ORIGINAL CONTRIBUTIONS

IN VITRO STUDY OF LANDOMYCIN E ANTITUMOR ACTIVITY

A.V. Korynevska[1], B.P. Matselyukh[2], R.S. Stoika[1,]*
[1]*Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv 79005, Ukraine*
[2]*D.K. Zabolotny Institute of Microbiology and Virusology, National Academy of Sciences of Ukraine, Kyiv 03627, Ukraine*

ИССЛЕДОВАНИЕ *IN VITRO* ПРОТИВООПУХОЛЕВОЙ АКТИВНОСТИ ЛАНДОМИЦИНА Е

А.В. Кориневская[1], Б.П. Мацелюх[2], Р.С. Стойка[1,]*
[1]*Институт биологии клетки НАН Украины, Львов, Украина*
[2]*Институт микробиологии и вирусологии им. Д.К. Заболотного НАН Украины, Киев, Украина*

Landomycin E (LE) was recently isolated from *Streptomyces globisporus* strain 1912 and its chemical structure was studied. However, its biological activity is still poorly understood. We performed *in vitro* investigation of antitumor activity of this new member of the angucyclin family of antibiotics. Dose-dependent effect of LE on growth and survival of different tumor cell lines was studied in comparison with such effect of other anticancer drugs (adriamycin, cisplatin, methotrexate, vincristine and fluorouracil). LE (2.5–10.0 μg/ml) inhibited growth of human colon adenocarcinoma cells of SW-480 line to a similar extent as adriamycin and stronger than fluorouracil, cisplatin and methotrexate did. IC_{50} for LE was found to be 1.28 μg/ml for human breast carcinoma cells of MCF-7 line, 6.68 μg/ml for human breast carcinoma cells of T47D line, 3.3 μg/ml for human colon adenocarcinoma cells of SW-480 line, 6 μg/ml for human colon adenocarcinoma cells of HT-29 line, 5 μg/ml for human mouth adenocarcinoma cells of KB line, 1.75 μg/ml for mouse leukemia cells of L1210 line, 2.68 μg/ml for mouse transformed fibroblasts of L929 line, 2.04 μg/ml for mouse embryo fibroblasts of NIH-3T3 line, 15.04 μg/ml for mink lung epithelial cells of CCL-64 line, and 2.68 μg/ml for mouse monocytes/macrophages of J774.2 line. Apoptotic DNA fragmentation was elicited under LE effect in L1210 leukemia cells. Investigation of various tumor cell lines showed the appearance of different cytomorphological changes characteristic for apoptosis (condensation of cytoplasm and nucleus, nucleus fragmentation, development of multiple vacuoles in the cytoplasm, and appearance of apoptotic protrudings of plasma membrane), although other ways of LE-induced cell death cannot be also excluded. These data may be helpful for further elucidation of cellular and molecular mechanisms of LE antineoplastic action.
***Key Words*: landomycin E, tumor cells, growth inhibition, apoptosis.**

Ландомицин Е (ЛЕ) недавно выделен из *Streptomyces globisporus* штамм 1912, его химическая структура установлена. Однако биологическая активность данного антибиотика изучена недостаточно. Мы провели *in vitro* исследование противоопухолевой активности этого нового представителя семейства ангуциклиновых антибиотиков. Дозозависимый эффект ЛЕ на рост и жизнеспособность различных линий опухолевых клеток изучали в сравнении с таким же эффектом других противоопухолевых препаратов (адриамицин, цисплатин, метотрексат, винкристин и флуороурацил). Ингибирование роста клеток линии SW-480 аденокарциномы прямой кишки ландомицином Е (2,5–10,0 μg/ml) было аналогично действию той же дозы адриамицина и значительно сильнее, чем эффекты флуороурацила, метотрексата и цисплатина. Показатель IC_{50} ЛЕ равен 1,28 μg/ml для клеток линии MCF-7 аденокарциномы молочной железы человека, 6,68 μg/ml – для клеток линии T47D карциномы молочной железы, 3,3 μg/ml – для клеток линии SW-480 аденокарциномы прямой кишки человека, 6 μg/ml – для клеток линии HT-29 аденокарциномы прямой кишки человека, 5 μg/ml – для клеток линии KB аденокарциномы гортани человека, 1,75 μg/ml – для клеток линии L1210 лейкемии мыши, 2,68 μg/ml – для мышиных трансформированных фибробластов линии L929, 2,04 μg/ml для мышиных эмбриональных фибробластов линии NIH-3T3, 15,04 μg/ml – для эпителиальных клеток линии CCL-64 легкого норки и 2,68 μg/ml – для мышиных макрофагов/моноцитов линии J774.2. При воздействии ЛЕ на клетки лейкемии мыши L1210 наблюдали апоптическую фрагментацию ДНК. В различных линиях опухолевых клеток выявлено наличие цитоморфологических изменений, характерных для апоптоза (конденсация цитоплазмы и ядра, фрагментация ядра, формирование множественных вакуолей в цитоплазме и апоптических изменений в плазматической мембране), хотя нельзя полностью исключить других путей гибели клеток под действием ЛЕ. Полученная информация может быть полезной для дальнейшего изучения клеточных и молекулярных механизмов антинеопластического действия ЛЕ.
***Ключевые слова:* ландомицин Е, раковые клетки, ингибирование роста, апоптоз.**

Appearance of malignant cell resistance to chemotherapy is a serious problem in oncology, because antitumor drug resistance develops in about 30% of cancer patients [29].That is why synthesis of new antitumor drugs, possessing diminished cytotoxity is an extremely actual task. Ineffectiveness of the chemotherapy may be caused by different reasons: 1) alterations inside tumor cells, namely, intensification of DNA reparation systems, expression of antiapoptotic and suppression of proapoptotic mechanisms; 2) changes in drug pharmacokinetics, namely, the inability of drug to reach target

Received: April 3, 2003.
* Correspondence: E-mail: stoika@biochem.lviv.ua
Abbreviations used: LE — landomycin E.

cells in adequate amounts and active form; 3) intensive drug excretion by specific transporting systems such as multidrug resistance system, and chemical modification of drug by cellular antioxidation systems.

Most chemotherapeutic drugs induce apoptosis in target cells [7, 15]. A series of antibiotics produced by different *Streptomyces* strains are active not only as antibacterial and antifungi agents, but also as antitumor drugs [3, 5, 23, 27]. The antibiotics of anthracycline and bleomycine group were shown to be the most effective drugs for tumor treatment [12, 13, 23].

Angucycline antibiotics belong to a large family of natural compounds produced by the actinomycetes. The landomycins belong to a subgroup of the angucycline family, whose molecule consists of an angular tetracyclic quinone, conjugated to a linear oligosaccharide. The cytostatic properties of various members of the landomycin family were found to depend on the length of their oligosaccharide chain [18, 24]. Landomycin A, the principal metabolite of *Streptomyces cyanogenus*, is a member possessing the longest glycan consisting of 6 saccharide residues [16, 26]. Flow cytometric study showed that landomycin A inhibited cell cycle progression from G1/S phase to S phase [10]. A potent antitumor activity of this antibiotic depends on its glycan moiety [23, 24].

Landomycin E (LE) is a novel representative of angucycline antibiotics. It is synthesized by *Streptomyces globisporus* strain 1912, growing in a soy–bean culture medium [2, 5, 19, 20]. It contains 3 saccharide residues (α–L–rhodinose–(1→3)–β–D–olivose–(1→4)–β–D–olivose) conjugated to an angular tetracyclic quinone. Antitumor action of LE was demonstrated against Guerin carcinoma in rats [6]. However, the mechanisms of its antineoplastic effect are still poorly known.

Here we studied the antineoplastic effect of LE using various mammalian tumor cell lines and compared the antitumor activity of LE with that of other antineoplastic drugs. LE was shown to be highly active for all studied tumor cell lines of both epithelial and mesenchymal origin. While pseudonormal and transformed fibroblasts were similarly sensitive to LE action, pseudonormal cells of epithelial origin (mink lung cells) were shown to be more resistant than tumor epithelial cells (human breast carcinoma cells of MCF–7 and T47D line, human colon adenocarcinoma cells of HT–29 and SW–480 lines and human mouth carcinoma cells of KB line). Cytomorphological and biochemical (DNA fragmentation) study of LE effect on tumor cell lines was also carried out. The obtained data demonstrated proapoptotic action of LE which suggests its potential role as a new antineoplastic drug.

MATERIALS AND METHODS

LE was prepared in the laboratory of Dr. B. Matselyukh at D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kyiv). Other antitumor drugs used in this study were adriamycine ("Farmitalia Carloerba"), methotrexate ("Ebeve"), cisplatin ("Bristol"), vincristine ("Richter"), and fluorouracil ("Darnitsa").

Cells were obtained from cell culture collections at Ludwig Institute for Cancer Research, Uppsala, Sweden (MCF–7, T47D, HT–29, SW–480, NIH–3T3, CCL–64), at William Harvey Institute, London, United Kingdom (J774.2) and at R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine, Kyiv, Ukraine (KB, L1210, L929, HT–1080). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (Sigma, USA), 50 μg/ml streptomycin (Sigma, USA), 50 units/ml penicillin (Sigma, USA) in 5% CO_2–containing humidified atmosphere at 37 °C.

For experiments cells were seeded into 24–well tissue culture plates (Becton–Dickinson, USA). The cytotoxic effect of antitumor drugs was studied under the inverted microscope (Biolam–P1, LOMO, Russia) after cell staining with trypan blue dye (0.1%) [14].

For the supravital staining of the cells, acridine orange (1.5 μg/ml, 30 min) was used [1, 11]. Romanowsky — Giemsa cell staining was performed as decribed in [4].

DNA fragmentation was studied using agarose gel electrophoresis [17]. Briefly, $5 \cdot 10^6$ cells were pelleted and resuspended in 50 μl of 20 mM EDTA/50 mM Tris–HCl, pH 7.5, centrifuged for 5 min at 1,600 g and pellets were resuspended in lysis buffer. SDS (final concentration 1%) (Serva, Germany,) and RNase A (final concentration 1 mg/ml (Sigma, USA) were added to each sample which were then incubated for 1 h at 37 °C. After that, proteinase K (final concentration 1 mg/ml, Boehringer Mannheim, Germany) was added to each sample which was then incubated for 1 h at 37 °C. Then 10 M ammonia acetate (50% of the sample volume) was added to each sample and DNA was precipitated with 2 volumes of ice–cold iso–propanol at –20 °C overnight. Samples were centrifuged for 30 min at 10 000 g, pellets were air dried, dissolved in TE buffer (10 μl/10^6 cells) and loaded into the dry wells of 1% (w.v) agarose gel. Electrophoresis was carried out in 1 mM EDTA/40 mM Tris–acetate buffer, pH 8.0 until the marker dye migrated 6–7 cm. Electrophoregrams were stained with ethydium bromide and screened in transilluminator under UV light and photographed.

Experiments were performed in triplicate and repeated 3 times. Significance of the difference in a typical experiment was assessed by Student's *t*–test. The level of significance was set at 0.05.

RESULTS

In order to estimate the effect of various anticancer drugs on growth and viability of different tumor cells the ratio N_{alive}/N_{total} was calculated. This indicator shows the number of alive (trypan blue negative) cells divided by the total number of cells counted at the end of their treatment with different concentrations of the studied drugs.

Dose–dependent effects of LE on N_{alive}/N_{total} ratio in human breast carcinoma cell lines (MCF–7 and T47D), and in human colon carcinoma cell lines (HT–29 and SW–480) are presented in Fig. 1. The IC_{50} which indicates drug concentration causing 50% decrease in N_{alive}/

N_{total} ratio was also calculated and presented in the figures. T47D cells were found to be 5 times more resistant to LE action comparing to MCF–7 cells (both lines were isolated from human breast carcinoma), while the difference between IC_{50} for human colon carcinoma cells of HT–29 and SW–480 lines was less distinct (6.0 and 3.3 μg/ml, correspondingly).

In Fig. 2 the results of testing LE action on epithelial cells of two other lines are presented. One can see that normal mink lung epithelial cells of CCL–64 line are 3 times more resistant to LE effect comparing to human mouth carcinoma cells of KB line sensitive to cisplatin action. However, when two different lines of mouse fibroblastic cells (transformed L929 line and normal embryonic NIH–3T3 line) were compared (Fig. 3), there was no big difference in their IC_{50} at LE action (2.68 and 2.04 μg/ml correspondingly).

The results of dose–dependent LE action on growth and viability of two more lines of cells of mesenchymal origin (mouse leukemia cells of L1210 line and mouse monocyte/macrophage J774.2 line) are presented in Fig. 4. Their IC_{50} under LE effect was 1.75 and 2.86 μg/ml correspondigly. Thus, high sensivity of leukemia cells (L1210 line) to LE action was noted here. Data on LE IC_{50} brought together from Fig. 1–4 and calculated in both μg/ml and molar concentrations are presented in the Table.

In the next series of experiments the effects of various anticancer drugs (including the LE) were compared using human colon carcinoma cells of SW–480 line as a test–system (Fig. 5). We did not find significant difference between the used drugs (adriamycine, fluorouracyl, LE, cisplatin, methotrexate and vincristin) when they were used in low doses (up to 1.0 μg/ml). However, when the concentration of drugs was increased to 2.5–

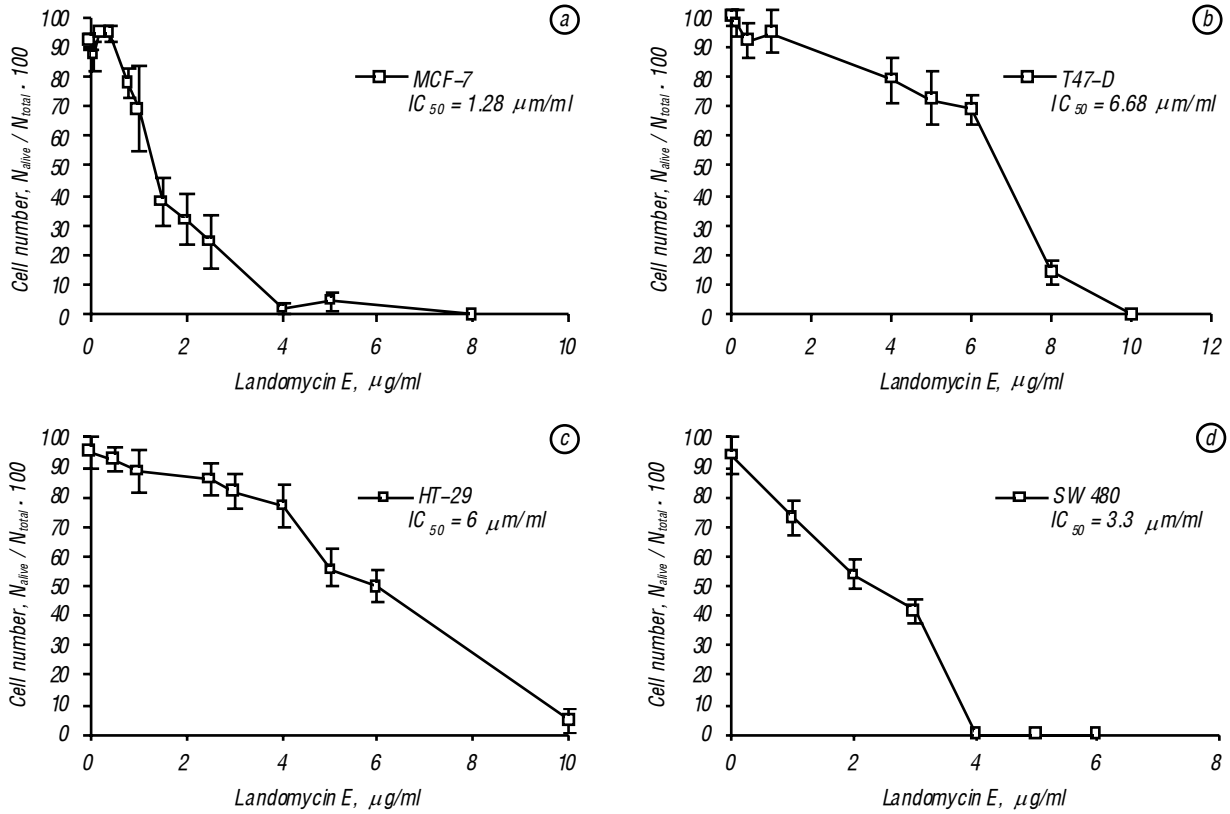

Fig. 1. Dose dependence of landomycin E effect on growth and survival (N_{alive}/N_{total}) of human breast adenocarcinoma cells of MCF–7 (*a*) and T47D (*b*) lines, and of human colorectal adenocarcinoma cells of HT29 (*c*) and SW–480 (*d*) lines (24 h treatment)

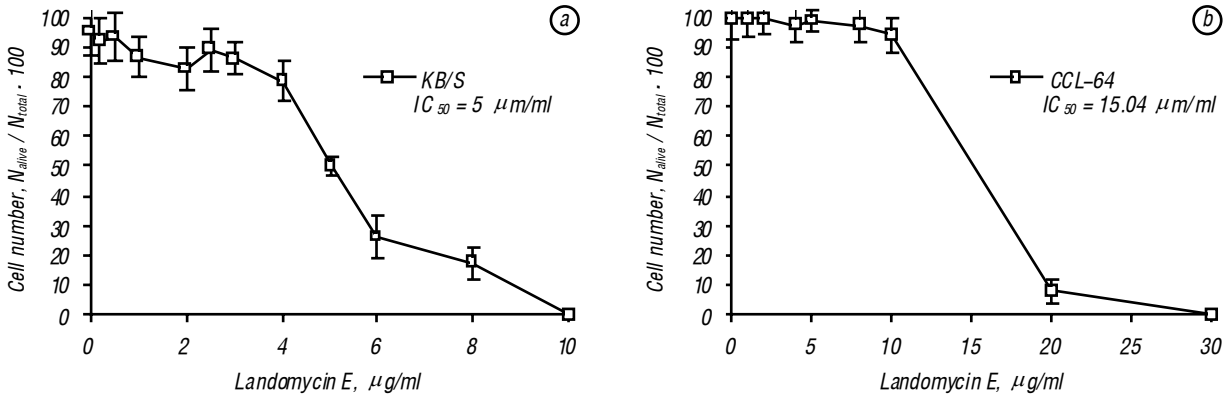

Fig. 2. Dose dependence of landomycin E effect on growth and survival (N_{alive}/N_{total}) of mouth carcinoma cells of KB line (*a*) and mink lung epithelial cells CCL–64 line (*b*) (24 h treatment)

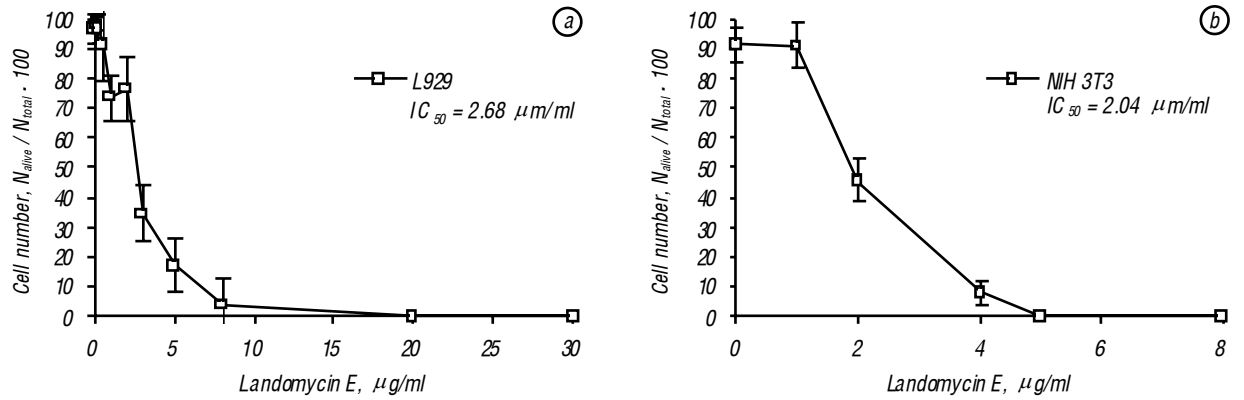

Fig. 3. Dose dependence of landomycin E effect on growth and survival (N_{alive}/N_{total}) of murine fibrosarcoma cells of L929 line (*a*) and of murine fibroblast–like cells of NIH–3T3 line (*b*) (24 h treatment)

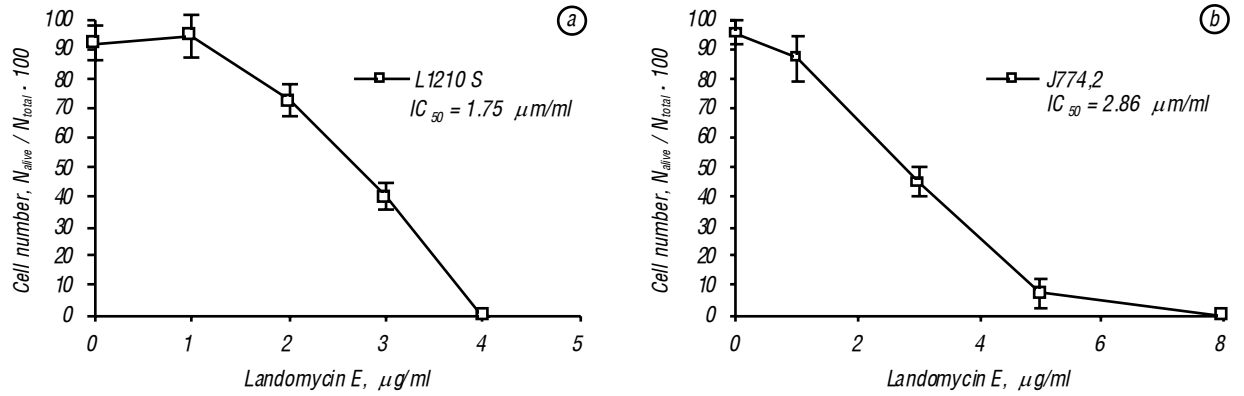

Fig. 4. Dose dependence of landomycin E effect on growth and survival (N_{alive}/N_{total}) of murine leukemia cells of L1210 line (*a*) and of murine macrophage/monocytes of J774.2 line (*b*) (24 h treatment)

Table. Characteristics of cytostatic and cytotoxic effects (cell number ratio N_{alive}/N_{total} was measured) of LE on different lines of human and animal cells (IC_{50} – inhibitory concentration of LE for its 50% effect; cell lines are noted in the "Results")

Cell line	LE concentrations	
	IC_{50}, μg/ml	IC_{50}, M · 10^{-6}
MCF–7	1.28	1.7
T47D	6.68	9.3
SW–480	3.30	4.6
HT–29	6.00	8.4
CCL–64	15.04	21.1
KB–S	5.00	7.0
L929	2.68	3.7
NIH 3T3	2.04	2.8
L1210	1.75	2.4
J774.2	2.68	4.0

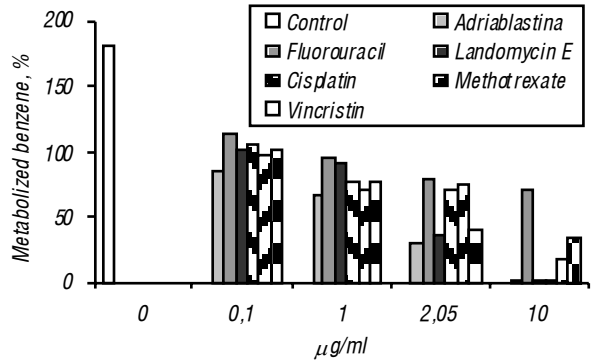

Fig. 5. Dose dependence of the effect of different anti–cancer drugs on human colorectal adenocarcinoma cells of SW–480 line (24 h treatment)

10.0 μg/ml, adriamycine and LE were shown to be the most effective tumor cell inhibitors. Molar concentrations for IC_{50} of anti–tumor agents were such: adriablastina — $1.7 \cdot 10^{-6}$ M < vincristin — $2.6 \cdot 10^{-6}$ M < cisplatin — $3.3 \cdot 10^{-6}$ M < landomycin E — $4.2 \cdot 10^{-6}$ M < metho–trexate — $5.1 \cdot 10^{-6}$ M < fluorouracil — $2.5 \cdot 10^{-5}$ M.

During the cytomorphological study of LE effect on different tumor cell lines the most prominent changes observed were the appearance of vacuoles in the cy–toplasm of LE–treated cells. One can see in Fig. 6 that LE in 2 μg/ml dose induced the appearance of big va–cuoles in human lung carcinoma cells of A549 line, while numerous small vacuoles were observed under the ef–fect of LE used in higher dose (4 μg/ml). In general, such effect of LE was confirmed when human mouth carcinoma cells of KB line were used (Fig. 7). The cy–toplasmic vacuoles could be also seen after the su–pravital cell staining with acridine orange (Fig. 8).

When mouse transformed fibroblasts of L929 line were cultured in the presence of 4 μg/ml LE, the most prominent changes observed were the appearing membrane protrudings which probably gave beginning to the apoptotic bodies (Fig. 9).

Specific internucleosomal DNA fragmentation is con–sidered to be one of the most cheracteristic biochemical indicator of apoptosis [7]. LE (2.5 μg/ml) was shown to induce intensive DNA fragmentation in murine leukemia cells of L1210 line (Fig. 10). It should be noted that these cells were also among the most sensitive to LE action, if IC_{50} indicator was taken into account.

Thus, LE posesses distinct inhibiting effect on cell growth and survival. At least partly, that effect is rea–

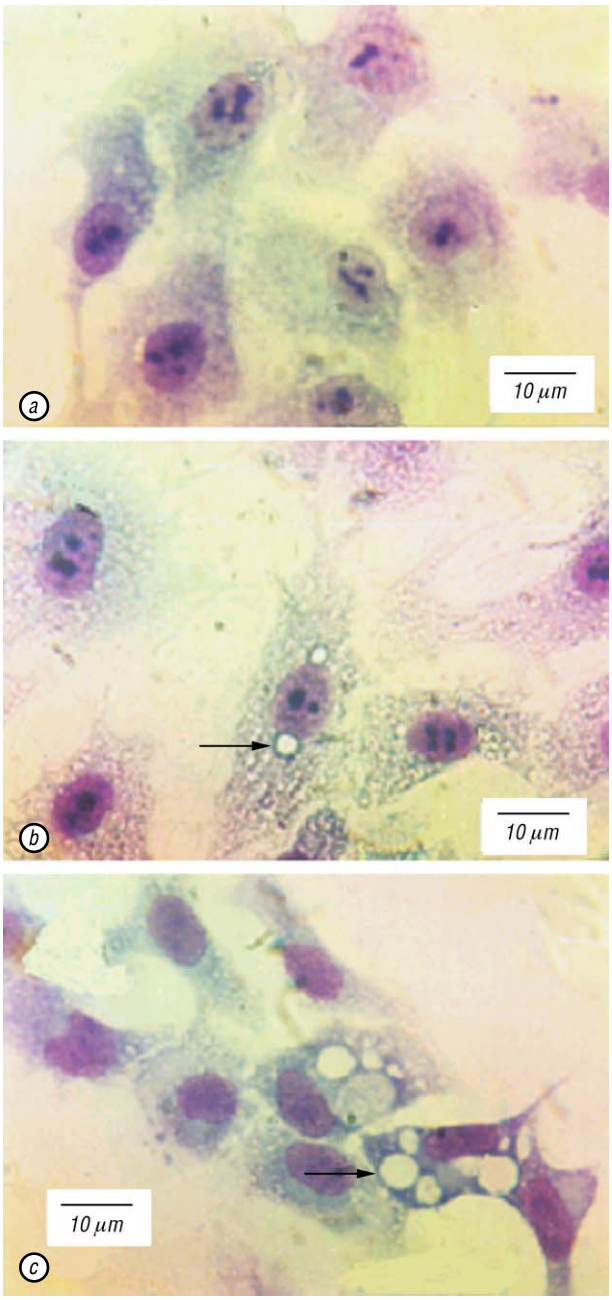

Fig. 6. Cytomorphological changes in human lung adenocarcinoma cells of A549 line (Romanowsky — Giemsa staining) under landomycin E effect (24 h treatment).
a — untreated; *b* — 2 μg/ml; *c* — 4 μg/ml; → — vacuoles

lized through typical apoptotic changes, such as the formation of cell membrane protrudings and cytoplasmic vacuoles, and the internucleosomal DNA fragmentation. These changes also lead to retardation of cellular growth and an increase in number of trypan blue–positive cells. Thus, an antineoplastic potential of LE is probable.

DISCUSSION

LE effect on tumor growth has been recently demonstarted in the *in vivo* experiments targeting Guerin carcinoma tumors in rats [6]. However, complex *in vitro* experiments are also needed to answer questions about cellular and molecular mechanisms of LE action. Here we used different approaches and lines of tumor cells to answer some of that questions.

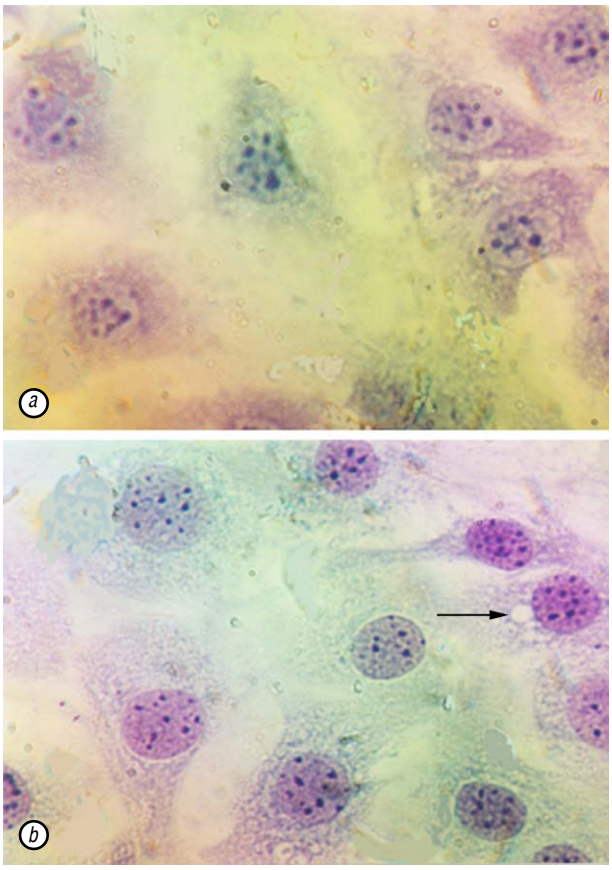

Fig. 7. Cytomorphological changes in human epidermal mouth carcinoma cells of KB line (Romanowsky — Giemsa staining) under landomycin E effect (24 h treatment).
a — untreated; *b* — 4 μg/ml; → — vacuoles

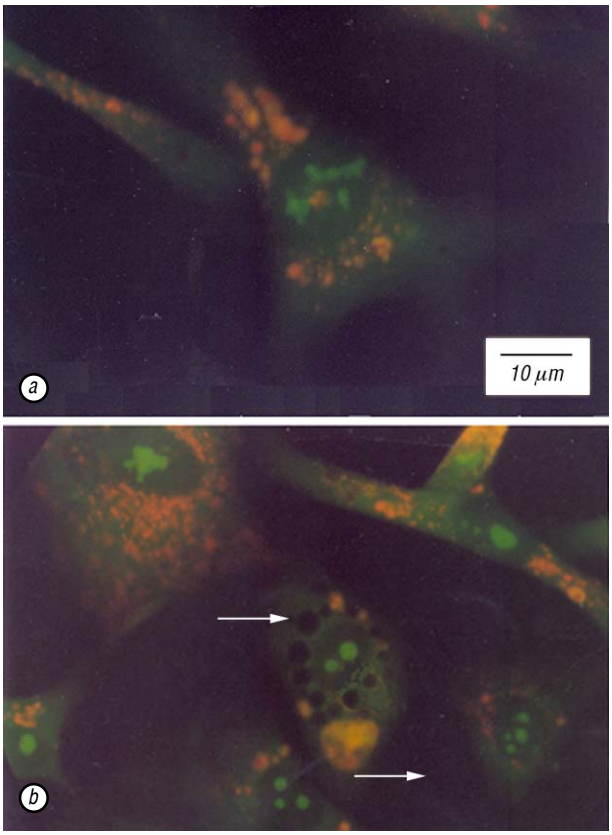

Fig. 8. Cytomorphological changes in human fibrosarcoma cells of HT–1080 line under landomycin E effect (48 h treatment, supravital staining with acridine–orange (1.5 μg/ml, 30 min)).
a — untreated; *b* — 4 μg/ml; → — vacuoles

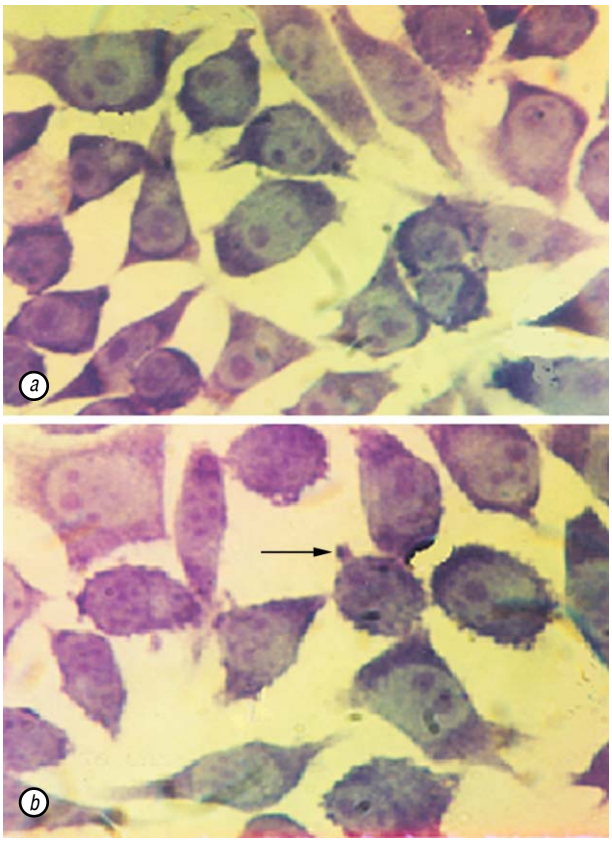

Fig. 9. Cytomorphological changes in mouse fibrosarcoma cells of L929 line (Romanowsky — Giemsa staining) under landomycin E effect (24 h treatment).
a — untreated; *b* — 4 mg/ml; → — membrane protrudings

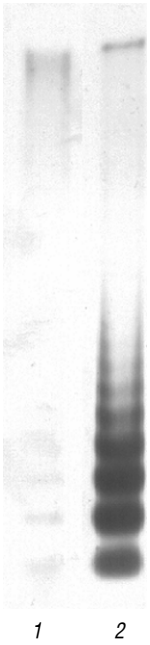

Fig. 10. Electrophoretic study of DNA fragmentation in murine leukemia cells of L1210 line treated with landomycin E (24 h treatment). Line *1* — untreated; line *2* — landomycin E (2.5 μg/ml)

We found that LE inhibited growth of various tumor cell lines in a dose–dependent manner. However, the character of LE effect and IC_{50} were shown to be cell line–specific. For example, the IC_{50} at LE action on human breast adenocarcinoma cells of MCF–7 and T47D lines differed considerably (1.28 and 6.68 μg/ml correspondingly). T47D cells were also shown to be more resistant than MCF–7 cells to growth inhibiting effect of the transforming growth factor β1 (TGF–β1) [21]. The explanation for such resistance could be that T47D cells lack normal expression of type II TGF–β1 receptors [21]. Earlier we suggested that TGF–β1 could mediate the action of some antitumor drugs and cytotoxic plant lectins, because these substances induced TGF–β1 production in different cancer cells [25, 28]. Human colon carcinoma cells of SW–480 line also have alterations in TGF–β signalling pathway (stop–codone mutation in the gene of Smad 4 signalling protein) comparing to another line (HT–29) of carcinoma cells of the same tissue and species origin [9, 22]. However, the results of our study showed that the resistance of HT–29 cells to LE action (based on the IC_{50} measurement) was higher than in SW–480 cells. Thus, the correlation between the increased resistance of tumor cells to the inhibiting actions of TGF–β and of LE is not a uniform property and that problem needs further studies.

Both transformed mouse fibroblasts of L929 line and normal mouse embryonic fibroblasts of NIH–3T3 line possessed similar sensitivity to LE action (IC_{50} equal 2.68 and 2.04 μg/ml correspondingly). However, the pseudo–normal cells of epithelial origin (mink lung epithelial cells of CCL–64 line) were more resistant to LE than all used cancer cell lines of the epithelial origin. It should be stressed that most mammalian tumors developed from the tissues of the epithelial origin [see 8]. Thus, our data suggest that LE is more effectively inhibiting cancer cells of the epithelial origin than normal cells of the same tissue origin, while it acts similarly on both tumor and normal cells of the mesenchymal origin.

The highest toxicity of LE was revealed when murine leukemia cells of L1210 line was used (IC_{50} equal 1.75 μg/ml). These data correlate with the results of study performed at the National Cancer Institute (NIH, USA) which showed that leukemic cells were the most sensitive to LE action [data are not presented]. We also found that the cytotoxic effect of LE on L1210 leukemic cells is accompanied by the intensive apoptotic DNA laddering in these target cells.

Cytomorphological investigation of various tumor cell lines showed that LE induced the appearance of different apoptotic phenomena (condensation of cytoplasm and nucleus, nucleus fragmentation, development of multiple vacuoles in the cytoplasm, and of the apoptotic protrudings of plasma membrane). These data suggest that LE induces apoptosis in different tumor cell lines and not only in L1210 leukemia cell line.

LE inhibited tumor cell growth in a dose–dependent manner and it was even more active than some other used anticancer drugs. Changes in target cell morphology elicited by Romanowsky — Giemsa staining, acridine orange supravital staining, and the intranucleosomal DNA fragmentation detected by electrophoresis suggest that LE–treated cells al least partly died by apoptosis.

In conclusion, LE was confirmed to be a perspective antitumor drug possessing growth inhibiting and pro–apoptotic activities towards various tumor cell lines. Although its highest cytotoxic and cytostatic activities were revealed

against the leukemia cells and other cells of mesenchymal origin, specific tumor cells of the epithelial origin may also be sensitive targets for LE antineoplastic action. Further studies on the molecular mechanisms of LE action on different tumor target cells are in progress.

ACKNOWLEDGMENTS

The authors thank Rostyslav Panchuk for his help in the manuscript design and Nataliya Kashchak for her comments on the manuscript text.

REFERENCES

1. **Zelenin AV.** Interaction of amino-derivatives of acrydine with the cell. Moskow: Nauka, 1971; 231 p (In Russian).

2. **Matselyukh BP, Lavrinchuk VYa.** Generation and characteristics of *Streptomyces globisporus 1912* mutants lacking landomycin E biosynthesis. Microbiol Zh 1999; **61**: 22–7 (In Ukrainian).

3. **Matselyukh BP, Konovalova TA, Polistchuk LV, Bambura OI**. Sensitivity of streptomycetes producing polyketyde antibiotics to landomycine A and E. Microbiol Zh 1998; **60**: 31–6 (In Russian).

4. **Pirs E.** Histochemistry. Moskow: Mir, 1962; 1078 p (In Russian).

5. **Polistchuk LV, Dehtyarenko TD, Stephanishin EE.** Plasmids of streptomycetes of globisporine group. Microbiol Zh 1985; **47**: 83–8.

6. **Polistchuk LV, Ganusevich P, Matselyukh BP.** The study of antitumor action of antibiotics produced by *Streptomyces globisporus 1912* on Guerin's carcinoma. Microbiol Zh 1996; **58**: 55–8.

7. **Filchenkov AA, Stoika RS.** Apoptosis and cancer. Kyiv: Morion, 1999; 184 p (In Russian).

8. **Matselyukh BP, Stoika RS, Bykorez AI.** Transforming growth factors. Kyiv: Naukova Dumka, 1994; 290 p (In Russian).

9. **Calonge MJ, Massague J.** Smad4/DPC4 silencing and hyperactive Ras jointly disrupt transforming growth factor-beta antiproliferative responses in colon cancer cells. J Biol Chem 1999; **274**: 33637–43.

10. **Crow RT, Rosenbaum B, Smith R, Ramos KS, Sulikowsky GA.** Landomycin A inhibits G_1/S cell cycle progression and induces apoptosis. Bioorg Med Chem Lett 1999; **9**: 1663–6.

11. **Darzynkiewicz Z, Kapuscinski J.** Acridine orange: a versatile probe of nucleic acids and other cell constituents. In: Flow Cytometry and Sorting. Melamed MR, Mullaney PF, Mendelsohn ML, eds. New York: John Wiley and Sons, 1990; 291–314 p.

12. **Depenbrock H, Bornschlegl S, Peter R, Rohr J, Schmid P, Schweighart P, Block T, Rastetter J, Hanauske A-R.** Assessment of antitumor activity of landomycin A (NSC 6399187-A). Ann Hematol 1996; **73** (Suppl II): A 80/316.

13. **Fujii I, Yutaka E.** Antracycline biosynthesis in *Streptomyces galilaeus*. Chem Reviews 1997; **97**: 2511–23.

14. **Grankvist K, Lernmark A, Taljedal IB.** Alloxan cytotoxicity *in vitro*. Microscope photometric analysis of Trypan Blue uptake by pancreatic islet cells in suspension. Biochem J 1977; **162**: 19–24.

15. **Hannun YA.** Apoptosis and the dilemma of cancer chemotherapy. Blood 1997: **89**: 1845–53.

16. **Henkel T, Rohr J, Beale J, Schwenen L.** Landonycines, new angucycline antibiotics from *Streptomyces sp*. J Antibiot 1990; **XLIII(**5): 492–503.

17. **Herrmann M, Lorenz HM, Voll R, Grunke M, Woith W, Kolden JR.** A rapid and simple method for the isolation of apoptotic DNA fragments. Nucl Acids Res 1994; **22**: 5506–7.

18. **Krohn K, Rohr J.** Angucyclines: total synthesis, new structures and biosynthetic studies of an emerging new class of antibiotics. Top Curr Chem 1997; **188**: 127–95.

19. **Matselyukh B, Polischuk L.** Characterization of antracyclinolike antibiotics coded by pSG 1912 plasmid of *Streptomyces sp*.1912. The 9-th Int. Symp. Biology of Actinomycetes. Moscow: VINITI, 1994; p. 111.

20. **Matselyukh B, Polischuk L, Rohr J.** Plasmid-induced synthesis of antibiotics in *Streptomyces*. Biology of Streptomycetes. — Ohrbeck: Osnabruek University, 1996, p. 38.

21. **Pouliot F, Labrie C.** Expression profile of agonistic Smads in human breast cancer cells: absense of regulation by estrogens. Int J Cancer 1999; **81**: 98–103.

22. **Preobrazhenska EV, Stoika RS**. Effects of TGF-β1, fluorouracil and cytotoxic lectins on HT-29 and SW-480 human colon cancer cells. Exp Oncol 2002; **24**: 188–93.

23. **Rohr J, Thiericke R.** Angucycline group antibiotics. Nat Prod Rep 1992: **9**: 103–37.

24. **Roush WR, Bennett CE, Roberts SE.** Studies and synthesis of landomycin A: synthesis and glycosidation reactions of L-rhodinosyl acetate derivatives. J Org Chem 2001; **66**: 6389–93.

25. **Stoika RS, Antonyuk VO, Yakymovych IA, Yakymovych M.Ya, Korchynsky OG, Preobrazhenska EV, Stasyk TV, Kashchak NI, Lutsik MD.** Tumor cell response to cytotoxic lectins and heat shock *in vitro*: study of possible involvement of transforming growth factor β1. Int J Med Environ 2000; **28**: 65–9.

26. **Tzefzer A, Fisher C, Stockert S, Westrich L, Kunzel E, Girresser U, Rohr J, Bechthold A.** Elucidation of the function of two glycosyltransferase genes (lanGt1 and lanGT4) involved in landomycin biosynthesis and generation of new oligosaccharide antibiotics. Chem Biol 2001; **8**: 1239–52.

27. **Weber S, Zolke C, Rohr J, Beale JM.** Investigation of the biosynthesis and structural revision of landomycin A. J Org Chem 1994; **59**: 4211–4.

28. **Yakymovych MYa, Yakymovych IA, Chekhun VF, Stoika RS.** Different production of TGF-b1 by cisplatin-sensitive and resistant L1210 cells treated with anti-cancer drugs and lectins. Exp Oncol 2001; **23**: 204–8.

29. **Young RC.** In: Drug resistance in cancer therapy. Kluwer, Dordrecht; 1989, 1–26.