საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 19(193), №1, 2025
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 19(193), no. 1, 2025

Human and Animal Physiology

Non-Lethal Simplified Assessment of the Therapeutic Value of the Innovative Adjunct Multicomponent Antitumor Drugs in the Framework of the Strongly Localized Combination Treatment and Proton Therapy of Cancer

Archil Chirakadze[*,,§], Nugzar Dvali[§§,#], Lali Gumberidze[##], Nino Sikharulidze[##], Nana Khuskivadze[§§], Teimuraz Chubinishvili[*], Tinatin Gelashvili[*], Giorgi Palavandishvili[θ], Khatuna Tserodze[θθ], Irina Khomeriki[Δ], Nikoloz Tavdishvili[§§], Irakli Nadiradze[ΔΔ], Davit Apkhazava[Ø], Ketevan Chigogidze[ØØ]**

[*] *Institute "Techinformi", Georgian Technical University, Tbilisi, Georgia*
[**] *Vladimer Chavchanidze Institute of Cybernetics, Georgian Technical University, Tbilisi, Georgia*
[§] *Elevter Andronikashvili Institute of Physics, Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia*
[§§] *Institute for Problems of Engineering Physics, Georgian Technical University, Tbilisi, Georgia*
[#] *Department of Science and Research, High Technology National Center of Georgia, Tbilisi, Georgia*
[##] *Department of Cerebral Circulation and Metabolism, Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia*
[θ] *Department of Sciences and Innovation, Georgian Technical University, Tbilisi, Georgia*
[θθ] *Department of Environmental Engineering and Ecology, Georgian Technical University, Tbilisi, Georgia*
[Δ] *Faculty of Informatics and Control Systems, Georgian Technical University, Tbilisi, Georgia*
[ΔΔ] *Department of Engineering Physics, Georgian Technical University, Tbilisi, Georgia*
[Ø] *International School of Medicine, ALTE Univerity, Tbilisi, Georgia*
[ØØ] *Caucasus School of Humanities and Social Sciences, Caucasus University, Tbilisi, Georgia*

(Presented by Academy Member Nodar Mitagvaria)

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, in the suffering and death 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 present 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.*

non-small cell lung cancer, anticancer treatment, strongly localized combined therapy, innovation, synergy, proliferation, apoptosis, proton therapy

The constant uncontrolled growth and aging of the population, as well as environmental degradation, cause a further increase in the number of cancer cases and deaths [1, 2]. 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 [3, 4]. According to the International Energy Agency (IEA) between 2023 and 2050, the total installed capacity of nuclear power plants should grow by almost 50% [5]. 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 and neutron therapy, which use the destructive effect of particle of different masses and energies (mainly, neutrons, electrons, α-particles. protons and heavy 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 [6]. Data from many researchers 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 [7, 8]. Many researchers forecasted the number of patients treated with proton therapy to reach approximately 400 thousand before 2023 and 500-550 thousand before 2026 [9]. Actually, the number of patients treated by proton therapy at the end of the year 2022 occurred to be about 300 thousand. However, the real spread of proton and heavy ion therapy was much slower, most likely due to their high cost, very high research intensity and extremely high demands 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 2024 [10].

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 very high financial costs, being a high-tech and extremely knowledge-intensive process. This results in unacceptably long delays in registration and application of new drugs, and in turn leads to the suffering and death of a thousands of patients. In addition, the financial risks and difficulty of obtaining funding for the most pioneering and innovative research increases dramatically from year to year. Moreover, the situation is complicated by new regulations and the increasingly stringent restrictions of laboratory animal testing. The reported study aimes 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 (NCI), about 700 anticancer drugs and drug combinations have been approved by FDA [11]. 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) [11].

Despite the well-known fact that innovative combinations are mostly more effective than the widely used mono-component drugs, it is impossible to test all promising combinations on laboratory animals, not to mention clinical trials. Therefore, we decided to limit our current research on testing the two most promising combinations listed in [11]. Taking into account our previous studies [12-14], the first step of the ongoing research was designed as an *in vitro* study of the efficacy and safety of

combinations based on mixtures of rubidium chloride, gemcitabine-cisplatin, carboplatin-paclitaxel, dimethylsulfoxide (DMSO), copper oxide nanoparticles water dispersion and standard saline solution, intended to be applied in the frame of the so called "strongly localized multicomponent combined cancer therapy" [12, 13].

Materials and Methods

All chemicals, reagents and solvents were obtained from Merck (India), Hi Media (India), Invitrogen (India), SRL (India) and Sigma-Aldrich (USA). Anticancer drugs gemcitabine, cisplatine, 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 assay. In 96-well microtiter culture plates, cells were planted 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 µM – 500 µM after an overnight incubation. The cells were then incubated for 48 h. Cisplatin and gemcetabine have been used as reference drugs. After the incubation period MTT (100 µl; 0.5 mg/ml) was added to each well and incubated in a humidified incubator containing 5% CO_2 at 37°C for 4 hrs. After discarding the supernatant the purple-colored formazan crystals formed in the wells ware dissolved in 100 µL 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.

Annexin V-FITC/PI staining for apoptosis assay. Induction of apoptosis was quantified via flow cytometric analysis of control and RbCl cells 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 µL of 1X binding buffer solution supplied within the kit.

Developed combinations. Eighteen combinations (1-18) containing various amount of water, saline, and copper oxide nanoparticles, and six combinations (19-24) containing the so called "blank" copper nanoparticles without DMSO, rubidium chloride, paclitaxel-carboplatine, gemcitabine-cisplatin and gemcitabine (see the Table) were prepared 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 µL of annexin V-FITC and 5 µL 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 RSV and RSA of the necrosis and apoptosis inducing capacity of the tested drug 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.

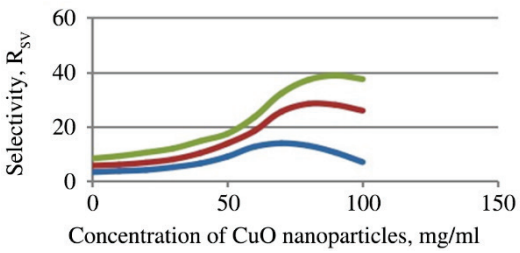

Figure. Dependence of the selectivity R_{SV} on the CuO nanoparticle content in the tested combinations based on (up to down): paclitaxel-carboplatine, gemcitabine-cisplatin, and gemcitabine.

Table. Composition of the developed and tested combinations

Component / Sample No	RbCl, mg	Water, Ml	Saline solution (0.9%), ml	DMSO (99.5%), ml	Gemcitabine, mg/	Gemcitabine,mg/ cisplatin, mg	Paclitaxel/ mg carrboplatin, mg	CuO, mg	Total volume of liquid, ml
1	300	5	2	3	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	

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 using visible light ovoscopy [13]. 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 viable hatched chicks in the exposed test group, N_C is the number of viable 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 the Figure. 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 hawing 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 1.4 to 2.9, 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 სიმსივნის საწინააღმდეგო დამხმარე პრეპარატების თერაპიული ღირებულების არალეტალური გამარტივებული შეფასება კიბოს ძლიერ ლოკალიზებული კომბინირებული მკურნალობისა და პროტონული თერაპიის ფარგლებში

ა. ჭირაქაძე[*,**,§], ნ. დვალი[§§,#], ლ. გუმბერიძე[##], ნ. სიხარულიძე[##], ნ. ხუსკივაძე[§§], თ. ჩუბინიშვილი[*], თ. გელაშვილი[*], გ. ფალავანდიშვილი[θ], ხ. წეროძე[θθ], ი. ხომერიკი[Δ], ნ. თავდიშვილი[§§], ი. ნადირაძე[ΔΔ], დ. აფხაზავა[Ø], ქ. ჩიგოგიძე[ØØ]

[*] საქართველოს ტექნიკური უნივერსიტეტი, ინსტიტუტი „ტექინფორმი“, თბილისი, საქართველო

[**] საქართველოს ტექნიკური უნივერსიტეტი, ვლადიმერ ჭავჭანიძის სახ. კიბერნეტიკის ინსტიტუტი, თბილისი, საქართველო

[§] ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, ელეფთერ ანდრონიკაშვილის სახ. ფიზიკის ინსტიტუტი, თბილისი, საქართველო

[§§] საქართველოს ტექნიკური უნივერსიტეტი, საინჟინრო-ფიზიკური პრობლემების ინსტიტუტი, თბილისი, საქართველო

[#] საქართველოს მაღალი ტექნოლოგიების ეროვნული ცენტრი, სამეცნიერო-კვლევით სამსახური, თბილისი, საქართველო

[##] ივანე ბერიტაშვილის სახ. ექსპერიმენტული ბიომედიცინის ცენტრი, თავის ტვინის სისხლის მიმოქცევის და მეტაბოლიზმის განყოფილება, თბილისი, საქართველო

[θ] საქართველოს ტექნიკური უნივერსიტეტი, მეცნიერებისა და ინოვაციების დეპარტამენტი, თბილისი, საქართველო

[θθ] საქართველოს ტექნიკური უნივერსიტეტი, გარემოსდაცვითი ინჟინერიის და ეკოლოგიის დეპარტამენტი, თბილისი, საქართველო

[Δ] საქართველოს ტექნიკური უნივერსიტეტი, ინფორმატიკის და მართვის სისტემების ფაკულტეტი, თბილისი, საქართველო

[ΔΔ] საქართველოს ტექნიკური უნივერსიტეტი, საინჟინრო ფიზიკის დეპარტამენტი, თბილისი, საქართველო

[Ø] ალტე უნივერსიტეტი, მედიცინის საერთაშორისო სკოლა, თბილისი, საქართველო

[ØØ] კავკასიის უნივერსიტეტი, კავკასიის ჰუმანიტარულ და სოციალურ მეცნიერებათა სკოლა, თბილისი, საქართველო

(წარმოდგენილია აკადემიის წევრის ნ. მითაგვარიას მიერ)

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 წინასწარმეტყველებდნენ. სავარაუდოდ, ამის მთავარი მიზეზი არის ნაწილაკებით თერაპიის მაღალი ღირებულება, კვლევის ინტენსივობა და სამედიცინო-საინჟინრო პერსონალის მიმართ მოთხოვნები. ყოველივე ზემოთქმული, ახალი მედიკამენტების რეგისტრაციისა და დანერგვის დაუშვებლად ხანგრძლივ ვადებთან ერთად, განაპირობებს ათასობით პაციენტის სიცოცხლის ხარისხის დაქვეითებას და სიკვდილს. ამასთანავე, პიონერული ინოვაციური პრეპარატების შემუშავების და ტესტირების ფინანსური რისკები და კვლევისთვის დაფინანსების მოპოვების სირთულე მკვეთრად იზრდება. ამრიგად, პროტონული თერაპიის ბიოლოგიური ეფექტურობისა და უსაფრთხოების მნიშვნელოვანი ზრდა არის დღევანდელი დღის მწვავე და გადაუდებელი საჭიროება, რათა უზრუნველყოფილ იქნეს პროტონული და მძიმე იონებით თერაპიის სწრაფი და მდგრადი განვითარება და მაღალი კონკურენტუნარიანობა. წარმოდგენილი კვლევა მიზნად ისახავს ლაბორატორიულ ცხოველებზე ტესტირებამდე გამარტივებული და სწრაფი არალეტალური მეთოდებით შეფასდეს კიბოს საწინააღმდეგო ახალი პრეპარატების თერაპიული ღირებულება, რათა მომავალში მნიშვნელოვნად შემცირდეს კიბოს საწინააღმდეგო ახალი პრეპარატების შემუშავების, ტესტირებისა და დანერგვის ხარჯები და ვადები.

REFERENCES

1. Chhikara B., Parang K. (2023) Global cancer statistics, 2022: the trends projection analysis. *Chemical Biology Letters,* 10, 1: 1-16.
2. Siegel R., Miller K., Sandeep N., Jemal A. (2023) Cancer statistics, 2022. CA: *Cancer Journal for Clinician,* 73, 1: 1-112.
3. Christodouleas J., Forrest R., Ainsley C., Tochner Z., Hahn S., Glatstein E. (2011) Short-term and long-term health risks of nuclear-power-plant accidents. *The New England Journal of Medicine,* 364: 2334-2341.
4. Kotenko K., Shinkarev S., Abramov I., Granovskaia E., Iatsenko V., Gavrilin I., Margulis I., Garetskaia O., Imanaka T., Khoshi M. (2012) Comparative analysis of the radionuclide composition in fallout after the Chernobyl and the Fukushima accidents. *Med. Tr. Prom. Ekol.,* 10: 1-5.
5. The World Nuclear Organization: Plans For New Reactors Worldwide. https://world-nuclear.org/information-library/current-and-future-generation/plans-for-new-reactors-worldwide.aspx/, accessed on August, 2, 2023.
6. Lane J., Slater G. Yang (2023) Image-guided proton therapy: a comprehensive review. *Cancers,* Basel. 29, 15, 9: 25-55.
7. Mizumoto M., Fuji H., Miyachi M., Soejima T., Yamamoto T., Aibe N., Demizu Y., Iwata H., Hashimoto T., Motegi A., Kawamura A., Terashima K., Fukushima T., Nakao T., Takada A., Sumi M., Oshima J., Moriwaki K., Nozaki M., Ishida Y., Sakurai H. (2021) Proton beam therapy for children and adolescents and young adults (AYAs): JASTRO and JSPHO Guidelines. *Cancer Treatment Reviews,* 98: 1-10.
8. Chang L., Shaaban Sh., Gogineni E., Page B., Quon H., Li H., Ger R. (2023) Daily head and neck treatment assessment for optimal proton therapy planning robustness. https://www.researchgate.net/publication/372613008_Daily_Head_and_Neck_Treatment_Assessment_for_Optimal_Proton_Therapy_Planning_Robustness, accessed on August, 22, 2023.
9. Particle Therapy Co-Operation Group (PTCOG), https://www.ptcog.site/, accessed on August, 22, 2023.
10. Particle Therapy Co-Operative Group. Statistics of patients treated in particle therapy facilities worldwide. https://ptcog.site/index.php/patient-statistics-2, accessed on April, 24, 2024.
11. NIH: National Cancer Institute https://www.cancer.gov/about-cancer/treatment/drugs/lung, accessed on April, 24, 2024.

12. Chirakadze A., Jishiashvili D., Buachidze Z., Gorgadze K., Shiolashvili Z., Jishiashvili A., Mitagvaria N., Lazrishvili I. (2018) New approaches to development of new nanomaterials for magnetic hyperthermia of cancer cells and prospectives of combined treatment of cancer in georgia. *Journal of Low Dimensional Systems,* 2, 1: 8-12.
13. Mitagvaria N., Chirakadze A., Chubinidze G., Dvali N., Chichua T., Khuskivadze N., Devdariani M., Gumberidze L., Kostiuchik N. (2023) Development and acute toxicity testing of anticancer drugs based on alkali metal solutions for treatment of non-small cell lung cancer. *Bull. Georg. Natl. Acad. Sci.,* **17,** 2: 142-147.
14. Chirakadze A., Chubinidze G., Bose M., Hatui L., Dvali N., Khuskivadze N., Bhattacharyya S., Pradhan R., Devdariani M., Gumberidze L., Davlianidze L., Kostiuchik N. (2023) Selective toxicity testing of Gemcitabine, DMSO, Rubidium and Cesium salts and saline solution compositions in A549 and NHDF cell lines. *Bull. Georg. Natl. Acad. Sci.,* **17,** 3: 93-99.

Received September, 2024