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Effect of complexes of natural oligoribonucleotides with D-mannitol on the viability of cell cultures of different origin

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Aim. Investigation of the effect of acidic and salt forms of natural yeast RNA oligoribonucleotides and their complexes with D-mannitol (ORN-D-M) on the viability of cell cultures of different origins. **Methods.** MTT assay, differential trypan blue staining. **Results.** A dose-dependent inhibition of the viability of mouse melanoma B16 cells and human glioblastoma U251 cells under the influence of ORN-D-M was established, as well as a dependence on the treatment time. U251 cells were more sensitive to the effect of the drug: at 48-hour treatment, the IC_{50} for this cell line was 1.0±0.3 mg/ml, whereas for B16 it was 2.5±0.4 mg/ml. At the same time, the inhibition of non-malignant canine kidney MDCK cells was observed only at high concentrations of the drug (IC_{50} — 6.1±1.1 mg/ml). The acidic form of ORN-D-M inhibited the viability of B16 and U251 cell cultures whereas the acidic form of ORN, salt forms of Na-ORN and Na-ORN-D-M showed a significantly lower inhibition activity. **Conclusions.** The acidic form of ORN-D-M has significant inhibitory activity against the malignant cells of mouse melanoma B16 and human glioblastoma U251. The acidic form of ORN-D-M is a promising drug for further antitumor studies *in vitro* and *in vivo*.

K e y w o r d s: natural oligoribonucleotides, ORN-D-M, anticancer activity, MTT assay.

Introduction

Nucleic acids and their derivatives are promising immunotherapeutic agents that have shown their effectiveness against different types of cancer. In the 1970s, the FDA approved the *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG) vaccine as an immunotherapeutic agent in the treatment regimen for the patients with bladder cancer, which is still used today [1, 2]; the clinical trials of this drug were also conducted in monotherapy and complex therapy of melanoma [3]. The studies of the phenomenon of BCG antitumor activity have shown, that the bacterial DNA, which is a component of the drug, initiates innate im-

munity responses, induces the synthesis of type I IFN, and enhances the activity of NK cells, which leads to inhibition of the tumor growth [4, 5]. Another type of therapeutics based on synthetic oligodeoxynucleotides rich in CpG motifs (CpG ODN) has shown itself not only as a potent immunoadjuvant but also as an antitumor agent that activates the receptor 9 (TLR9) in immune cells [6]. However, there is little data on the use of oligoribonucleotide-based therapy in the fight against cancer. One example is the use of artificially synthesized ORN as an agonist for TLR 7/8, which induced apoptosis in the colon carcinoma cells [7]. Another instance is sodium nucleinate — a sodium salt of low molecular weight yeast RNA with impurities of proteins and DNA, which showed its effectiveness in the combination therapy of melanoma with an immunocorrective effect of the CD3+ and CD4+ lymphocytes number, and was applied in the combined therapy on the late cancer stages [8, 23].

Natural oligoribonucleotides obtained from total yeast RNA in complex with D-mannitol (ORN-D-M) have pronounced anti-inflammatory and antioxidant activity, as well as immunoprotective effect on the innate immune system, that was shown in various models of inflammation, including influenza virus-induced [9,10] and acute hepatotoxicity [11]. It is known that tumor-associated inflammation is not just an epiphenomenon, but it is currently considered the main factor in the malignant progression [12], therefore, the anti-inflammatory agents take place in cancer therapy [13, 14]. The combination of anti-inflammatory and immunoprotective effects in one pill makes ORN-D-M a promising agent for anticancer therapy. Additionally, the drug is produced on the basis of a cheap natural substance — yeast ribonucleic acid, which is a significant advantage in contrast to the expensive chemotherapy drugs. Therefore, we investigated the effect of ORN-D-M and its derivatives on the viability of cell cultures of various etiologies.

Materials and Methods

For research, we used the acidic form of commercial yeast RNA (Goodwill Associates Inc., USA), registered as a substance in the Ministry of Health of Ukraine (Registration certificate № UA/2810/01/01, order of the Ministry of Health of Ukraine № 777 dated 04/06/2020), which, according to the mass spectrometric analysis is low-molecular-weight oligoribonucleotides (ORN) with a dominant fraction of 4–6 b.p [9, 15]. As a complex of oligoribonucleotides with D-mannitol (ORN-D-M) we used the drug Nuclex from "Valartin Pharma" Ukraine, which consists of yeast RNA from Goodwill Associates Inc., USA, and D-mannitol (D-M). Sodium salts of oligoribonucleotides (Na-ORN) and oligoribonucleotides modified with D-mannitol (Na-ORN-D-M) were obtained by titrating the corresponding acidic forms of ORN and ORN-D-M with NaOH to pH 7.0.

Cultivation of cells. The objects of the study were the mouse melanoma tumor B16 cell line, human glioblastoma U251 cell line and non-tumor canine kidney MDCK cell line (obtained from the collection of the Institute of Molecular Biology and Genetics). The B16 and MDCK cells were cultured in RPMI (Gibco, USA) and U251 in DMEM (Gibco, USA) supplemented with FBS (Gibco, USA) to a final concentration of 10 %, penicillin (100 U/mL) and strep-

tomycin (100 μg /mL) (Sigma-Aldrich, USA) at 37 °C in a humidified incubator with 5 % CO_2. When the cells reached 70–90 % confluency, they were reseeded at a dilution of 1:3–1:5. To detach the cells from the surface of the culture flask, they were washed with Versene and incubated in 0.05 mg/mL trypsin (Gibco, USA) for 10 min at room temperature. After that, the cells were resuspended and transferred to a culture medium. The culture was re-cultivated every 2–3 days.

MTT assay. The assessment of cell viability under the conditions of substances treatment was carried out by the MTT technique. For this, the cells were seeded in duplexes on a 96-well plate with a density of $8x10^3$ cells/well and left overnight for attachment. Later, the culture medium was replaced by a serum-free medium with the dissolved investigated drug and incubated for 12, 24, 48 hours. We used such concentrations of drugs: ORN-D-M, Na-ORN, Na-ORN-D-M — 0.6–10 mg/mL; ORN and D-M were used at the corresponding concentrations to its relative content (2.5 ORN : 1 D-M) in the ORN-D-M (Table 1).

Table 1. **Relative content of ORN and D-M in the ORN-D-M used in the experiments**

ORN-D-M, mg/mL	0.6	1.2	2.5	5	10
ORN, mg/mL	0.4	0.9	1.8	3.6	7.2
D-M, mg/mL	0.2	0.3	0.7	1.4	2.8

The influence of the medium pH on the viability of cells was determined by the incubation of cells for 48 hours in a medium with the appropriate pH achieved by titration with HCl. After the treatment, MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.75 mg/mL) (Sigma-Aldrich, USA) was added to each well for the last 4 hours. During this time, the reagent was regenerated by oxidoreductases present in living cells. Crystals of the reduction product — formazan — were dissolved in DMSO. The concentration of formazan in the wells was determined by a microphotometer ELx800 Absorbance Microplate Reader (BioTek Instruments Inc., USA) on 570 nm wavelength [16]. The number of viable cells (in percent) was determined by the ratio of the absorbance value between treated and control untreated cells (O.D. of treated group * 100 % / O.D. of untreated group). A comparison of the drug effect on the cell viability was carried out by the concentration of half-maximal inhibition, which causes inhibition of the viability of 50 % of cells. IC_{50} values were obtained using the method of extrapolation of linear regression data on a graph of the percentage of the viable cells versus the dose (the concentration is given in decimal logarithm) using the GraphPad Prism 8.0.1 program [17].

Differential trypan blue staining. The number of live and dead cells was assessed using a Horyaev chamber by staining trypsinized experimental cells with 0.4 % trypan blue. This dye is able to penetrate dead cells through a damaged cell membrane [18].

Statistical analysis. The results were analyzed and illustrated using GraphPad Prism 8.0.1 software (GraphPad Software, USA). Values are expressed as the mean (M) of at least three independent experiments ± standard deviation (SD). Statistical significance was established when comparing the control and experimental groups using Student's t-test.

Results and Discussion

During the ORN-D-M treatment of malignant cells B16 and U251, a dose-dependent inhibition of viability was established using the MTT assay, as well as a dependence on the time of treatment (Fig. 1, Table 2). The inhibition was considered reliable if the percentage of viable cells was less than 70 % [19]. The most obvious effect was found after long-term incubation: 48-hour treatment with the drug at a concentration of 2.5 mg/mL caused a decrease in viability by on average 25 % compared to the same dose during the 12-hour treatment. U251 was more sensitive to ORN-D-M treatment: the IC_{50} value at 48-hour treatment was 2.5 times lower than for B16. Treatment of the non-malignant MDCK showed the decreased cell viability at the highest concentration of 10 mg/mL. The use of lower concentrations of ORN-D-M and increasing the treatment time did not reveal a significant effect. Additionally, non-malignant cells were the least sensitive to the activity of the drug, as the IC_{50} index of ORN-D-M was 2.5–6 times higher compared to the malignant cell lines (Table 2). As the 48-hour treatment caused the most pronounced effect, this incubation time was chosen for further experiments.

Because the oligoribonucleotides presented in the drug have acidic properties, the dissolving of this drug can cause a decrease in the nutrient medium pH. Therefore, we studied the effect of different forms of these drugs at concentrations from 0.6 to 10 mg/mL on the pH of RPMI and DMEM media, on which the B16, U251, and MDCK cells were respectively grown (Fig. 2). It was shown that ORN drugs at concentration >5 mg/mL caused a significant decrease in pH towards the acidic side, whereas all other drugs did not significantly affect the pH of RPMI and DMEM media, which can lead to a significant effect on the growth of these cell cultures. It is important to emphasize that after the addition of the acidic form of ORN-D-M to the investigated media (RPMI and DMEM) their pH was 7.2–7.8, which is optimal for cell growth (Fig. 2).

Hereafter, we investigated the sensitivity of these cell cultures to the fluctuations in the pH of the nutrient medium with the addition of the studied drugs and their components, namely ORN, ORN-D-M, their sodium salts and D-M. Firstly, we analyzed a decrease in the pH of the nutrient media after the addition of the aforementioned drugs at concentrations 0.6–10 mg/mL

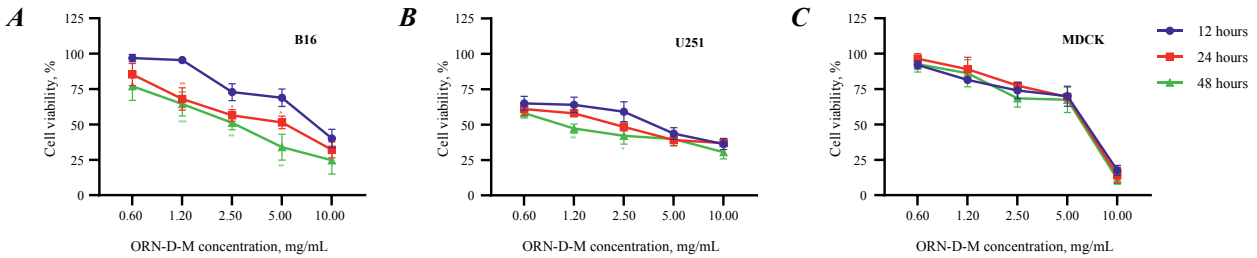

Fig. 1. Dependence of cell viability of mouse melanoma (B16), human glioblastoma (U251), and non-malignant canine kidney (MDCK) lines on the dose and time of ORN-D-M treatment. MTT assay results are presented as M±SD (n = 6). * — $p \leq 0.05$; ** — $p \leq 0.01$; *** — $p \leq 0.001$ — compared to the 12-hour treatment

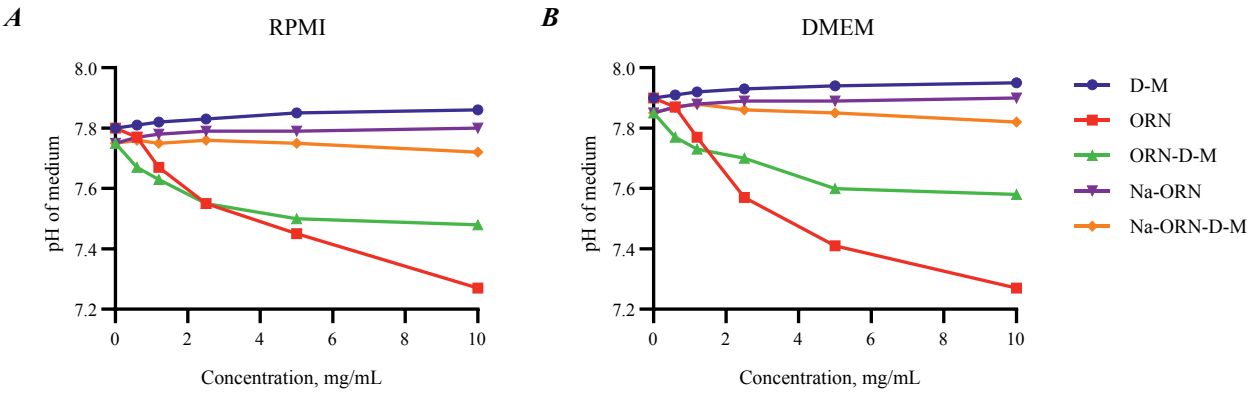

Fig. 2. The effect of different forms of ORN at concentrations 0.6-10 mg/mL on the pH of RPMI and DMEM media.

and established that after adding the drug at a concentration <5 mg/ml, the media pH decreased but it was not critical for cell growth. ORN and D-M were studied in the concentration range corresponding to their relative content in ORN-D-M — 2.5 ORN : 1 D-M. That is, ORN was investigated in the range 0.4–7.2 mg/mL, and D-M — in the range 0.2–2.8 mg/mL, since these amounts are contained in 0.6–10 mg of ORN-D-M.

To determine the effect of this slight decrease in pH on the growth of cell cultures, the medium was titrated to the appropriate pH values with HCl and the cells were incubated for 48 hours. Additionally, to study the effect of D-M, nutrient media were acidified to pH levels observed after the addition of ORN-D-M in different concentrations. It was shown that acidification of the media to pH 6.8 had almost no effect on the growth of the U251 culture, as the cell viability was >90 %. Whereas, the ORN-D-M treatment (5 mg/mL) of malignant cells leads to significantly decreased cell viability by 50 % compared to the HCl acidification group. A similar trend was found when studying the effect of ORN-D-M (5 mg/mL) on the B16 cells, although B16 cells were more sensitive to acidification of the medium, and at these conditions their viability decreased by 80 %. There was also an effect of the ORN on the viability of these cell cultures under the same conditions, but this effect was insignificant compared to the ORN-D-M. All studied drugs at concentration <5 mg/ml slightly decreased the viability of the MDCK cells with decreasing acidity of the medium caused by the addition of HCl. However, an increase of the concentration of the drugs to 10 mg/mL led to the medium acidification pH 6.1 and a catastrophic reduction in the cell viability (Fig. 3).

Therefore, it was established that the decrease in pH of the medium after adding the ORN-D-M was critical for the cells only at a concentration of 10 mg/mL (pH 6.1); at lower concentrations, acidification of the medium did not have a negative effect on the cell viability. The ORN have significantly less inhibitory potential than in the complex with D-M, while D-M does not affect cell growth.

Additionally, for the investigation of the ORN-D-M effect on cancer cells in a neutral

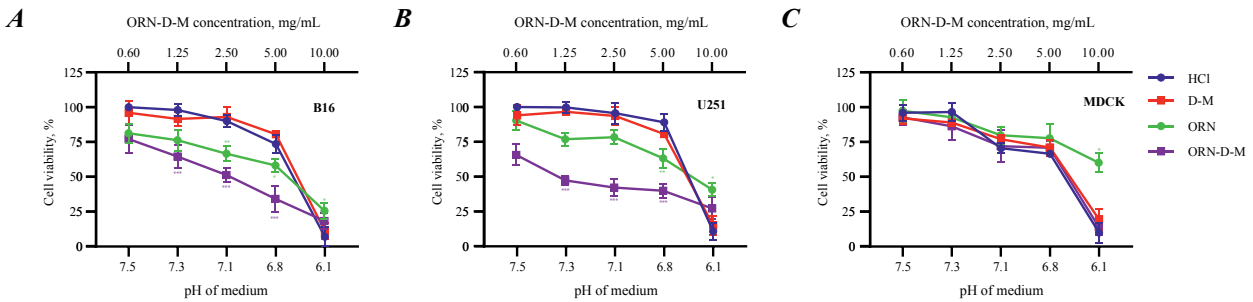

Fig. 3. Comparison of cell viability of mouse melanoma (B16), human glioblastoma (U251), and non-malignant canine kidney (MDCK) lines after 48-hour treatment with: ORN-D-M at different concentrations; ORN at the corresponding concentrations to its relative content (2.5 ORN : 1 D-M) in the amount of ORN-D-M indicated on the upper X-axis; D-M at the corresponding concentrations to its relative content (2.5 ORN : 1 D-M) in the amount of ORN-D-M indicated on the upper X-axis and with acidification of medium to the pH indicated on the lower X-axis; after acidification of the medium with HCl to the pH values observed after the addition of the appropriate amount of ORN-D-M indicated on the upper X-axis. MTT assay results are presented as M±SD (n = 6). * — $p \leq 0.05$; ** — $p \leq 0.01$; *** — $p \leq 0.001$ — compared to the HCl treatment group.

medium, the pH of the media was adjusted by NaOH after the addition of ORN-D-M, or ORN, and a 48-hour treatment was conducted. As well, the effect of D-M on cancer cells in a neutral medium was studied. In this case, we found that general trends of dose-dependent inhibition of the cancer cell viability by the ORN-D-M and ORN were maintained, and D-M in a neutral medium did not inhibit cell growth (Fig. 4).

Furthermore, we investigated the activity of the sodium salt form of the drugs — Na-ORN-D-M and Na-ORN — on the viability of the studied cell lines. This compound was used in the combined therapy of different types of cancer [20]. However, unlike acidic, salt forms

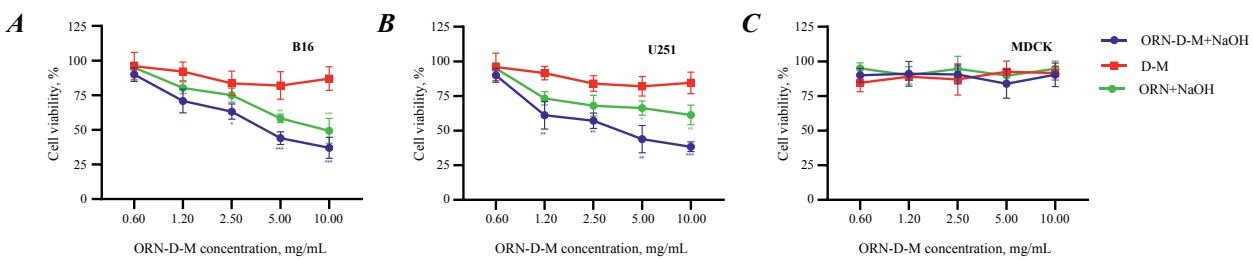

Fig. 4. Comparison of cell viability of the mouse melanoma (B16), human glioblastoma (U251), and non-malignant canine kidney (MDCK) lines in neutral pH medium adjusted by NaOH after addition of ORN-D-M at different concentrations; ORN at the corresponding concentrations to its relative content (2.5 ORN : 1 D-M) in the amount of ORN-D-M indicated on the X-axis; and D-M at the corresponding concentrations to its relative content (2.5 ORN : 1 D-M) in the amount of ORN-D-M indicated on the X-axis. MTT assay results for 48-hour treatment are presented as M±SD (n = 6). * — $p \leq 0.05$; ** — $p \leq 0.01$; *** — $p \leq 0.001$ — compared to the D-M treatment group.

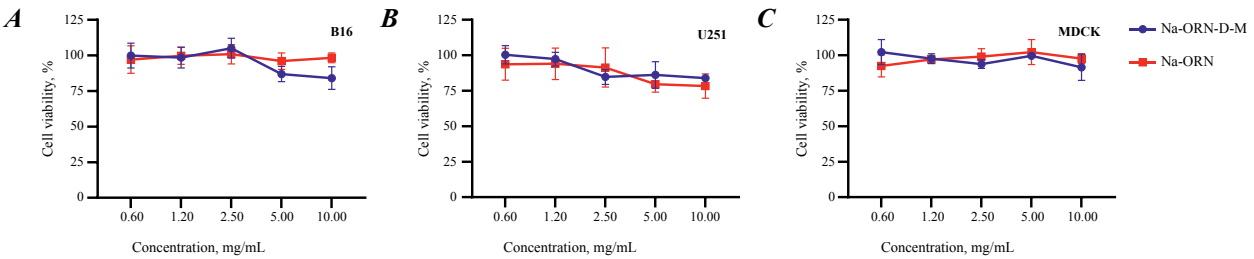

Fig. 5. Comparison of cell viability of the mouse melanoma (B16), human glioblastoma (U251), and non-malignant canine kidney (MDCK) lines on the dose of Na-ORN-D-M and Na-ORN salts after 48-hour treatment. The MTT assay results are presented as M±SD (n = 6).

of ORN-D-M did not inhibit cell growth in the studied concentration range (Fig. 5).

We showed that the inhibitory properties of ORN-D-M were preserved even at neutral pH. However, the sodium salt of the compound did not cause inhibition.

Table 2. **IC_{50} values for the studied drugs under the treatment of different cell lines**

Substance	Cell line	Mean IC_{50} ± SD, mg/mL
ORN-D-M	B16	2.5±0.4
	U251	1.0±0.3
	MDCK	6.1±1.1
ORN	B16	7.2±0.5
	U251	9.4±0.4
	MDCK	>10
D-M	B16	>5
	U251	>5
	MDCK	>5
Na-ORN-D-M	B16	>10
	U251	>10
	MDCK	>10
Na-ORN	B16	>10
	U251	>10
	MDCK	>10

Mouse melanoma — B16, human glioblastoma — U251, canine kidney — MDCK

It is known that the MTT assay reflects the activity of cellular oxidoreductases, which, in addition to cell damage and death, can be affected by oxidative stress, cellular metabolic and energy disturbances [21]. However, some substances are capable of directly reacting with the MTT reagent in a cell-free medium [22]. Therefore, we performed a differential count of live and dead cells using trypan blue staining after treatment with ORN-D-M in concentrations: 2.5–5 mg/mL for B16 and 1–2 mg/mL for U251. Using this method, the dose-dependent and time-dependent statistically significant decrease in the number of living cells during ORN-D-M treatment was confirmed, as well as an increase in the percentage of dead cells: for the B16 line during 48-hour treatment under the condition of using the highest concentration of the drug, and for the U251 line — in all groups (Fig. 6). The IC_{50} value after 48-hour treatment was in the range of 2.5–5 mg/mL for B16 and 1–2 mg/mL for U251, which correlates with the data obtained using the MTT assay. Thus, it was established that the nature of this drug activity is manifested in both reducing the proliferation of cells and promoting their death.

Based on the obtained results, it was proved that ORN-D-M shows a dose-dependent and

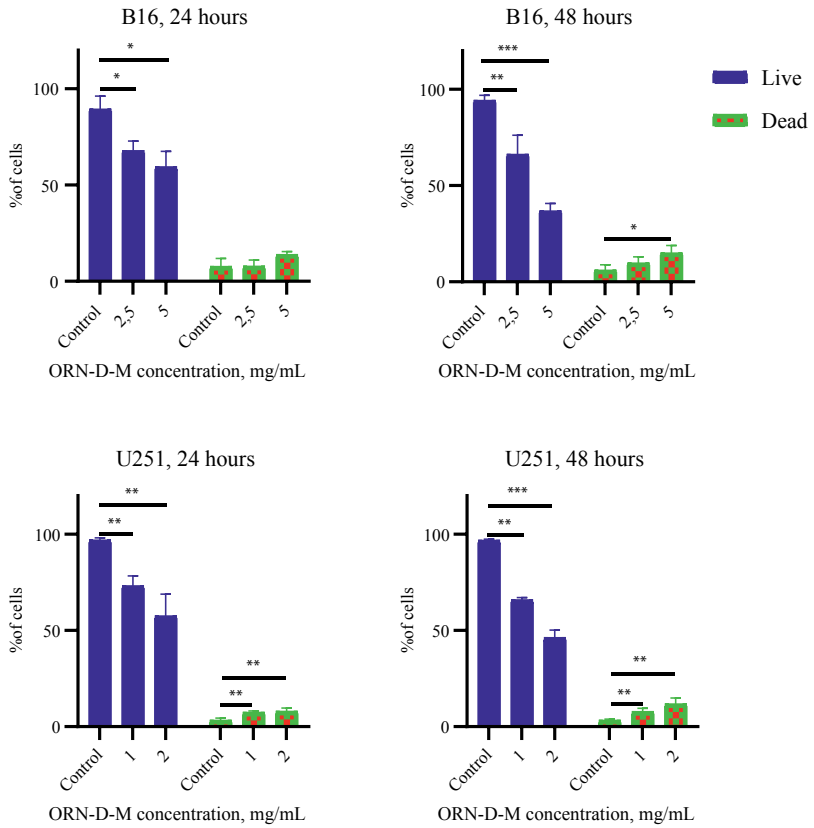

Fig. 6. The percentage ratio of live and dead cells number of mouse melanoma (B16) and human glioblastoma (U251) under conditions of 24- and 48-hour ORN-D-M treatment (total number of control intact cells was taken as 100 %). The results are presented as M±SD (n = 6). Statistical significance compared to the control untreated group: * — $p \leq 0.05$; ** — $p \leq 0.01$; *** — $p \leq 0.001$

time-dependent inhibition of the viability of tumor cells of different origins –mouse melanoma B16 and human glioblastoma U251 (Table 2). At the same time, ORN-D-M had a lower level of viability inhibition of the non-malignant canine kidney MDCK line — the IC_{50} was 2.5–6 times higher compared to the values for the malignant cells. It was established that RNA components of this drug have lower inhibitory properties. This indicates that the binding between D-mannitol and oligoribonucleotides (5 mg/mL) leads not only to a significant decrease in the acidity of the environment, but also to an increase in the inhibitory activity of this acidic form of RNA against the studied tumor cells. At the same time, the salt forms of complexes of the RNA with D-mannitol do not have such activity, even at very high concentration of 10 mg/mL (Table 2.). Previously we have shown that the ORN-D-M at concentrations up to 5 mg/mL does not have a cytotoxic effect on MDCK and RK-13 cell cultures, thus the ORN-D-M inhibition of the growth of tumor cell lines B16 and U251 at concentrations up to 5 mg/mL may indicate a specific antitumor effect of this drug [23, 24]. According to the literature, natural nucleic acids do not exhibit cytotoxic activity against tumor cells *in vitro*, as it was shown in the study of the effect of a salt form of DNA from

salmon milt on Ehrlich ascites carcinoma cells, although an antitumor effect of this drug was observed in the same animal model [25]. However, some salt forms of synthetic nucleic acids have direct inhibitory activity. For instance, the treatment of different glioma cell lines by a synthetic analog of double-stranded viral RNA — polyinosinic-polycytidylic acid (Poly (I:C)) showed the viability inhibition only in the fourth-grade astrocytoma line [26]. Synthetic analogue of a salt form of CpG ODN inhibits the viability of breast cancer cell lines — MB231 and SKBR3 with an IC_{50} of about 1.8 mg/mL [27]. It can be assumed that acidic forms of these drugs could have more effective anti-cancer activity, especially in combination with D-mannitol, which we plan to study in the future.

The antitumor activity of nucleic acids is associated with their influence on the immune defense, namely, their ability to act as the agonists for Toll-like receptors, which are mainly expressed in the cells of the innate immune system. The activation of these receptors leads to the initiation of T-cell immunity, contributes to the maturation of dendritic cells, and induces the secretion of cytokines, followed by the formation of tumor-specific T-helpers and cytotoxic T-lymphocytes [28]. Therefore, the study of the mechanism of the inhibitory action of acidic forms of nucleic acids, their complexes with D-mannitol and especially ORN-D-M, on malignant cells is an important issue for further research.

Conclusion

Our studies established that the acidic forms of natural oligoribonucleotides in complex with D-mannitol, unlike their salt forms, show an inhibitory effect against some tumor cell lines, namely the mouse melanoma B16, and human glioblastoma U251. At the same time, non-malignant MDCK cells are less sensitive to the inhibitory action of the drug.

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REFERENCES

1. *Guallar-Garrido S, Julián E.* Bacillus Calmette-Guérin (BCG) Therapy for Bladder Cancer: An Update. *Immunotargets Ther.* 2020; **9**:1–11.
2. *Babjuk M, Böhle A, Burger M, et al., and Zigeuner R.* EAU Guidelines on Non-Muscle-invasive Urothelial Carcinoma of the Bladder: Update 2016. *Eur Urol.* 2017; **71**(3):447–61.
3. *Kremenovic M, Schenk M, Lee DJ.* Clinical and molecular insights into BCG immunotherapy for melanoma. *J Intern Med.* 2020; **288**(6):625–40.
4. *Tokunaga T, Yamamoto H, Shimada S, et al., and Sudo T.* Antitumor activity of deoxyribonucleic acid fraction from *Mycobacterium bovis* BCG. I. Isolation, physicochemical characterization, and antitumor activity. *J Natl Cancer Inst.* 1984; **72**(4): 955–62.
5. *Yamamoto S, Yamamoto T, Kataoka T, et al., and Tokunaga T.* Unique palindromic sequences in synthetic oligonucleotides are required to induce IFN [correction of INF] and augment IFN-mediated [correction of INF] natural killer activity. *J Immunol.* 1992; **148**(12):4072–6.
6. *Wang H, Rayburn E, Zhang R.* Synthetic oligodeoxynucleotides containing deoxycytidyl-deoxyguanosine dinucleotides (CpG ODNs) and modified analogs as novel anticancer therapeutics. *Curr Pharm Des.* 2005; **11**(22):2889–907.
7. *Komura F, Okuzumi K, Takahashi Y, et al., and Nishikawa M.* Development of RNA/DNA Hydrogel

Targeting Toll-Like Receptor 7/8 for Sustained RNA Release and Potent Immune Activation. *Molecules.* 2020; **25**(3):728.
8. *Pavlova IN, Konopatskova OM, Ekaterynushkin DA.* Immunocorrection in patients with melanoma. *Rus Biotherap J.* 2016; **15**(1):82–3.
9. *Tkachuk Z.* Multiantivirus Compound, Composition and Method for Treatment of Virus Diseases. U.S. Patent 20,120,232,129, 16 April 2013.
10. *Melnichuk N, Kashuba V, Rybalko S, Tkachuk Z.* Complexes of Oligoribonucleotides with d-Mannitol Modulate the Innate Immune Response to Influenza A Virus H1N1 (A/FM/1/47) In Vivo. *Pharmaceuticals (Basel).* 2018; **11**(3):73.
11. *Marchyshak T, Yakovenko T, Shmarakov I, Tkachuk Z.* The Potential Protective Effect of Oligoribonucleotides-d-Mannitol Complexes against Thioacetamide-Induced Hepatotoxicity in Mice. *Pharmaceuticals (Basel).* 2018; **11**(3):77.
12. *Landskron G, De la Fuente M, Thuwajit P, et al., and Hermoso MA. Chronic inflammation and cytokines in the tumor microenvironment. J Immunol Res.* 2014; **2014:149185.**
13. *Rayburn ER, Ezell SJ, Zhang R.* Anti-Inflammatory Agents for Cancer Therapy. *Mol Cell Pharmacol.* 2009; **1**(1):29–43.
14. *Jana NR.* NSAIDs and apoptosis. *Cell Mol Life Sci.* 2008; **65**(9):1295–301.
15. *Tkachuk ZY, Levchenko SM.* Mass spectrometric analysis of oligoribonucleotides of total yeast RNA. *Dopov Nac akad nauk Ukr.* 2021; **2**:115–22.
16. *Mosmann T.* Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983; **65**(1–2):55–63.
17. *Motulsky HJ.* Equation: log(inhibitor) vs. normalized response. *GraphPad Curve Fitting Guide.* Accessed 11 March 2023. Retrieved from https://www.graphpad.com/guides/prism/8/curve-fitting/reg_dr_inhibit_normalized.htm
18. *Riss TL, Moravec RA, Niles AL, et al., and Minor L. Cell Viability Assays. 2013 May 1 [updated 2016 Jul 1]. In: Markossian S, Grossman A, Brimacombe K, et al., and Xu X, editors.* Assay Guidance Manual [Internet]. Bethesda (MD): Eli Lilly & Company and the National Center for Advancing Translational Sciences; 2004-.
19. *Zhernov YV.* Analysis of cytotoxicity of humic substances of peloids. *Bull Samara Sci Cen RAS.* 2011; **13**(1–8):1996–8.
20. *Stepanenko AA, Dmitrenko VV.* Pitfalls of the MTT assay: Direct and off-target effects of inhibitors can result in over/underestimation of cell viability. *Gene.* 2015; **574**(2):193–203.
21. *Shoemaker M, Cohen I, Campbell M.* Reduction of MTT by aqueous herbal extracts in the absence of cells. *J Ethnopharmacol.* 2004; **93**(2–3):381–4.
22. *Shalimov SA, Keĭsevich LV, Volchenskova II, et al., and Evtushenko GV.* Use of polyplatillen in the treatment of stage IV malignant neoplasms. *Klin Khir (1962).* 1992; **1**:40–1.
23. *Tkachuk ZYu, Rybalko SL, Zharkova LD, et al., and Semernikova LI.* Anti-influenza effect of Nuclex. *Rep NASU.* 2010; **9**:191–6.
24. *Tkachuk ZYu, Rybalko SL, Dyadyun DB, Starosila DB.* Antiherpetic activity of Nuclex. *Rep NASU.* 2011; **4**:162–8.
25. *Fedyanina LN, Besednova NN, Aminin DL, et al., and Kalenyk TK.* Experimental study of the antitumor activity of DNA from salmon milt and some of its mechanisms. *Far Eastern Med J.* 2006; **3**:59–62.
26. *Dick RS, Hubbell HR.* Sensitivities of human glioma cell lines to interferons and double-stranded RNAs individually and in synergistic combinations. *J Neurooncol.* 1987; **5**(4):331–8.
27. *Taghavi Pourianazar N, Gunduz U.* CpG oligodeoxynucleotide-loaded PAMAM dendrimer-coated magnetic nanoparticles promote apoptosis in breast cancer cells. *Biomed Pharmacother.* 2016; *78*:81–91.
28. *Xun Y, Yang H, Kaminska B, You H.* Toll-like receptors and toll-like receptor-targeted immunotherapy against glioma. *J Hematol Oncol.* 2021; **14**(1):176.

Вплив природних олігорибонуклеотидів у комплексі з D-манітолом на життєздатність культур клітин різного походження

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Мета. Вивчити вплив кислих і солевих форм природних олігорибонуклеотидів дріжджової РНК, та їх комп-

лексів з D-манітолом (OPH-D-M) на життєздатність культур клітин різного походження. **Методи.** MTT-аналіз, диференційне фарбування трипановим синім. **Результати.** Встановлено дозозалежне інгібування життєздатності клітин меланоми миші B16 та гліобластоми людини U251 під впливом кислої форми OPH-D-M, а також залежність від часу обробки. Клітинна лінія U251 виявилася більш чутливою до впливу цього препарату: при 48-годинній обробці показник IC_{50} для цієї лінії становив 1,0±0,3 мг/мл, тоді як для B16 — 2,5±0,4 мг/мл. Разом з цим, інгібування незлоякісних клітин нирки собаки MDCK спостерігалося лише при застосуванні високих концентрацій препарату (IC_{50} – 6,1±1,1 мг/мл). Препарат OPH найяскравіше реалізував свою інгібуючу активність на життєздатність культур клітин B16 і U251 лише у комплексі з D-манітолом і в кислій формі. Тоді як його складова OPH в кислій формі, сольова форма Na-OPH і сольова форма в комплексі з D-манітолом Na- ORN-D-M, показували значно нижчу інгібуючу активність. **Висновки.** Кисла форма дріжджових олігорибонуклеотидів у комплексі з D-манітолом має суттєву інгібуючу активністю щодо життєздатності злоякісних клітин мишачої меланоми B16 та гліобластоми людини U251, що робить цей препарат перспективним для подальших досліджень його протипухлинної дії *in vitro* і *in vivo*.

Ключові слова: природні олігорибонуклеотиди, OPH-D-M, протипухлинна активність, MTT-тест.

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