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Study of cytotoxicity of complex of Poly-γ-glutamic acid from *Bacillus licheniformis* and Doxorubicin in tumor cell lines

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Abstract: The development of carrier-antitumor complexes is aimed at reducing toxicity to normal cells and increasing antitumor activity. The aim of this paper was to study the antioxidant activity and cytotoxicity of the complex of poly-γ-glutamic acid with Doxorubicin in different ratios on different cultures of tumor cells in comparison with the toxicity of Doxorubicin alone.

Poly-γ-glutamic acid (PGA) is anionic biopolymer produced by *Bacillus licheniformis* M 20G. Cell lines were A549, LL, P3HR1, MDBK. Antioxidant activity was determined with DPPH-test and cytotoxicity with MTT-test.

PGA-Dox complexes showed antioxidant activity up to 25%. The complex showed at least two times higher toxicity compared with Doxorubicin against A549 cells after 48 h: the CC_{50} was 1.5 μg/ml for Doxorubicin and 0.31 - 0.72 μg/ml for PGA-Dox. In P3HR1 cells the CC_{50} for Doxorubicin was 0.68 μg/ml, and in the PGA-Dox complex 0.31 - 0.68 μg/ml. In Lewis carcinoma cells (LL), the complexes were more effective after 24 h (the CC_{50} were 0.92 - 1.06 μg/ml for PGA-Dox, 1.14 μg/ml for Dox). For non-tumor cells of MDBK Doxorubicin was as toxic as for tumor cells (CC_{50} was 1.24 μg/ml), and the toxicity of the PGA-Dox complexes were significantly lower (3.38-68.40 μg/ml).

The results presented in this work demonstrated that the PGA-Dox complexes exhibited greater toxicity to tumor cells than Doxorubicin and were less toxic to cells of non-tumor origin.

Keywords: PGA-Dox complexes; tumor cells; cytotoxicity; antioxidant activity.

Introduction

The main principle of chemotherapy of cancers is to suppress the mitotic and metabolic processes of tumor cells. However, some healthy tissues are also sensitive to the toxic effects of chemotherapeutic drugs, which leads to the development of side effects. One of the problems in the treatment of tumors is the targeted delivery of cytotoxic antitumor drugs. The development of carrier-antitumor complexes is aimed at reducing toxicity to normal cells and increasing antitumor activity. Promising is the use of poly-γ-glutamine acid to create systems of target antitumor agents. The formation of conjugates occurs due to free carboxyl groups in the side branches of the polymer, which act as points of conjugation with drugs, thus making the latter more soluble and easier to supply to target tissues. The formed complexes are quickly transported to the sites of tumor localization and release the active substance. The decomposition product of the polymer is glutamic acid, which enters the normal metabolism of cells and does not have a toxic effect on the macroorganism. Bacteria of the genus Bacillus are known producers of poly-γ-glutamic acid. Due to these properties, non-pathogenicity for animals and manufacturability in cultivation, producer strains can be used in the creation of targeted drug delivery systems.

The **aim** of this paper was to study the antioxidant activity and cytotoxicity of the complex of doxorubicin - poly-γ-glutamic acid (PGA-Dox) in different ratios on different cultures of tumor cells in comparison with the toxicity of doxorubicin alone.

Complexes of poly-γ-glutamic acid with doxorubicin (PGA-Dox):

	Concentrations, mg/ml	
Complex	**Dox**	**PGA**
PGA-Dox_1	1	2
PGA-Dox_2	0.666	2
PGA-Dox_3	0.4	2

Results and discussion

Insoluble precipitate was observed in PGA-Dox complexes №1 and №2, which was not taken for the study. Accordingly, the concentration of DOX in these drugs was recalculated according to the calibration curve of DOX and it was found that in PGA-Dox_1 its concentration was 0.404 mg/ml, in PGA-Dox_2 - 0.393 mg/ml. The drugs were investigated in the range of ten-fold concentrations. The cytotoxicity of the drugs was studied in human A549 tumor cell lines (lung adenocarcinoma), Lewis lung carcinoma cells (LL) and P3HR1 (human B-lympoma), as well as in non-tumor cell line MDBK (bull kidney cells). Cytotoxicity was measured by MTT. Toxicity was analyzed after 24 h and 48 h.

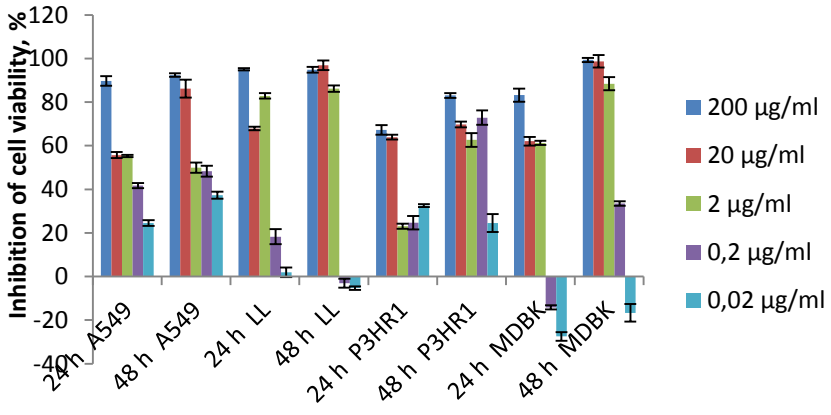


Fig. 1. Cytotoxicity of Doxorubicin

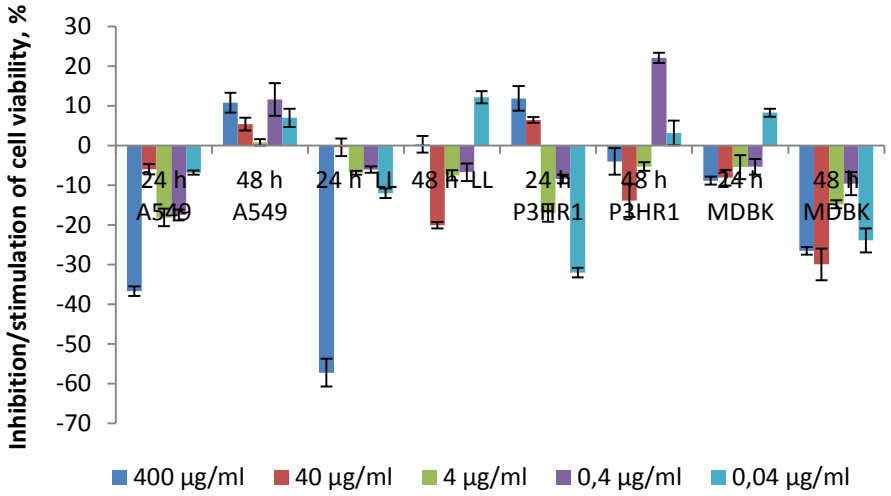


Fig. 2. Cytotoxicity of PGA

Doxorubicin was toxic to all cells studied at almost the same level (Fig. 1)

PGA did not show significant toxicity, and in some concentrations increased the proliferative activity of cells (Fig. 2)

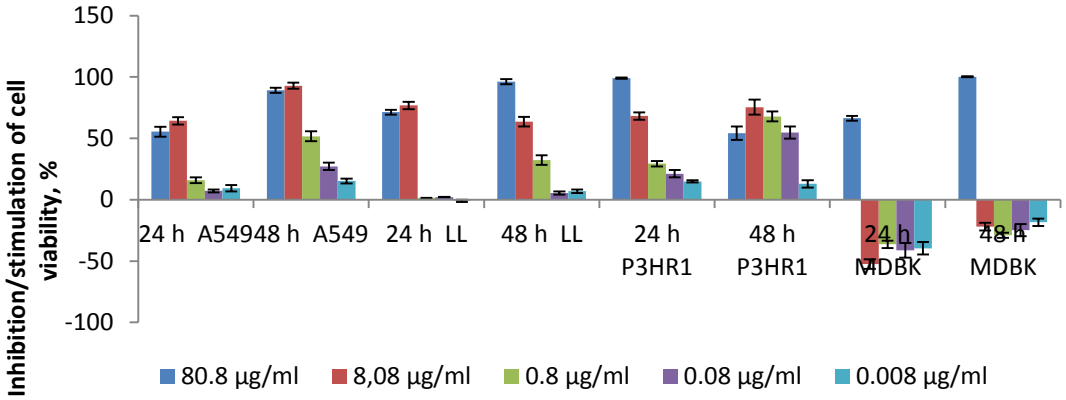


Fig. 3. Cytotoxicity of the PGA-Dox_1

PGA-Dox_1 showed toxicity against tumor cells after 24 and 48 h (Fig.3)

PGA-Dox_2 showed greater toxicity against A549 and P3HR1 cells after 48 h, the level of cell inhibition was higher than after 24 h (Fig 4).

Both complexes showed lower cytotoxicity in MDBK non-tumor cells.

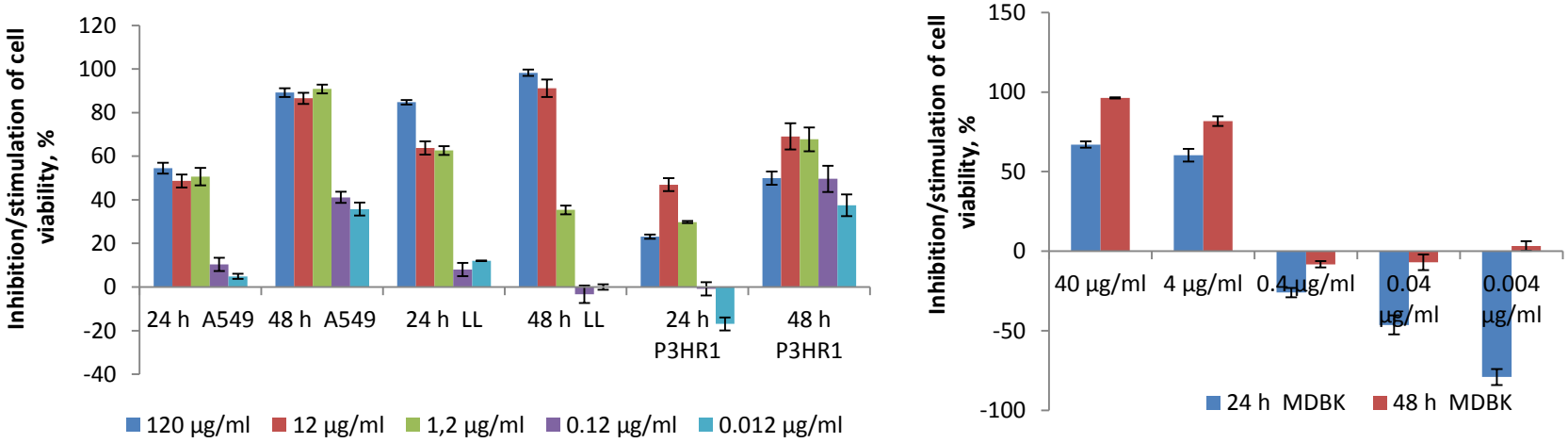
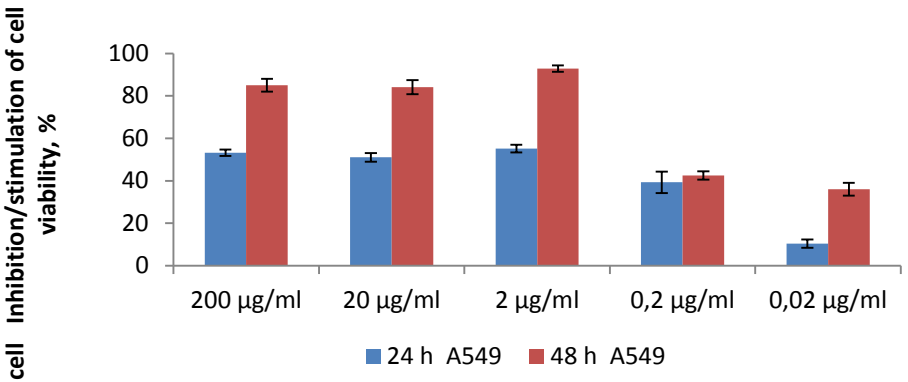
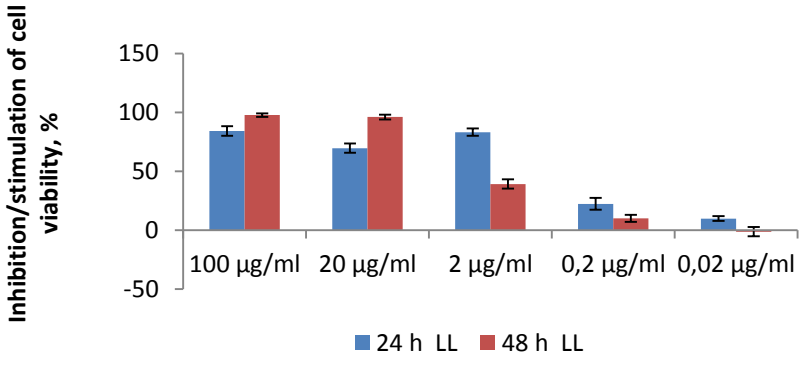
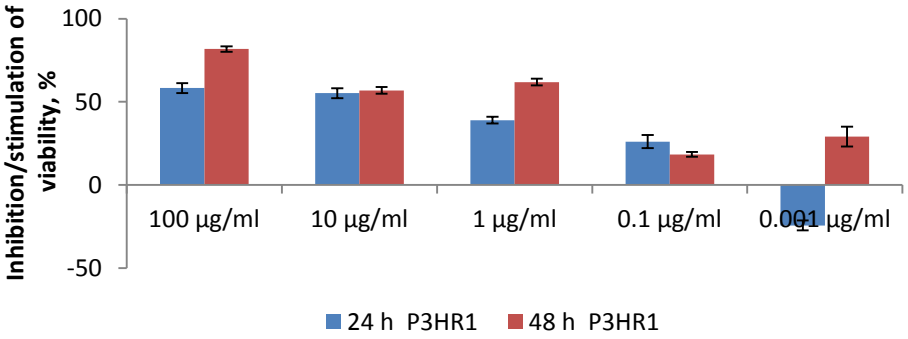


Fig. 4. Cytotoxicity of the PGA-Dox_2







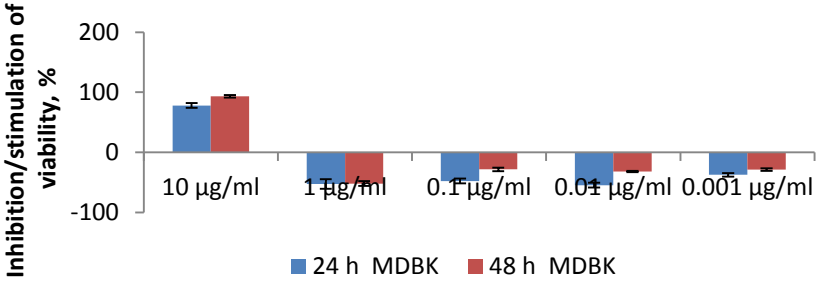


Fig. 5. Cytotoxicity of the PGA-Dox_3

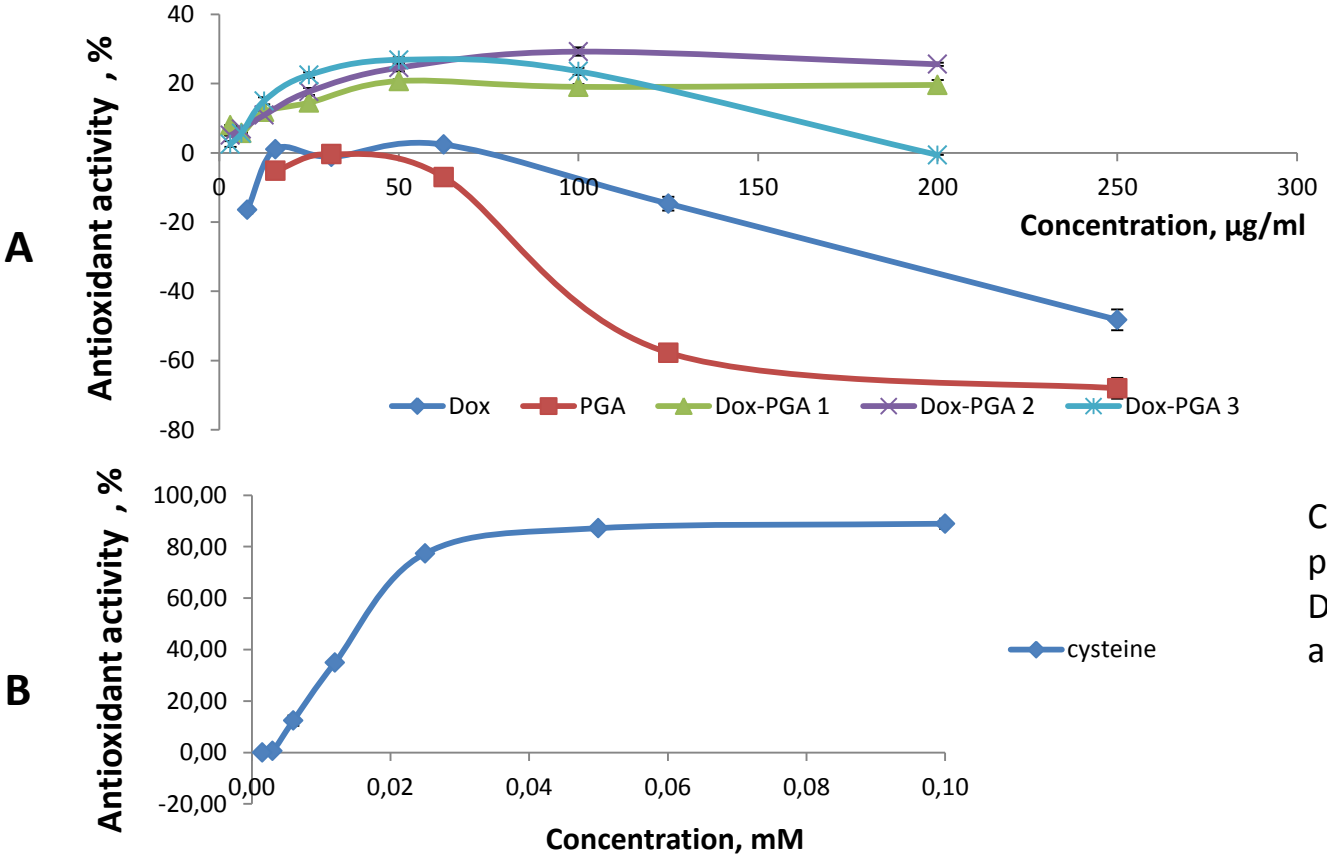
PGA-Dox_3 showed higher toxicity in epithelial tumor cells than in lymphoblastoid cells (Fig.5) The complex showed greater toxicity after 48 h in A549 and P3HR1 cells that means its prolonged action. PGA-Dox_3 was less cytotoxic in MDBK cells.

CC_{50} of studied drugs

Index	Cells	Dox		PGA		PGA-Dox_1		PGA-Dox_2		PGA-Dox_3	
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
CC_{50}, µg/ml	A549	1.33	1.5	-	-	1.33	0.72	1.18	0.31	1.33	0.48
	LL	1.14	1.24	-	-	5.34	5.26	0.92	5.6	1.06	8.5
	P3HR1	12.36	0.68	-	-	4.8	0.35	9.9	0.37	7.15	0.68
	MDBK	1.73	1.024	-	-	68.4	50.35	3.36	2.54	7.9	6.8

DOX showed toxicity in all cell lines in the range 0.68 – 1.73 µg/ml. The PGA-Dox complexes showed greater toxicity and more efficient delivery and prolonged action in the culture of lung adenocarcinoma cells A549 and B-cell lymphoma P3HR1 and after 48 h the toxicity was greater than after 24 h. In human Lewis carcinoma cell culture, the toxicity of the complexes did not significantly exceed the toxicity of DOX. Reduction of PGA-Dox toxicity after 48 h was observed, which may indicate its "detoxification" in this culture.

The complexes were analyzed for antioxidant properties. Antioxidant activity was determined by DPPH-test. It was shown that all complexes posses up to 25% of antioxidant activity. DOX and PGA did not have this activity.



Cysteine served a positive control in DPPH-test of antioxidant activity.

Fig. 6. Antioxidant activity of studied complexes (A), control – cysteine (B)

Conclusions

The analysis of cytotoxicity of the studied complexes against tumor and non-tumor cells showed that PGA bonded to DOX molecules promoted the release of the latter in cells and prolonged action. The lower number of DOX molecules per PGA-Dox complex was more effective against tumor cells and less toxic in non-tumor cells. Obtained data indicate that the most promising is the PGA-Dox_2 complex that was less toxic to non-tumor cells while the same toxic as DOX. This complex was even more toxic in cell cultures of A549 and B-cell lymphoma P3HR1. The marked effects can be linked to the antioxidant activity that all complexes posses, however an addition study needs to prove this.