



UDC: 616-006+616.4+615.3

STUDY OF INHIBITION OF B16F10 MELANOMA GROWTH IN MICE BY LANDOMYCIN A IN COMPARISON TO DOXORUBICIN

L. V. Lehka¹, R. R. Panchuk¹, N. R. Skorokhyd¹,
Yu. S. Kozak³, J. Rohr², R. S. Stoika^{1,3}

¹*Institute of Cell Biology, NAS of Ukraine, 14–16, Drahomanov St., Lviv 79005, Ukraine
e-mail: lilyalehka@gmail.com*

²*Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky
789 S. Limestone St., Lexington, Kentucky, 40536-0596, USA*

³*Ivan Franko National University of Lviv, 4, Hrushevskiyi St., Lviv 79005, Ukraine*

Landomycin A is a new antibiotic of angucycline group with antineoplastic activity against tumor cells of different origin. In this work, we investigated the effects of landomycin A on the growth of B16F10 mouse melanoma. It was elucidated that studied antibiotic possessed a significant dose-dependent cytotoxic activity against these melanoma cells *in vitro*, and its $LC_{50} = 2 \mu\text{M}$ was 5 times lower than that of the doxorubicin, anticancer drug widely used for treatment of solid tumors. Landomycin A at concentration of 10 mg/kg body weight did not cause pathological changes, mortality or general toxic symptoms in intact mice of C57black/6 line. No significant changes in the body weight and hematological parameters of animals treated with landomycin A as compared to the control group were observed. While B16F10 melanoma maintained rapid growth *in vivo* with a continual increase in tumor volume, landomycin A effectively inhibited tumor growth without a marked myelosuppressive effects or cardio- and hepatotoxicity that are characteristic for doxorubicin action. These results suggest a perspective of landomycin A application in chemotherapy of malignant tumors.

Keywords: landomycin A, melanoma, tumor volume, oxidative therapy.

INTRODUCTION

A cutaneous melanoma is a highly malignant tumor derived from melanocytes, the pigment-producing cells in skin epidermis [14]. If being early diagnosed and surgically removed while localized in the outermost skin layer, melanoma is potentially curable. When tumor cells have spread to distant lymph nodes or metastasized (stage IV), they become refractory to common chemotherapies and, therefore, incurable. The prognosis for patients with stage IV metastatic melanoma is very poor, with an expected median survival of only 6 to 9 months [7]. The rapid increase in the incidence of malignant melanomas has not been associated with any improved therapeutic options over the years [2].

Landomycin A (LA) is a new angucycline antibiotic with potential antitumor action. It consists of benzoanthracene tetracyclic aglycone and hexasaccharide chain that comprise two repetitive trisaccharide subunits (α -L-rhodinose-(1-3)- β -D-olivose-(1-4)- β -D-olivose) joined by O-glycoside bond (Fig. 1).

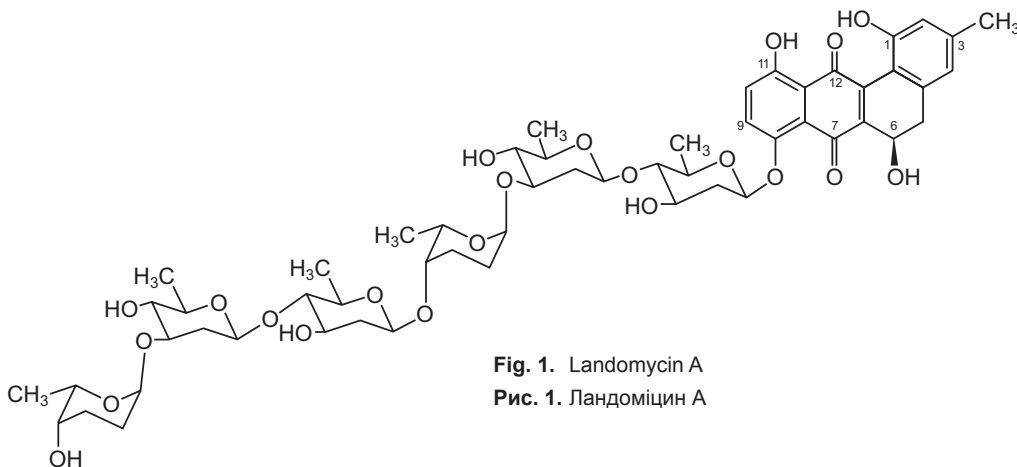


Fig. 1. Landomycin A
Рис. 1. Ландоміцин А

LA exhibits a strong cytotoxic effect towards tumor cells of different origin and induces early apoptosis in target cells. Our finding that landomycin A induces oxidative stress in cancer cell lines *in vitro* provides a possible mechanism of its antineoplastic activity [8]. It is known that cancer cells have increased ROS steady state level, and they are likely to be more vulnerable to damage by further ROS insults induced by exogenous agents [4]. Thus, manipulating ROS levels by redox modulation could be a way to kill cancer cells selectively without causing a significant toxicity to normal cells. Melanoma is a unique type of cancer with melanin biosynthesis that produces high level of ROS and oxidative stress [10, 15]. Thus, melanoma cells are expected to be very sensitive to therapeutic strategies whose application leads to induce cancer cell death through ROS production.

In this study, we evaluated the effects of landomycin A *in vitro* and *in vivo* on the growth of mouse melanoma cells.

MATERIALS AND METHODS

Landomycin A (99% purity according to thin-layer chromatography) was obtained in the laboratory of professor Jurgen Rohr (Department of Pharmaceutical Sciences, University of Kentucky, USA). Doxorubicin hydrochloride (Dx, Pfizer, New York, NY) was bought in local pharmacy.

Cell culture. B16F10 cell line (mouse melanoma) used in this study was obtained from the collection of the Institute of Cancer Research at Medical University of Vienna (Austria). Cells were grown in RPMI-1640 medium (Sigma, USA) supplemented with 10% fetal bovine serum (Sigma, USA) and 50 μ g/ml gentamicin (Sigma, Missouri, USA) in 5% CO₂-containing humidified atmosphere at 37 °C. Cells were re-seeded every two days at the rate of 2.5×10^5 cell/ml of culture medium [1].

Cytotoxicity assay. Cytotoxicity was measured using Trypan Blue exclusion assay. Briefly, exponentially growing B16F10 cells at 1×10^5 cells/well density were treated

with various concentrations of studied drug and cultivated in a 24-well tissue culture plate (Greiner Bio One, Frickenhausen, Germany). After 24 h incubation, the number of cells was calculated in the hemocytometer chamber by counting of the number of dead cells using 0.1% Trypan Blue dye that stains dead cells with damaged membrane in blue, whereas alive cells remain unstained [5].

Animals. Studies of the biological activity of LA were conducted using male C57black/6 mice Kent at animal facility of the Institute of Cell Biology, NAS of Ukraine (Lviv, Ukraine). All *in vivo* experiments were conducted in accordance with the international principles of the European Convention for protection of vertebrate animals under a control of the Bio-Ethics Committee of the above mentioned institution (Protocol N 9/2015 from 1.09.2015 of the BioEthics committee of the Institute of Cell Biology, NAS of Ukraine). Animals weighing 18–25 g were kept at a temperature of 22 ± 2 °C and with photo cycle of 12-hour light/12-hour dark. Water and food pellets were provided *ad libitum*.

Testing of LA *in vivo* toxicity. To define the toxicity of studied compound three experimental groups of male C57black/6 mice were used. Animals were distributed according to the study design: 1st group – control, intact animals, 2nd group – animals treated intraperitoneally (IP) with LA (10 mg/kg), 3rd group – mice treated IP with Dx (cumulative dose 10 mg/kg). There were three mice used in each group. During the period of study, the animals were weighed every day. Clinical symptoms were evaluated in mice daily.

Blood cell formula. For blood sampling amputation of small part of mouse tail was cut with pumping of ~100 μ l of blood in a test tube, followed by immediate disinfection of a wound with 70% alcohol. For counting of red blood cells, 5 μ l of blood were dissolved in 5 ml of isotonic NaCl solution (1:1000 dilution), while for leukocyte, 5 μ l of blood were dissolved in 95 μ l of 3% acetic acid solution (1:20 dilution). Erythrocytes and leukocytes were counted under the Evolution 300 Trino microscope (Delta Optical, Mińsk Mazowiecki, Poland) and calculated by standard formulas, described in [11]. For blood smear preparation, 3 μ l of blood were put at the edge of a slide, and then spread for 1.5 cm using another narrow polished slide, placed at a 45° angle. The obtained smears were dried at room temperature, then fixed with absolute methanol, and later rehydrated by subsequent washing in ethanol solutions with decreasing concentration (96%, 75%, 50%, 25%, 12.5%). Finally, the smears were washed with distilled water, stained with Giemsa dye and air-dried, after which they were ready for analysis of leukogram. Counting of leukocytes was performed under Evolution 300 Trino microscope (Delta Optical, Mińsk Mazowiecki, Poland) using 90 \times oil immersion objective. Cell counting was always done using the same system – half of cells were counted in the upper half part of the smear, and the rest 50% of cells were counted on the lower part of the smear. A percentage of certain types of white blood cells in each smear was determined after counting of at least 300 cells.

Tumor implantation. Tumor inoculation was done by a subcutaneous injection of B16F10 cells suspension diluted with sterile 1-x phosphate-buffered saline (PBS) in an amount of 1 mln per one animal. The viability and number of cells stained with 0.1% Trypan Blue were checked by cell counting in the haemocytometric chamber. The vitality of melanoma cells used for transplantation was not less than 98%.

Animals were distributed according to study design: 1st group (n = 5) – intact animals, 2nd group (n = 5) – control animals with B16F10 melanoma, 3rd group (n = 5) – animals treated IP with LA (cumulative dose 10 mg/kg), 4th group (n = 3) – mice treated IP with Dx (cumulative dose 10 mg/kg).

LA and Dx were administered 5 times in dose of 2 mg/kg every 72 hours. Injections of studied compounds were performed on 10th day after tumor inoculation. The length, width, and height of tumors were measured every three days with calipers. Experiments were terminated on 22th day, when tumor volumes in the control group reached the clinical endpoint (2500 mm³ or become necrotic) in accordance with animal ethics guidelines. Tumor volume was calculated as: $Vol = \frac{1}{2} L_{width} * L_{length} * L_{height}$ [12].

Aspartate and alanine aminotransferase activities. For determination of aspartate aminotransferase activity (AST), 10 μ l of blood serum were mixed with 100 μ l of substrate solution (2 mM α -ketoglutaric acid; 0.2 M D,L-aspartate in 0.1 M phosphate buffer pH 7.4), while in control tube 10 μ l of distilled water were added instead of blood serum. The tubes were placed for 60 min at 37 °C, and then 100 μ l of 1 mM solution of 2,4-dinitrophenylhydrazine was added to the samples and left for 20 min at RT. Then 1 ml of 0.4 M sodium hydroxide solution was added to each sample for extra 10 min, and optical density of samples was measured using ThermoSpectronic spectrophotometer (Helios, Great Britain) at 540 nm wavelength. For measuring alanine aminotransferase (ALT) activity, the procedure was identical except substrate solution (2 mM α -ketoglutaric acid; 0,2 M D,L-alanine in phosphate buffer pH 7.4).

Statistical analysis. *In vitro* experiments were performed in triplicate for each variant. For statistical analysis, standard variation data within a group were calculated together with a statistical reliability of differences between two groups of data assessed by t-test. The level of significance was set at 0.05.

RESULTS

To determine the effect of LA on growth of *B16F10* mouse melanoma *in vitro*, tumor cells were treated with studied drug at various concentrations (1, 2, 4, 6 μ M) and examined by cell counting using Trypan Blue assay on 24 hour after LA addition to the culture medium. Compared with control group, cell density in groups treated with LA decreased significantly. Thus, LA effectively inhibits the growth of B16F10 cells (Fig. 2, A). It was shown that LA suppressed proliferation of melanoma cells in a dose-dependent manner. LC₅₀ value (the concentration of compound which cause a death of 50% cells compared to the control) was 2 μ M, which is about 5 times lower than for doxorubicin (IC₅₀ for Dx on B16F10 cells = 10 μ M, Fig. 2, B). Doxorubicin is considered to be gold chemotherapy standard and it is widely used for treatment of solid tumors [16].

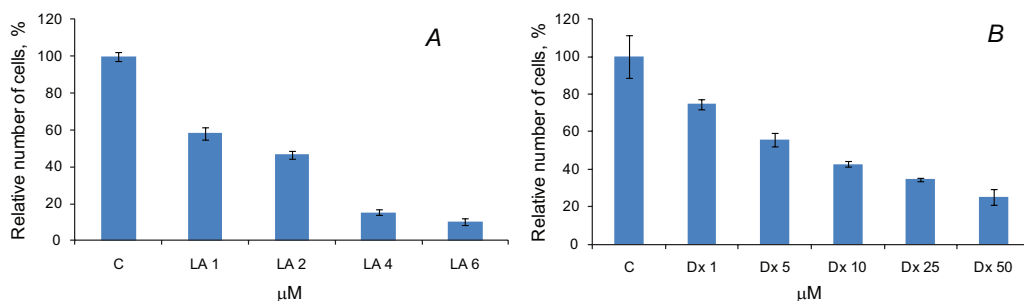


Fig. 2. Cytotoxic effect of landomycin A (LA, A) and doxorubicin (Dx, B) on B16F10 mouse melanoma cell line, 24 hours treatment

Рис. 2. Цитотоксичний ефект ландоміцину А (ЛА, А) і доксорубіцину (Дх, В) на клітинах лінії В16F10 мишачої меланоми, 24 год інкубації

The next step in our work was studying LA toxicity in male C57black/6 mice. This experiment was designed to investigate potential side effects of one-time IP administration of studied antibiotic in a dose of 10 mg/kg body weight. Fig. 3 demonstrates the results of weighing mice of control group.

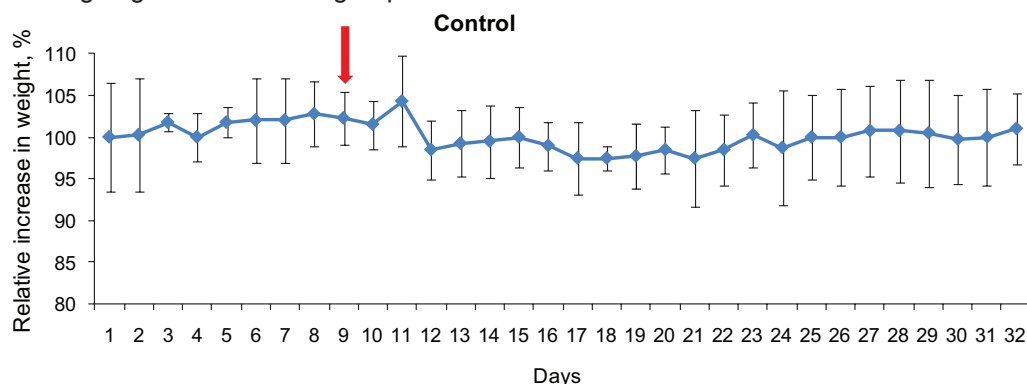


Fig. 3. Dynamics of *C57black/6* mice body weight (control group) during the experiment. ↓ – day of blood collection, n = 3

Рис. 3. Динаміка маси тіла мишей *C57black/6* (контрольна група) протягом експерименту. ↓ – день забору крові, n = 3

It was shown that their body weight did not change during the experiment term. We did not reveal any pathological changes in the LA-treated mice, as compared to the control group of animals. No mortality or significant changes were observed in the body weight of mice that received LA (Fig. 4). A similar experiment, as above, was done with Dx. It was revealed (Fig. 5) that already after the first injection of Dx, the weight of experimental animals decreased by 8% in comparison to control group. Starting from the 9th day after the injection of last Dx dose the further decrease of weight of experimental animals took place. Mice of this group in 3 days lost about 16% of their body weight. Although the weight of animals did not return to the initial value, suggesting general toxic effect of this drug.

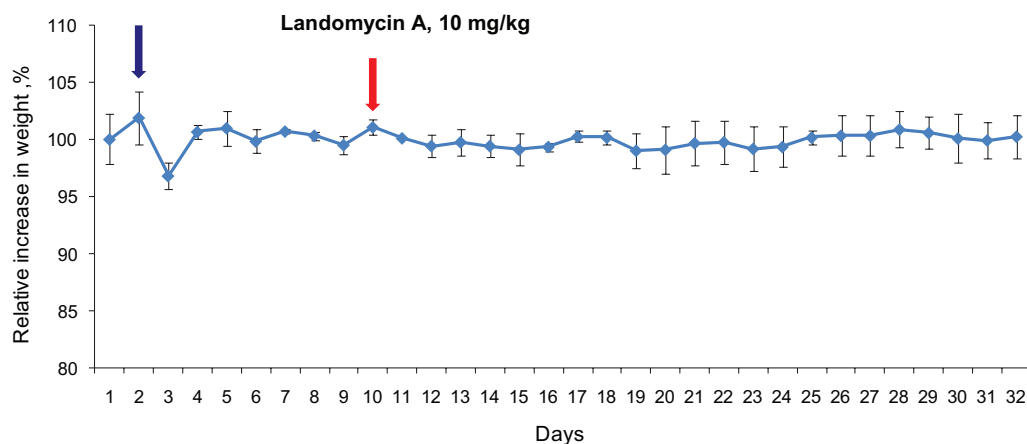


Fig. 4. Dynamics of *C57black/6* mice body weight during the experiment. ↓ – day of blood collection, ↓ – drug administration, n = 3

Рис. 4. Динаміка маси тіла мишей *C57black/6* протягом експерименту. ↓ – день забору крові, ↓ – день введення речовини, n = 3

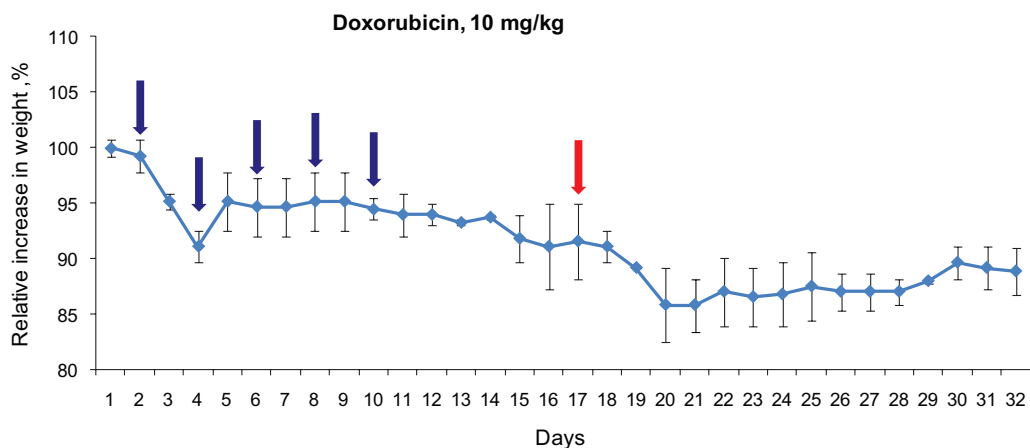


Fig. 5. Dynamics of *C57black/6* mice body weight during the experiment. ↓ – day of blood collection, ↓ – drug administration, n = 3

Рис. 5. Динаміка маси тіла мишей *C57black/6* протягом експерименту. ↓ – день забору крові, ↓ – день введення речовини, n = 3

The hematological profile (number of red and white blood cells as well as a leukogram) in the experimental animals was also studied. After LA administration, no significant changes in the peripheral blood cells were observed in comparing to animals of control group (Table 1).

Table 1. Number of white, red blood cells and peripheral blood leukogram of mice on 7th day after the last injection of landomycin A and Doxorubicin

Таблиця 1. Загальна кількість лейкоцитів та еритроцитів і лейкограма периферичної крові мишей трьох дослідних груп через 7 днів після останньої ін'єкції ландоміцину А і доксорубіцину

Blood cells	Control	Landomycin A	Doxorubicin
White blood cells ($\times 10^3/\mu\text{l}$)	14.9 \pm 2.8	15.9 \pm 2.1	10.6 \pm 1.9*
Red blood cells ($\times 10^6/\mu\text{l}$)	9.5 \pm 0.7	9.1 \pm 0.6	8.7 \pm 0.6*
Neutrophils with ring-shaped nuclei, RSN (%)	1.6 \pm 0.4	1.9 \pm 1.6	4.2 \pm 0.6
Neutrophils with segmented nuclei, SN (%)	22.0 \pm 1.4	24.6 \pm 4.5	31.0 \pm 1.2
Small lymphocytes, SL (%)	72.0 \pm 1,4	70.3 \pm 4.7	58.6 \pm 0.9
Big lymphocytes, BL (%)	2.2 \pm 0.8	1.9 \pm 0.4	2.5 \pm 0.4
Monocytes, M (%)	1.3 \pm 1.0	1.3 \pm 1.5	3.4 \pm 0.7*

Comments: * – statistically significant changes $p < 0.05$ related to control

Примітки: * – статистично достовірні зміни $P < 0,05$ порівняно з контролем

In case of mice receiving the same dose of Dx, changes in a blood parameters were more marked and typical for this compound: mielosuppressive effect including leukopenia

(29% decrease in the amount of white blood cells compared to control group), erythrope-
nia (10% reduction in red blood cells number), diminution of SL percentage (from $72.0 \pm 1.4\%$ in untreated mice to $58.6 \pm 0.9\%$) and increase in monocytes amount (from $1.3 \pm 1.0\%$ to $3.4 \pm 0.7\%$ were found (Table 1). These results prove low toxic nature of LA, which is a primary criterion and important step in development of anticancer drugs.

B16F10 cells formed large, aggressive tumors in C57black/6 mice. To determine whether LA limits melanoma growth *in vivo*, the IP injection of LA (cumulative dose 10 mg/kg) was carried out in B16F10 melanoma-bearing mice. As a result (Fig 6), the mean of tumor volume was significantly less (742 mm^3) compared with that in control group ($3,126 \text{ mm}^3$) on 22th day when control mice were sacrificed. We also used Dx as a positive control in the same dose (cumulative dose 10 mg/kg). Group of mice treated with Dx also did show a decrease in tumor volume ($1,878 \text{ mm}^3$ comparing with $3,126 \text{ mm}^3$ in control), however the tumor size in Dx-treated mice was 153% larger compared to LA in the same concentration.

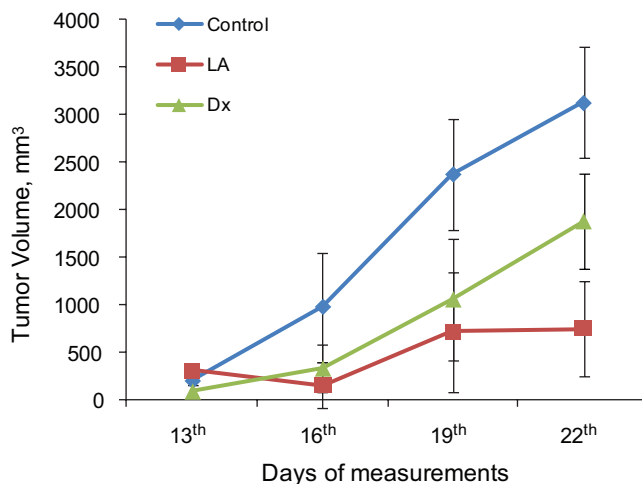


Fig. 6. Changes in tumor volume in B16F10 melanoma-bearing mice treated with Landomycin A (LA) and Doxorubicin (Dx)

Рис. 6. Зміни об'єму пухлини у мишей із меланою В16F10, що одержували ландоміцин А (ЛА) та доксорубіцин (Дх)

The hematological profile was also analyzed in B16F10 melanoma-bearing animals. The growth of B16F10 melanoma was characterized by a significant increase in the level of SN (from $20.0 \pm 4.5\%$ to $57.0 \pm 16.2\%$) and marked increase in the level of WBC (from $6.2 \pm 0.9\%$ to $13.5 \pm 1.7\%$). Regarding the leukogram in a group treated with LA, it was revealed a restoration of these indicators found in control group, thus, suggesting a therapeutic effect of this drug (Table 2).

The amount of SL is another important indicator that was decreased in tumor-bearing animals (from $78.0 \pm 5.1\%$ in intact mice to $31.9 \pm 4.1\%$). LA partially restored this value to control level – from $31.9 \pm 4.1\%$ to $54.0 \pm 10.6\%$.

Similar influence on above-mentioned indices was found at Dx action in the analogous dose (Table 2). At the same time, we detected myelosuppression in the experimental animals which is a negative side effect of this antibiotic that affecting proliferation

of bone marrow cells, thus, leading to anemia. The amount of RBC decreased by almost on 50% compared to intact control (from $10 \pm 0.9 \times 10^6/\mu\text{l}$ to $5.8 \pm 0.3 \times 10^6/\mu\text{l}$) and 20% compared to mice with B16F10 melanoma ($7.3 \pm 0.3 \times 10^6/\mu\text{l}$ to $5.8 \pm 0.3 \times 10^6/\mu\text{l}$). A significant increase (from $1.9 \pm 0.8\%$ in intact control to $9.5 \pm 2.6\%$) in a group treated with Dx was observed in the number of monocytes.

Table 2. Number of white, red blood cells and peripheral blood leucogram of intact mice and with B16F10 melanoma on 10th day after the last injection of landomycin A and doxorubicin

Таблиця 2. Загальна кількість лейкоцитів та еритроцитів і лейкограма периферичної крові інтактних мишей і тварин з меланою B16F10 на 10-й день після останньої ін'єкції ландоміцину А і доксорубіцину

Blood cells	Control (intact animals)	Control B16F10 melanoma	Doxorubicin (cumulative dose 10 mg/kg)	Landomycin A (cumulative dose 10 mg/kg)
White blood cells ($\times 10^3/\mu\text{l}$)	6.2 \pm 0.9	13.5 \pm 1.7 [#]	8.2 \pm 2.4 [*]	7.4 \pm 0.9 [*]
Red blood cells ($\times 10^6/\mu\text{l}$)	10.0 \pm 0.9	7.3 \pm 1.4	5.8 \pm 0.3 ^{***}	7.2 \pm 0.5
Neutrophils with ring-shaped nuclei, RSN (%)	0.5 \pm 0.2	3.6 \pm 1.3	2.9 \pm 1.8	1.0 \pm 0.5
Neutrophils with segmented nuclei, SN (%)	20.0 \pm 4.5	57.2 \pm 1.2 [#]	38.1 \pm 15.6	38.4 \pm 9.3 ^{**}
Small lymphocytes, SL (%)	78.0 \pm 5.1	31.9 \pm 4.1 [#]	42.7 \pm 16.8	54.0 \pm 10.6 [*]
Big lymphocytes, BL (%)	2.3 \pm 0.7	5.7 \pm 4.7	6.6 \pm 2.0	3.0 \pm 2.0
Monocytes, M (%)	1.9 \pm 0.8	4.2 \pm 0.8	9.5 \pm 2.6 ^{**}	3.7 \pm 1.5

Comments: * – statistically significant changes $p < 0.05$ related to control (B16F10 melanoma); ** – $p < 0.01$ related to control B16F10 melanoma; *** – $p < 0.001$ related to control B16F10 melanoma; # – $p < 0.05$ related to control (intact animals)

Примітки: * – статистично достовірні зміни $P < 0,05$ порівняно з контролем (B16F10 меланома); ** – $p < 0,01$ порівняно з контролем (B16F10 меланома); *** – $p < 0,001$ порівняно з контролем (B16F10 меланома); # – $p < 0,05$ порівняно з контролем (інтактні тварини)

The activity of aspartate and alanine aminotransferases (AST and ALT) in blood serum of LA, Dx treated mice was measured and AST/ALT correlation (De Ritis ratio) were calculated for evaluating the effect of these drugs on liver and heart metabolism (Table 3). It was shown that Dx increased in ALT/AST ratio (De Ritis ratio) from 1.3 ± 0.1 (normal value in healthy mice) to 1.8 ± 0.1 indicating hepato- and cardiotoxicity induced under treatment [3]. This index also remained within normal limits (1.2 ± 0.2) in animals with B16F10 melanoma without treatment. In case of LA application, De Ritis ratio increased insignificantly compared to control groups and showed 1.5 ± 0.1 .

A therapeutic selectivity and avoiding resistance to drugs are two important issues in the anticancer therapy. Strategies for improving therapeutic selectivity depend significantly on understanding of the biological difference between tumor and normal cells.

Table 3. Impact of landomycin A and doxorubicin on ALT/AST levels in blood serum of B16F10 melanoma bearing mice on 10th day after last injection of drugs

Таблиця 3. Вплив ландоміцину А і доксорубіцину на рівень АСТ/АЛТ у сироватці тварин з меланою B16F10 на 10-й день після останньої ін'єкції речовин

Variant of experiment	Aspartate aminotransferase (AST), units/ml×100	Alanine aminotransferase (ALT), units/ml×100	De Ritis ratio
Control (intact)	1.68±0.00	1.33±0.00	1.3±0.1
Control (B16F10 Melanoma)	2.54±0.01	2.08±0.01	1.2±0.2
Doxorubicin treatment	1.83±0.01	1.03±0.00	1.8±0.1
Landomycin A treatment	1.83±0.01	1.22±0.01	1.5±0.1*

Comments: * – statistically significant changes $p < 0.05$ related to intact control

Примітки: * – статистично достовірні зміни $P < 0,05$ порівняно з інтактним контролем

Tumor cells, compared to normal ones are under big oxidative stress related to the oncogenic transformation and alterations in metabolic activity [13]. Exogenous agents that rapidly increase ROS generation will move the redox equilibrium and induce tumor cells death. In contrast, normal cells are less sensitive to agents that induce an oxidative stress due to low level of ROS production and high antioxidant capacity. Previously we have shown that under LA action *in vitro*, the level of ROS, mainly H₂O₂, had increased several times, comparing to control level already at the 1st hour after start of LA addition to the culture medium [8]. It is well established that high level of ROS, like H₂O₂, induce apoptosis in a wide variety of tumor cells via activating the caspase cascade [9]. We suppose that such early and rapid generation of ROS, accompanied with caspases activation allows LA to inhibit growth of B16F10 melanoma both *in vitro* and *in vivo* with much lower negative side effects than such effects of Dx.

CONCLUSION

In this study, we have shown that LA possessed antineoplastic effect towards B16F10 melanoma cells *in vitro*, and that effect was even stronger than such of Dx used as a positive control. LA's action was not accompanied by cachexy in experimental animals and it didn't cause hematotoxic effects found at Dx's action. Our data on measurement of AST/ALT ratio testify to the lack of cardio- and hepatotoxicity under LA treatment. LA effectively inhibited growth of B16F10 melanoma *in vivo* without significant myelosuppressive effects.

Taking into consideration these results, investigation of action of LA in the other experimental tumor models that might be especially sensitive to this drug are desired, as well as studying the molecular mechanisms of its therapeutic activity.

ACKNOWLEDGEMENTS

We thank Prof. W. Berger from the Institute of Cancer Research at Medical University of Vienna for providing B16F10 melanoma cell line.

This work was partially supported by grants awarded to L. Lehka by West-Ukrainian BioMedical Research Centre (Ukraine-USA) in 2013–2014, and 2015–2016.

1. Adams R. **Laboratory techniques in biochemistry and molecular biology**. Elsevier, 1990. 16–94 p.
2. Atallah E., Flaherty L. Treatment of metastatic malignant melanoma. **Curr. Treat. Options Oncol**, 2005; 6(3): 185–193.
3. Botros M., Kenneth S. The De Ritis Ratio: The Test of Time. **Clin. Biochem. Rev**, 2013; 34: 117–130.
4. Chiara G., Isaac S., Harris M., Tak M. Modulation of oxidative stress as an anticancer strategy. **Nat. Rev. Drug Discov**, 2013; 12: 931–947.
5. Freshney R.I. **Culture of animal cells: a manual of basic technique and specialized applications**: Wiley-Blackwell, 2010. 768 p.
6. Han S., Hong C., Kim H., Lyu S. Anti-cancer effects of enteric-coated polymers containing mistletoe lectin in murine melanoma cells *in vitro* and *in vivo*. **Mol. Cell Biochem**, 2015; 15: 1–15.
7. Korn E.L., Liu P.Y., Lee S.J. Meta-analysis of phase II cooperative group trials in metastatic stage IV melanoma to determine progression-free and overall survival benchmarks for future phase II trials. **J. Clin. Oncol**, 2008; 26: 527–34.
8. Lehka L.V., Panchuk R.R., Berger W. et al. The role of reactive oxygen species in apoptosis of tumor cells induced by landomycin A. **Ukrainian Biochemical Journal**, 2015; 87(5): 72–82.
9. Matsura T., Kai M., Fujii Y., Yamada H. Hydrogen Peroxide-induced Apoptosis Requires Caspase-3 Activation in HL-60 Cells. **Free Rad. Res**, 1998; 30: 73–83.
10. Meyskens F.L., Jr. Farmer P., Fruehauf J.P. Redox regulation in human melanocytes and melanoma. **Pigment Cell Res**, 2001; 14: 148–54.
11. Ronin V.S., Utevsky N.L. **Textbook for classes on the methods of clinical laboratory investigations**. 4th ed. Moscow: Medicine, 1989. 335 p. (In Russian).
12. Tomayko M., Reynolds P. Determination of subcutaneous tumor size in athymic (nude) mice. **Cancer Chemotherapy and Pharmacology**, 1989; 24(3): 148–154.
13. Toyokuni S., Okamoto K., Yodoi J., Hiai H. Persistent oxidative stress in cancer. **FEBS Letters**, 1995; 358: 1–3.
14. White N., Gillian K., Butler P., Burnstock G. An *in vivo* model of melanoma: treatment with ATP. **Purinergic Signal**, 2009; 5(3): 327–333.
15. Wittgen H.G., Van Kempen L.C. Reactive oxygen species in melanoma and its therapeutic implications. **Melanoma Res**, 2007; 17: 400–9.
16. Zhang X., Teodoro J., Nadeau Jay. Intratumoral gold-doxorubicin is effective in treating melanoma in mice. **Nanomedicine: Nanotechnology, Biology, and Medicine**, 2015; 20: 1–11.

ДОСЛІДЖЕННЯ ІНГІБУВАННЯ РОСТУ МЕЛАНОМИ В16F10 ЛАНДОМІЦИНОМ А ПОРІВНЯНО З ДОКСОРУБІЦИНОМ У МИШЕЙ

Л. В. Легка¹, Р. Р. Панчук¹, Н. Р. Скорохід¹,
Ю. С. Козак³, Ю. Рор², Р. С. Стойка^{1,3}

¹Інститут біології клітини НАН України, вул. Драгоманова, 14/16, Львів 79005, Україна
e-mail: lilyalehka@gmail.com

²Відділ фармацевтичних наук, Фармацевтичний коледж, Університет Кентуккі
вул. Лаймстоун, 789 S, Лексінгтон, Кентуккі 40536-0596, США

³Львівський національний університет імені Івана Франка
вул. Грушевського, 4, Львів 79005, Україна

Ландоміцин А – новий антибіотик ангуциклінового ряду з антинеопластичною активністю щодо пухлинних клітин різного генезу. У цій роботі ми досліджували вплив ландоміцину А на ріст мишачої меланоми лінії В16F10. Було з'ясовано, що досліджуваний антибіотик має виражену дозозалежну цитотоксичну активність щодо клітин цієї лінії *in vitro* зі значенням LC_{50} 2 мкМ, що є у 5 разів нижчим, ніж для протипухлинного антибіотика доксорубіцину, який широко використовують для лікування солідних пухлин. Ландоміцин А у дозі 10 мг/кг маси тіла не спричиняв патологічних змін, смертності або симптомів токсичності в інтактних мишей лінії C57black/6. Не спостерігали істотних змін ваги та гематологічних показників у тварин, яким вводили ландоміцин А, порівняно з тваринами контрольної групи. Незважаючи на те, що для В16F10 меланоми *in vivo* характерний швидкий ріст і постійне збільшення об'єму пухлини, ландоміцин А ефективно пригнічував ріст пухлини без вираженого мієлосупресивного ефекту, кардіо- і гепатотоксичності, які властиві доксорубіцину. Отримані результати доводять перспективність застосування ландоміцину А в хіміотерапії злоякісних пухлин.

Ключові слова: ландоміцин А, меланома, об'єм пухлини, оксидативна терапія.

ИССЛЕДОВАНИЕ ИНГИБИРОВАНИЯ РОСТА МЕЛАНОМЫ В16F10 ЛАНДОМИЦИНОМ А ПО СРАВНЕНИЮ С ДОКСОРУБИЦИНОМ У МЫШЕЙ

Л. В. Легка¹, Р. Р. Панчук¹, Н. Р. Скорохід¹,
Ю. С. Козак³, Ю. Рор², Р. С. Стойка^{1,3}

¹Институт биологии клетки, НАН Украины, ул. Драгоманова, 14/16, Львов 79005, Украина
e-mail: lilyalehka@gmail.com

²Отдел фармацевтических наук, Фармацевтический колледж, Университет Кентукки
ул. Лаймстоун, 789 S, Лексингтон, Кентукки 40536-0596, США

³Львовский национальный университет имени Ивана Франка
ул. Грушевского, 4, Львов 79005, Украина

Ландоміцин А – новий антибіотик ангуциклінового ряду з антинеопластичною активністю в отношении опухольових кліток различного генеза. В этой работе мы исследовали влияние ландоміцина А на рост мышинои меланоми линии В16F10. Было выяснено, что исследуемый антибіотик имеет выраженную дозозависимую цитотоксическую активність на этой линии кліток *in vitro* со значением

LC₅₀ 2 мкм, что в 5 раз ниже, чем для доксорубина, противоопухолевого антибиотика, который широко используют для лечения солидных опухолей. Ландомицин А в дозе 10 мг/кг массы тела не вызывал патологических изменений, смертности или симптомов токсичности у интактных мышей линии C57black/6. Не наблюдали существенных изменений веса и гематологических показателей у животных, которым вводили ландомицин А, по сравнению с животными контрольной группы. Несмотря на то, что для B16F10 меланомы *in vivo* характерен быстрый рост и постоянное увеличение объема опухоли, ландомицин А эффективно подавлял рост опухоли без выраженного миелосупрессивного эффекта, кардио- и гепатотоксичности, которые свойственны доксорубину. Полученные результаты доказывают перспективность применения ландомицина А в химиотерапии злокачественных опухолей.

Ключевые слова: ландомицин А, меланома, объем опухоли, оксидативная терапия.

Одержано: 09.02.2016