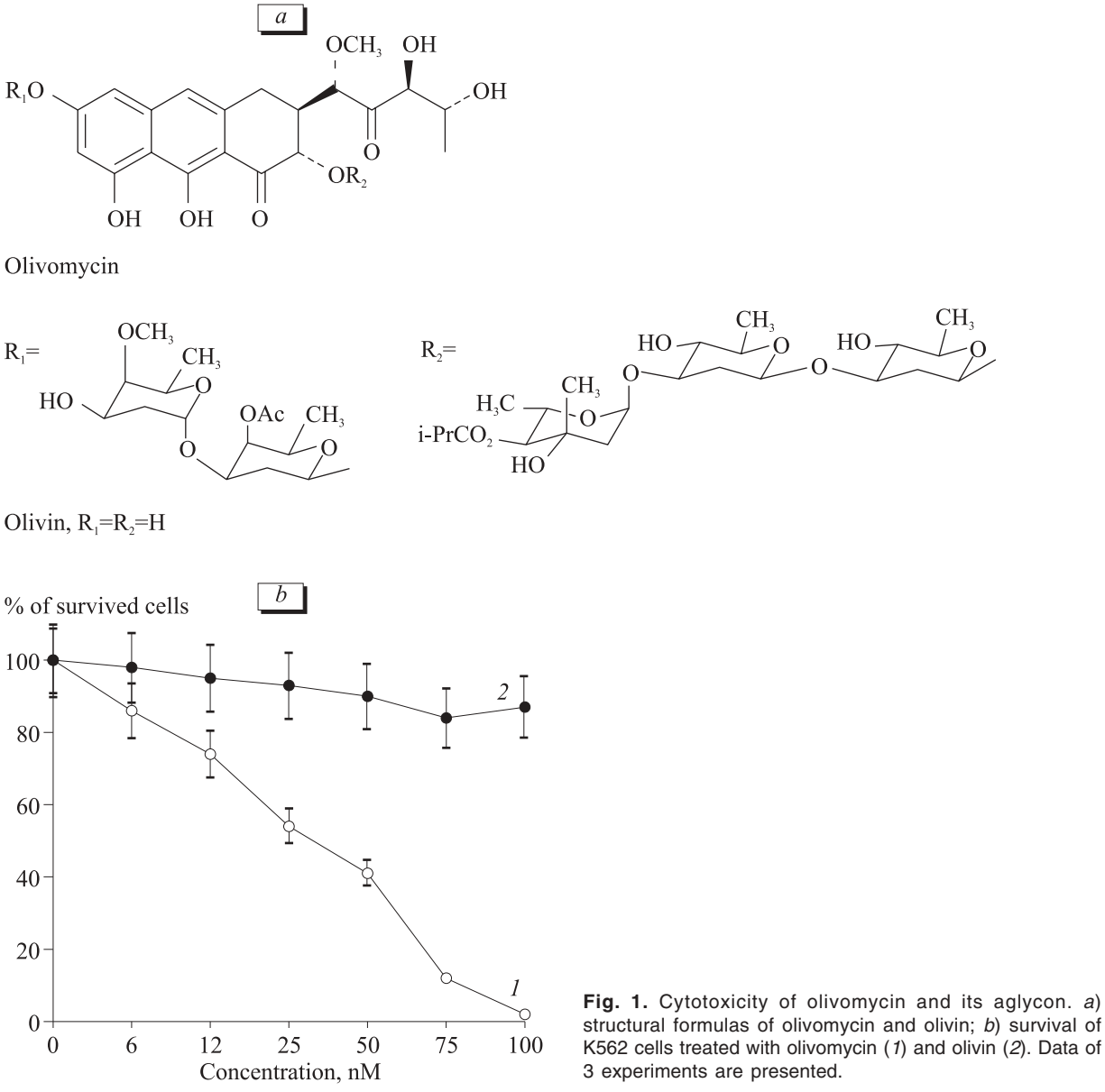


Fig. 1. Cytotoxicity of olivomycin and its aglycon. *a*) structural formulas of olivomycin and olivin; *b*) survival of K562 cells treated with olivomycin (*1*) and olivin (*2*). Data of 3 experiments are presented.

treated with mitoxanthrone (0.5 μM) or 5-fluorouracyl (5-FU, 3 μM) for 24 h. In transcription inhibition experiments, 100 nM OM or 5 μM olivin were added simultaneously with these chemicals. After incubation the cells were lyzed in a buffer containing 1 mM $MgCl_2$, 250 mM Tris-HCl (pH 7.4), 0.02% NP-40, and 0.2% *o*-nitrophenyl-β-D-galactopyranoside (GS substrate) and incubated at 37°C for 30 min. GS activity (optical density of lysates at λ=414 nm) was standardized by total concentration of protein in lysates (specific activity). Regulation of transcription (activation index) was expressed as the ratio of specific GS activity in cells treated with antitumor drugs to specific enzyme activity in intact cells. The data were statistically processed using Student's *t* test.

RESULTS

Olivomycin in nanomolar concentrations caused death of K562 cells (Fig. 1, *b*); the dose decreasing the survival by 50% (IC_{50}) was about 30 nM. OM in the same concentrations (IC_{50}=25-50 nM) caused death of HepG2, A2780, A549, MCF-7, BE(2)C, and HCT116 cells. Olivin aglycon was virtually nontoxic (Fig. 1, *b*). Hence, the absence of carbohydrate chains abolished OM cytotoxicity.

The next step was to clear out whether OM retained its activity towards cell resistant to other antitumor drugs. We previously showed that OM cytotoxicity was the same for HCT116 cells expressing proapoptotic p53 protein and substrains with deletion of both alleles of *p53* gene [9]. OM caused death of A2780 and A2780/DDP4 cells, its cytotoxic effect was more pronounced in cells resistant to cisplatin. Cisplatin IC_{50} for A2780 cells is 1 μg/ml, for cisplatin-resistant variant 4 μg/ml. Olivomycin IC_{50} is 25-50 nM for A2780 cells and 12.5-25.0 nM for A2780/DDP4 cells. Hence, the development of cell resistance to cisplatin is not paralleled by a decrease in their sensitivity to OM. Together with the data on similar toxicity of OM for FEMX human melanoma resistant to the growth-inhibitory effect of dexamethasone [4,9], the data on higher OM activity for cells selected by cisplatin resistance extends the therapeutic range of OM.

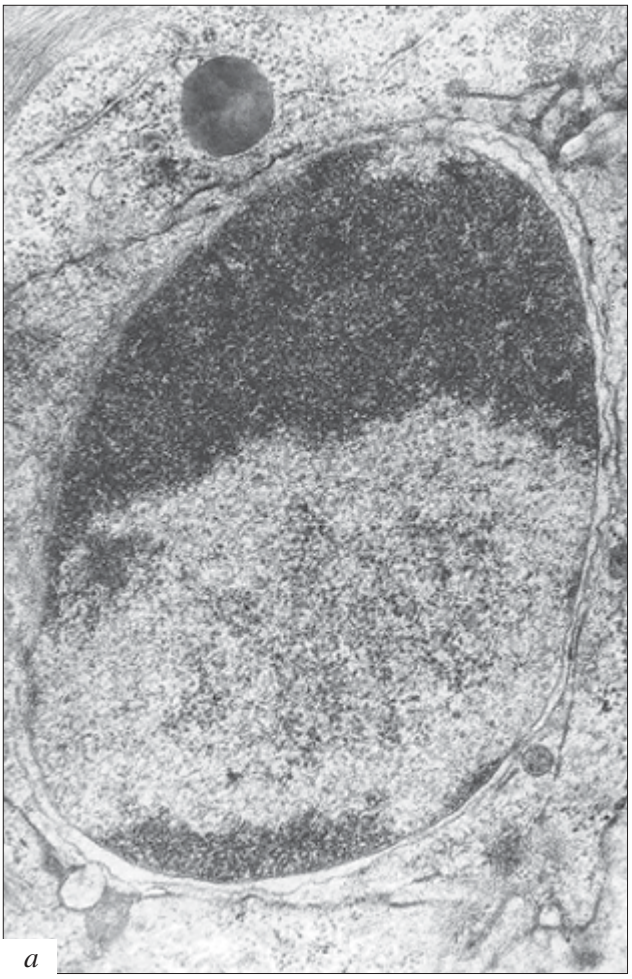
The study of the mechanisms of cell death under the effect of OM showed that the drug induced apoptosis. Within the first few hours OM caused changes characteristic of apoptosis in the cell nucleus: marginal condensation of chromatin and nucleus disintegration (Fig. 2, *a*, *b*). Longer (24-48 h) treatment led to internucleosomal DNA fragmentation, detected by electrophoresis as a series of bands differing by molecular weight by about 180 b. p. (Fig. 2, *c*). After 48-h exposure the appearance of low-molecular fragments was paralleled by degradation of high-molecular-weight pool of DNA molecules. Cell death after OM treatment was associated with pronounced DNA disintegration.

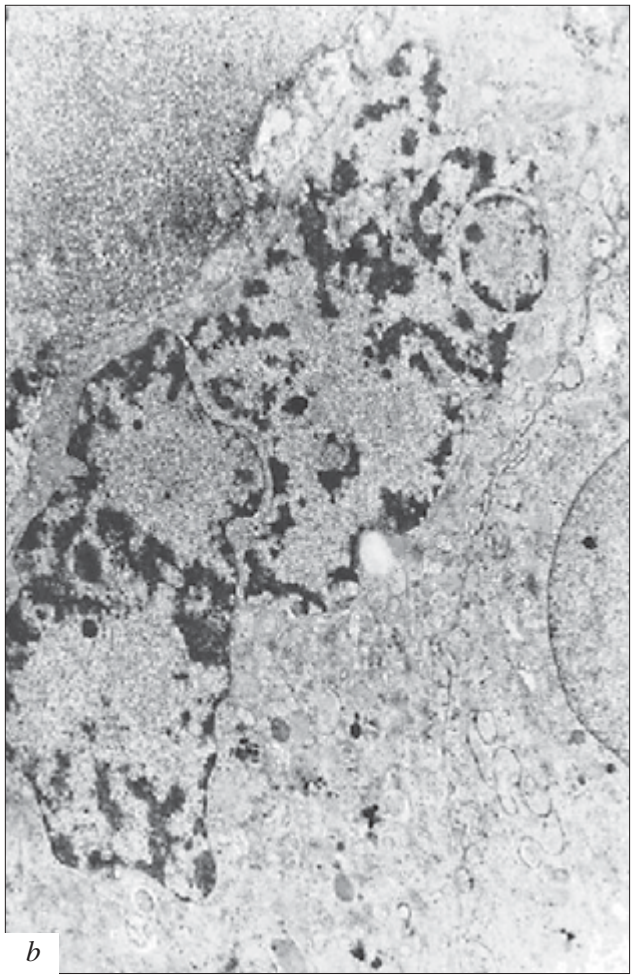
Olivomycin interaction with DNA suggests that the drug can regulate transcription. We studied the effect of OM on p53-dependent transcription. For induction of p53 the cells were treated with antitumor drugs mitoxanthrone, 5-FU, cytosar, and etoposide. The blocking effect of OM appeared at OM concentration of 25 nM and increased dose-dependently. OM in a concentration of 100 nM completely abolished GS transcription activated by 5-FU (Fig. 3, *3* and *4*; $p<0.05$) or mitoxanthrone (Fig. 3, *5* and *6*; $p<0.05$). Olivomycin in concentrations of 50-100 nM inhibited transcription of the reporter gene induced by cytosar and etoposide (data not shown). OM prevented not only inducible (caused by p53 inductors), but even basal (detected in intact cultures) activity of the promotor (Fig. 3, *1* and *2*; $p<0.05$). These results suggest that OM is a "universal" inhibitor of transcription, in contrast to, *e. g.*, DNA-binding antibiotic ecteinascidine 743, blocking inducible, but not basal transcription [6]. Olivin did not modify transcription even in a concentration of 5 μM.

Hence, OM is characterized by the following important effects: it causes apoptosis of human tumor cell in different tissues; it is toxic for cells resistant to some xenobiotics; it suppresses gene transcription.

Olivomycin produced these effects after short-term treatment in nanomolar concentrations, which confirms high antitumor activity of the preparation. Since cytotoxicity and antitranscription effect of OM manifest within the same range of concentrations, we can hypothesize that cell death after OM treatment is determined by due to its capacity to inhibit transcription. Presumably, OM is not a gene-specific blocker of transcription and its inhibitory effect in different domains of the genome is a result of DNA binding, impairment of its architectonics, and prevention of matrix synthesis. Our data on OM blocking of DNA synthesis in a cell-free system confirm this hypothesis. This does not rule out the involvement of chromatin into inhibition of transcription in cells under the effect of OM. Further study of the mechanisms of antitranscription effect of OM will show whether the drug prevents interaction between transcription factors and DNA and formation of multicomponent protein complexes with RNA polymerases at the regulatory sites of genes, whether it blocks transcription initiation, RNA elongation, and/or modulates other processes associated with gene transcription.

Olivin (aglycon) caused virtually no cell death and did not block transcription. Biological inertness of olivin can be explained by its poor association with DNA. Hydrolysis of sugar residues in OM molecule considerably weakens interaction of the molecule with DNA [7].





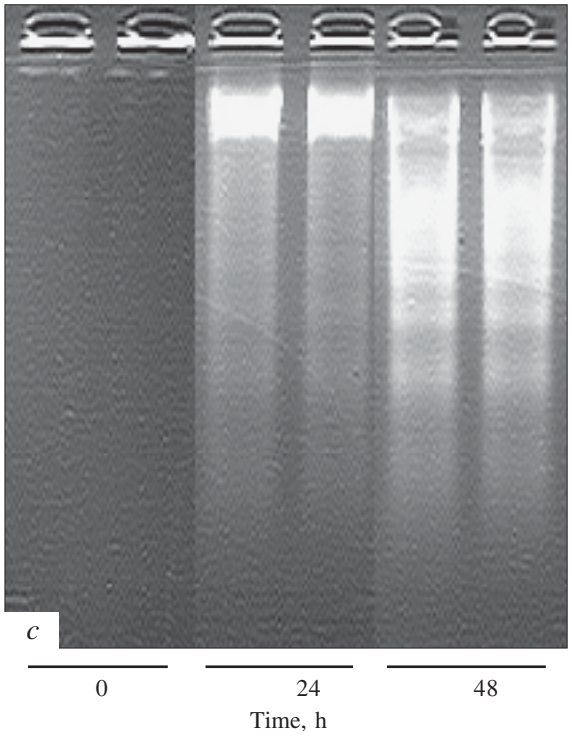


Fig. 2. Apoptosis under the effect of olivomycin. *a*, *b*) HCT116 cells, 100 nM olivomycin, 4 h; *c*) K562 cells, 100 nM olivomycin; *a*) marginal condensation of chromatin, ×4000; *b*) degradation of nuclear material into large fragments, ×20,000; *c*) fragmentation of genome DNA.

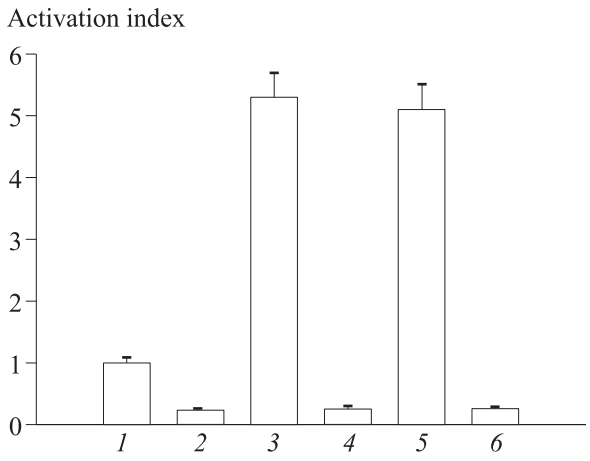


Fig. 3. Inhibition of p53-dependent transcription under the effect of olivomycin. *1*) intact HCT116WafConALacZ cells; *2*) olivomycin; *3*) 5-fluorouracyl; *4*) 5-fluorouracyl+olivomycin; *5*) mitoxanthrone; *6*) mitoxanthrone+olivomycin. Data of 5 experiments are presented. $p<0.05$ between groups *2* and *1*; *4* and *3*; *6* and *5*.

Hence, strong interaction between the drug and DNA is essential for suppressing gene transcription and induction of cell death. Hydrogen bonds between aglycon and guanine 2-amino group and between sugar hydroxyl groups and DNA carbohydrate-phosphate backbone can provide the needed strength [2].

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