

Assessment of the Therapeutic Value of the Innovative Multicomponent Antitumor Drugs

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Abstract. The most modern and advanced method of treating cancerous tumors is undoubtedly the charged particle therapy which should be mostly concentrated in the tumor area due to the maximum dissipation of the energy of charged particles in the region of the Bragg peak. The real spread of hadron therapy is much slower than expected, most likely due to its extremely high cost, research intensity and requirements to medical and engineering staff. All this results in unacceptably long delays in registration and implementation of new innovative drugs, and in turn, results in the suffering and death of ten of thousands of patients. In addition, the financial risks and difficulty of obtaining funding for the most pioneering and innovative research increases dramatically. Therefore, a significant increase in the biological effectiveness and safety of proton therapy is an acute and urgent need of the current day to provide the rapid and sustainable development of proton and heavy ion therapy. The reported study aims to investigate the possibility of simplified and rapid in vitro forecasting of the potency of new anticancer drugs. © 2025 Bull. Georg. Natl. Acad. Sci.

Keywords: non-small cell lung cancer, anticancer treatment, therapeutic value, innovation

Introduction

The constant uncontrolled growth and aging of the population, as well as environmental degradation, cause a further increase in the number of cancers and deaths (Chhikara, & Parang, 2023; Siegel et al., 2023). This trend is particularly evident in underdeveloped and developing countries and in statistical analysis of child and adolescent morbidity and mortality from certain forms of

cancer. Alarming expectations are also associated with the experience of nuclear weapons testing and incidents at nuclear power plants. All these expectations are exacerbated by the active intensification of planning, construction and exploitation of new nuclear power plants and power units in at least 15 world countries (Christodouleas et al., 2011; Kotenko et al., 2012). According to the International Energy Agency (IEA) between 2023 and 2050, the total installed capacity of nuclear power

plants should grow by almost 50% (World Nuclear Organization). The growth of nuclear energy will mainly be ensured by the rapid development of this industry in Asia (India, China, Korea, Bangladesh and others).

The most modern and advanced method of treating cancerous tumors is undoubtedly the charged particle therapy, which uses the destructive effect of particle of different masses and energies (mainly, electrons, α -particles, proton and ions) on the DNA of tissue cells, and this effect should be mostly concentrated in the tumor area due to the maximum dissipation of the energy of charged particles in the region of the Bragg peak (Lane et al., 2023). Numerous research data show that proton therapy is especially preferable in the treatment of cancers in children and adolescents, as well as tumors in the eye, neck and head areas (Mizumoto et al., 2021; Chang, (n.d.)).

Many researchers forecasted the number of patients treated by proton therapy to reach approximately 400 thousand before 2023 and 500-550 thousand before 2026 (Particle Therapy Co-Operation Group). Actually, the number of patients treated by proton therapy at the end of year 2022 occurred to be about 320 thousand. However, the real spread of hadron therapy was much slower, most likely due to its high cost, very high research intensity and very high requirements for medical and engineering staff. Therefore, a significant increase in the biological effectiveness and safety of proton therapy is an acute and urgent need of the current day. This assumption is also consistent with data on the number of proton therapy facilities put in operation during the period from 1969 to 2023 (Particle Therapy Co-Operative Group).

Development, testing (on cell cultures and laboratory animals) and clinical trials of innovative multicomponent antitumor combinations, which can be successfully used in conjunction with proton therapy to dramatically increase the effectiveness and safety of combination therapy, require a lot of time and significant financial costs, being high-tech

and extremely knowledge-intensive process. All these results in unacceptably long delays in registration and application of new drugs, and in turn leads to the suffering and death of a significant number of patients. In addition, the financial risks and difficulty of obtaining funding for the most pioneering and innovative research increases dramatically. Moreover, the situation is complicated by new regulations and the increasingly stringent restrictions of laboratory animal testing.

The reported study aimed to investigate the possibility of simplified and rapid in vitro forecasting of the potency of new anticancer drugs. According to the data of the National Cancer Institute about 700 anticancer drugs and drug combinations are approved by FDA. Among over 85 anticancer approved by FDA for treatment of the Non-Small Cell Lung Cancer, only two are drug combinations (namely, carboplatin-paclitaxel (Taxol) and gemcitabine-cisplatin).

Despite the widely known fact that innovative combinations are mostly more effective than the widely used mono-component drugs, it is quite obvious that it is completely impossible to test all promising combinations, and their safety on laboratory animals, not to mention clinical trials. Therefore, we decided to limit our current research on testing the two combinations listed in (National Cancer Institute). Taking into our previous results (Chirakadze et al., 2023; Mitagvaria et al., 2023; Chirakadze et al., 2018) the first step of the ongoing research is the in vitro study of the efficacy and safety of combinations based on mixtures of rubidium chloride, gemcitabine-cisplatin, carboplatin-paclitaxel, copper oxide nanoparticles water dispersion and standard saline solution, designed to be applied in the frame of the so called “strongly localized multicomponent combined cancer therapy”.

Materials and Methods

Chemicals, reagents and solvents were obtained from Merck (India), Hi Media (India), Invitrogen (India), SRL (India) and Sigma-Aldrich (USA).

Anticancer drugs gemcitabine, cisplatin, carboplatin and paclitaxel were purchased from PSP and Pharmaco (Georgia).

Cell culture and maintenance: Human lung cancer cell line A549 and human Primary Normal Human Dermal Fibroblasts (NHDF) were obtained from ATCC, USA.

MTT cell proliferation and Annexin V-FITC apoptosis assays. Cells were planted in 96-well microtiter culture plates, at a density of 0.5×10^5 cells per well. The medium was taken out and replaced with fresh one containing various concentrations of RbCl, ranging from 0 μ M - 500 μ M after an overnight incubation. The cells were then incubated for 48 h. Cisplatin and gemcitabine have been used as reference drugs. After the incubation period MTT (100 μ l; 0.5 mg/ml) was added to each well and incubated in a humidified incubator containing 5% CO₂ at 37°C for 4 hrs. After discarding the supernatant the

purple-colored formazan crystals formed in the wells were dissolved in 100 μ L DMSO per well and the absorbance was measured at 490 nm using microplate reader.

Each treatment group's percentage of cell viability in comparison to the control group has been calculated. Induction of apoptosis was quantified via flow cytometric analysis of control and test using the Annexin V-FITC apoptosis detection kit according to the manufacturer's protocol (BD Bioscience). Briefly, post treatment cells were harvested with 1X Trypsin and washed in ice cold 1x PBS followed by re-suspending in 100 μ L of 1X binding buffer solution supplied within the kit.

Eighteen combinations (1-18) containing various amount of water, saline, and copper oxide nanoparticles, and six combinations (19-24) containing so called "blank" copper nanoparticles (without DMSO, rubidium chloride, paclitaxel-carboplatine, gemcitabine-cisplatin, gemcitabine)

Table1. Composition of the developed and tested combinations

Component Sample No	RbCl, mg	Water, Ml	Saline solution (0.9%), ml	Gemci- tabine, mg/	Gemcita- bine,mg/ cisplatin, mg	Paclitaxel/mg carrbopla-tin, mg	CuO, mg	Total volume of liquid, ml
1	300	5	2	300	-	-	-	10
2		0.91	6.09		-	-	200	
3		1.82	5.18		-	-	400	
4		2.73	4.27		-	-	600	
5		3.64	3.36		-	-	800	
6		4.55	2.45		-	-	1000	
7		5	2	-	270/30	-	-	
8		0.91	6.09	-		-	200	
9		1.82	5.18	-		-	400	
10		2.73	4.27	-		-	600	
11		3.64	3.36	-		-	800	
12		4.55	2.45	-		-	1000	
13		5	2	-	-	200/100	-	
14		0.91	6.09	-	-		200	
15		1.82	5.18	-	-		400	
16		2.73	4.27	-	-		600	
17		3.64	3.36	-	-		800	
18		4.55	2.45	-	-		1000	
19	-	7	3	-	-	-	-	
20	-			-	-	-	200	
21	-			-	-	-	400	
22	-			-	-	-	600	
23	-			-	-	-	800	
24	-			-	-	-	1000	

were prepared (Table 1) using standard equipment for mechanical and ultrasonic mixing.

The “blank” solutions were tested to evaluate the influence of copper oxide nanoparticles on the efficacy and toxicity of the developed combinations. Finally, cells were incubated with 5 μ L of annexin V-FITC and 5 μ L of PI for 15 min at room temperature in dark before acquiring data using BD FACS Verse flow cytometer (BD Biosciences, San Jose, CA). Annexin V/FITC positive cells were regarded as apoptotic cells analyzed using Cell Quest Software (BD Biosciences). Water dispersion of CuO nanoparticles (22 weight%, 20-50 nm) was procured from “Nanografi Nanotechnologies” (Turkey). Ratios R_{SV} and R_{SA} of the necrosis and apoptosis inducing capacity of the tested drugs in healthy cells versus cancer cells, showing the selectivity to cancer cells A549 in comparison to healthy NHDF cells, was calculated and considered as the measure of the efficacy and safety of each combination.

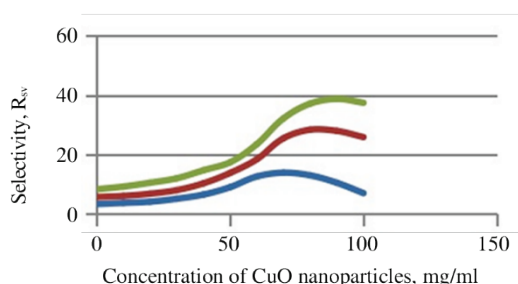


Figure. Dependence of the selectivity R_{SV} on the CuO nanoparticle content in the tested combinations based on (up to down): paclitaxel-carboplatine, gemcitabine-cisplatin, and gemcitabine.

Testing of the acute toxicity of innovative multi-component combinations. Acute toxicity is one of the main characteristics of the safety of drugs. Testing of acute toxicity was carried out on chick embryos accompanied by visible light ovoscopy.

The Acute toxicity index TI of the test samples to chick embryos was calculated according to formula (1):

$$TI (\%) = (1 - N_T / N_C \text{ after treatment}) \cdot 100 \quad (1)$$

where N_T is the number of hatched chicks in the exposed test group, N_C is the number of hatched chicks in control group and N_T/N_C is the viability of exposed embryos in comparison to unexposed embryos.

Experimental results. Data on R_{SV} , measured by MTT testing, are given in Fig. 1. They clearly show that paclitaxel-carboplatin and gemcitabine-cisplatin based combinations are up to three-times more effective than the gemcitabine based combination. Compositions 1-18 reveal the non-monotonous dependence in the content of copper oxide nanoparticles having smooth maximums at 60-90 mg/ml. Data on R_{SA} , measured by Annexin V-FITC/PI assay, are in good coincidence with results of MTT assay. Testing of the “blank” combinations containing copper oxide nanoparticles showed that their anticancer selectivity R_{SV} depends monotonically on the content of nanoparticles and their selectivity varies from 1 to 1.6, so that they can cause the observed effects only due to the synergistic super additive positive or negative interaction with the active components of combinations. Study of the acute toxicity showed that all the developed combinations have practically the same acute toxicity to embryos as the gemcitabine based combination 1. Thus, the studied combinations based on rubidium chloride, gemcitabine, carboplatin, cisplatin, paclitaxel and DMSO should be considered as highly promising for the development of new drugs for treatment of lung cancers (especially, the non-small cell lung cancer).

ადამიანისა და ცხოველთა ფიზიოლოგია

ინოვაციური მრავალკომპონენტიანი ანტისიმსივნური პრეპარატების თერაპიული ღირებულების შეფასება

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საქართველოს ტექნიკური უნივერსიტეტი, მეცნიერების და ინოვაციების დეპარტამენტი, თბილისი, საქართველო

საქართველოს ტექნიკური უნივერსიტეტი, გარემოსდაცვითი ინჟინერიის და ეკოლოგიის დეპარტამენტი, თბილისი, საქართველო

ავთვისებიანი სიმსივნეების მკურნალობის ყველაზე თანამედროვე და მოწინავე მეთოდი უდავოდ არის დამუხტული ნაწილაკებით თერაპია, რომელიც ძირითადად კონცენტრირებული უნდა იყოს სიმსივნის მიდამოში, ბრეგის პიკის (დამუხტული ნაწილაკების ენერგიის მაქსიმალური გაფანტვის) არეში. დამუხტული ნაწილაკებით თერაპიის ობიექტების რაოდენობის რეალური ზრდა და გლობალური გავრცელება ბევრად უფრო ნელა მიმდინარეობს, ვიდრე ამას დარგის წამყვანი ექსპერტები პროგნოზირებდნენ. სავარაუდოდ, ამის მთავარი მიზეზი არის ადრონული თერაპიის მაღალი ღირებულება, კვლევის ძალიან მაღალი ინტენსივობა და სამედიცინო-საინჟინრო პერსონალის მიმართ ძალიან მაღალი მოთხოვნები. ყოველივე ზემოთქმული, ახალი მედიკამენტების რეგისტრაციისა და დანერგვის დაუშვებლად ხანგრძლივ ვადებთან ერთად, განაპირობებს ათიათასობით პაციენტის ტანჯვასა და სიკვდილს. ამასთანავე, მკვეთრად იზრდება ახალი პრეპარატების შემუშავების და ტესტირების ფინანსური რისკები და კვლევის დაფინანსების მოპოვების სირთულე. ამრიგად, პროტონული თერაპიის ბიოლოგიური ეფექტურობისა და უსაფრთხოების მნიშვნელოვანი ზრდა არის დღევანდელი დღის მწვავე და გადაუდებელი საჭიროება, რათა უზრუნველყოფილ იქნეს პროტონული და მძიმე იონური თერაპიის სწრაფი და მდგრადი განვითარება. წარმოდგენილი კვლევის მიზანია ლაბორატორიულ ცხოველებზე ტესტირებამდე გამარტივებული და სწრაფი არალეტალური მეთოდებით კიბოს საწინააღმდეგო ახალი პრეპარატების თერაპიული ღირებულების შეფასება, რათა მომავალში მნიშვნელოვნად შემცირდეს ახალი ანტისიმსივნური პრეპარატების შემუშავების, ტესტირებისა და დანერგვის ხარჯები და ვადები.

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