საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 17, №2, 2023
BULLETIN OF THE GEORGIAN NATIONAL ACADEMY OF SCIENCES, vol. 17, no. 2, 2023

Human and Animal Physiology

Development and Acute Toxicity Testing of Anticancer Drugs Based on Alkali Metal Solutions for Treatment of Non-Small Cell Lung Cancer

N. Mitagvaria[*,,§], A. Chirakadze[**,#,θ], G. Chubinidze[**], N. Dvali[**], T. Chichua[**], N. Khuskivadze[**], M. Devdariani[§], L. Gumberidze[§], N. Kostiuchik[**]**

[*]*Academy Member, National Academy of Sciences of Georgia, Tbilisi, Georgia*
[**]*Center for Synthesis and Research of Chemotherapeutic Drugs, Caucasus International University, Tbilisi, Georgia*
[§]*Department of Cerebral Circulation and Metabolism, Ivane Beritashvili Center for Experimental Biomedicine, Tbilisi, Georgia*
[#]*Department of Computing Engineering Elements and Nanomaterials, Vladimer Chavchanidze Institute of Cybernetics, Georgian Technical University, Tbilisi, Georgia*
[θ]*Department of Condensed Matter Physics, Elevter Andronikashvili Institute of Physics, Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia*

The concept of multicomponent strongly localized combined therapy of malignant tumors was first proposed and scientifically substantiated at the end of the last decade. Gemcitabine, cisplatin and carboplatin are among the most effective drugs against the non-small cell lung cancer (NSCLC), although quite often they are not always sufficiently effective and safe for patients. Therefore, scientists are constantly looking for safer and more effective chemotherapeutic approaches and drugs against NSCLC. Cesium (Cs) and Rubidium (Rb) salts (chlorides and carbonates) are easily soluble in water and other non-hazardous solvents and putative cancer treatment compounds. Taking into account the rather conflicting data from various studies, special attention should be paid to the study of acute and chronic toxicity of cesium and rubidium preparations and of their interaction with other antitumor modalities. Of particular interest is also the study of cesium and rubidium bio-kinetics in bird embryos and laboratory animals, as well as in the human body. *© 2023 Bull. Georg. Natl. Acad. Sci.*

anticancer drugs, non-small cell lung cancer, high pH therapy, combined strongly localized combined therapy, acute toxicity, alkaline metals, MTT, visible light ovoscopy

The concept of multicomponent strongly localized combined therapy of malignant tumors including various widely used and novel anticancer treatments was first proposed and scientifically substantiated at the end of the last decade [1,2]. This concept has acquired a particular importance in Georgia and the South Caucasus as a whole due to founding of a modern proton therapy center in Kutaisi [3-5]. Gemcitabine, cisplatin and carboplatin are among the most effective drugs against the non-small cell lung cancer (NSCLC), although quite often they are not always sufficiently effec-

tive and safe for patients [6,7]. One of the modern approaches is the implementation of the so-called "high pH hypothesis" suggesting that alkali metal salts create a highly alkaline intracellular environment and can cause death of cancer cells, whereas heavier elements can have a more pronounced effect [8]. Cesium (Cs) and Rubidium (Rb), the heaviest alkali metals occurring in nature in stable form, are putative cancer treatment compounds [8,9]. Cesium chloride based preparations are among the most widely used alternate antitumor modalities in the United States, although the over-the-counter use can cause undesirable, dangerous and even lethal side effects [10,11]. Some researchers, taking into account the rather conflicting data from various studies, are of the opinion that this issue should be studied more intensively. Special attention should be paid to the study of acute and chronic toxicity of cesium and rubidium preparations, to their interaction with other antitumor modalities and to cesium and rubidium bio-kinetics in bird embryos and laboratory animals, as well as in the human body [12-14].

Purpose and object of the research. The main purpose of the reported research was the preliminary complex study of cytotoxicity of cesium chloride, rubidium chloride, cesium carbonate and rubidium carbonate solutions to cancer and healthy human cell cultures (by testing of cell viability) and chick embryos (testing of mortality of embryos) in comparison to gemcitabine solutions in standard saline solution and in DMSO (dimethyl sulfoxide)/ water solution. Another aim was the analysis of the results obtained and assessment of the minimum required concentration, dose and time of exposure to the tested anticancer formulations. At the beginning, MTT testing [15] was carried out and TI (toxicity index) to chick embryos was measured.

Experimental Study. Materials and Methods

Cesium chloride, cesium carbonate, rubidium chloride and rubidium carbonate (purity ≥ 99.8 %) were dissolved in double distilled and deionized water and the resulting solutions were hermetically sealed in 2 ml polypropylene tubes, 80 ml in total. The anticancer drug "AqVitabin" (lyophilisate for infusion solution) containing gemcitabine (as gemcitabine hydrochloride) 200.0 mg (228.0 mg), mannitol 200.0 mg, sodium acetate anhydrous 12.5 mg and concentrated hydrochloric acid/sodium hydroxide q. s. up to pH 2.7-3.3 was dissolved in the standard saline solution or DMSO solution for injection. Finally, four test and two control samples with molar concentration 100 mM were hermetically sealed and stored in optimal conditions to be tested in the laboratories of "Binfosol Private Limited" (MTT assay, non-small cell lung cancer NSCLC A549 cell culture and normal human dermal Fibroblast NHDF cell culture) and in the laboratory of the Caucasus International University with participation of researchers of the Georgian Technical University and Ivane Beritashvili Center for Experimental Biomedicine using visible light ovoscopy of chick embryos. The Acute toxicity index TI of the test samples to chick embryos was calculated according to formula (1):

$$TI\ (\%) = (1 - NT / NC\ \text{after treatment}) \bullet 100, \quad (1)$$

where NT is the number of hatched chicks in the test group, NC is the number of hatched chicks in control group and NT/NC is the viability of the exposed embryos.

Experimental results. Results of MTT testing are represented by charts given in figures 1- 6. The image of computer controlled visible ovoscope and the typical images of the treated chick embryos are given in figures 7-12. MTT testing [15] of the developed and control drug preparations was carried out in the concentration range (0-500) μM. with three replications (N = 3). An important characteristic of the drug effectiveness is the LC50 index, showing the concentration causing the death of the half of exposed cells at a fixed dose. The

LC50 values of four test and two control drug samples and their ratio for two cell cultured are given in Table. Each value represents mean ± SEM, N=3. One-way ANOVA was followed by Dunnett multiple comparison test and ($P<0.001$) was interpreted as statistically significant, as compared with the control. The data of MTT testing clearly show, that RbCl and CsCl based preparations ($R \geq 8.8$ and

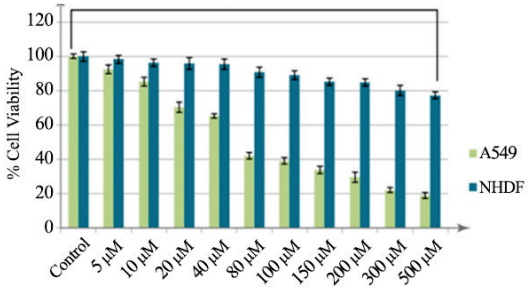

Fig. 1. Dependence of cell viability on rubidium chloride concentration.

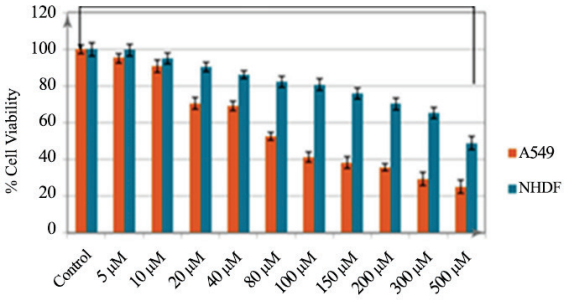

Fig. 2. Dependence of cell viability on cesium chloride concentration.

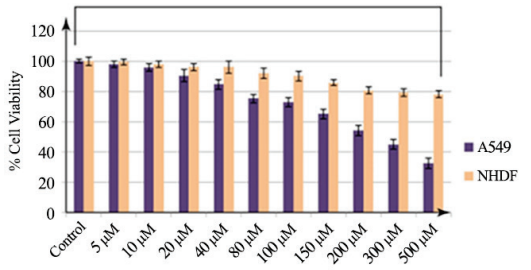

Fig. 3. Dependence of cell viability on rubidium carbonate concentration.

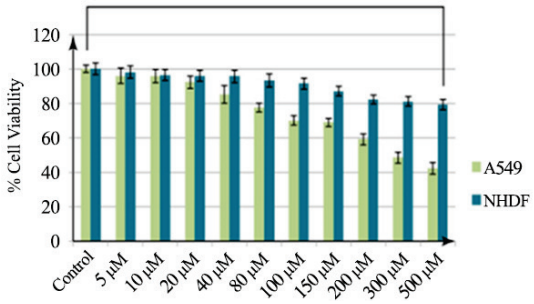

Fig. 4. Dependence of cell viability on cesium carbonate concentration.

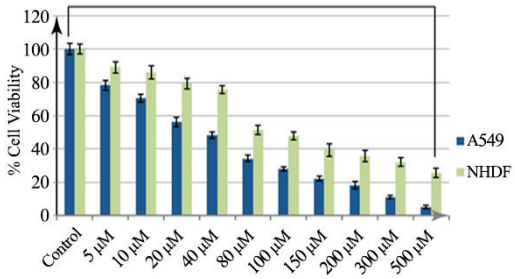

Fig. 5. Dependence of cell viability on gemcitabine + DMSO concentration.

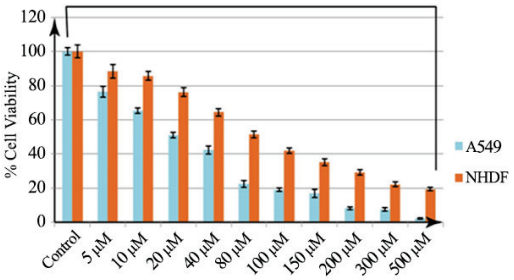

Fig. 6. Dependence of cell viability on gemcitabine concentration.

Fig. 7. 50 embryo capacity computer controlled incubator with a visible light ovoscope.

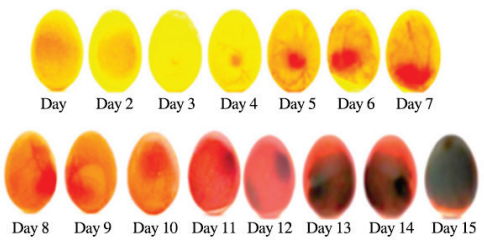

Fig. 8. Typical ovoscopy images of a normally developing chick embryo.

R = 5.5) are significantly more selective to cancer cells than gemcitabine based preparations (R = 3.6 and R = 3.2), while selectivity of Rb2CO3 and Cs2CO3 based preparations (R ≥ 2.2 and R ≥ 1.8) seems to be close to selectivity of gemcitabine based preparations. Polynomial extrapolation of the data shows that at concentrations close or above 1.5 mM the selectivity of all test samples should be considerably higher compared to selectivity of gemcitabine based preparations.

Table. LC_{50} index, square mediate error SEM and ratio of indices for A549 and NHDFcells

Drug sample	A549	NHDF	R
RbCl	56.82 ± 3.51	≥ 500	≥ 8.8
CsCl	84.19 ± 2.89	464 ± 3.46	5.5
Rb_2CO_3	225 ± 3.06	≥ 500	≥ 2.2
$Cs_2 CO_3$	271± 2.65	≥ 500	≥ 1.8
Gemcitabine + DMSO	6,258 ± 1.26	22.64 ± 3.72	3.6
Gemcitabine	8.22 ± 2.05	26.39 ± 4.22	3.2

Obviously, chlorides are less toxic than carbonates, while cesium compounds are less toxic than rubidium compounds. It is also important that ovoscopy makes it possible to determine which vital organs and systems of embryos, to what extent and in what sequence, are affected by toxic effects, which is useful for a deeper understanding of the mechanism of toxicity and its prevention or reducing.

Discussion and Conclusions

Rubidium and cesium based preparations are much less toxic to NHDF cells than the gemcitabine based ones. Despite the relatively low cytotoxicity of the cesium and rubidium based solutions to A549 cells their extremely low cytotoxicity to NHDF cells provides significantly better selectivity to cancer cells which, in turn, can provide their significantly better efficacy and safety at higher concentrations compared to gemcitabine. Thus, MTT testing of the developed preparations and

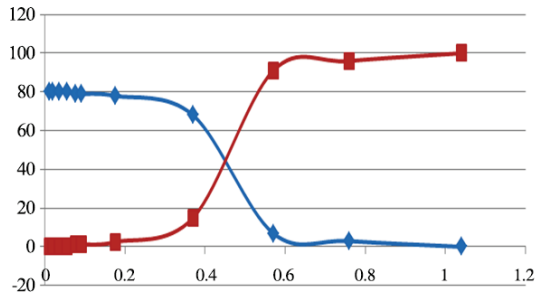

Fig. 9. CsCl toxicity index and exposed cell viability.

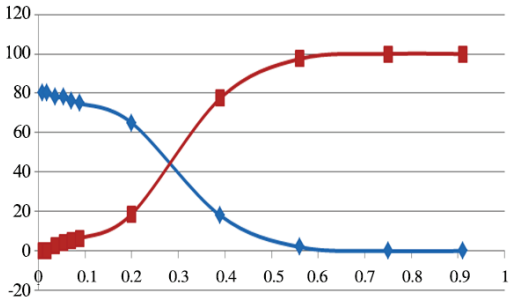

Fig. 10. RbCl toxicity index and exposed cell viability.

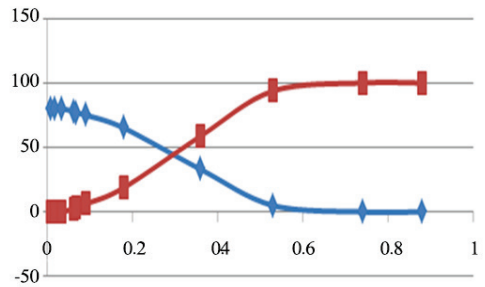

Fig. 11. Cs_2CO_3 toxicity index and exposed cell viaability.

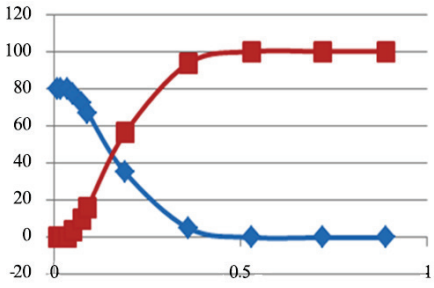

Fig. 12. Rb_2CO_3 toxicity index and exposed Cell viability.

gemcitabine at concentrations up to 1000-1500 μM are necessary. The results of MTT assay, the visible light ovoscopy and the long term monitoring showed that ranking of the developed cesium and rubidium based preparations according their selectivity should be as follows: 1) RbCl, 2) CsCl, 3) Rb_2CO_3 and 4) Cs_2CO_3. To characterize the developed preparations with higher accuracy and reliability and for a better understanding of mechanisms of action the following tests should be carried out: AnnexinV/PI test, Caspase 3 expression level test, DAPI staining chromosomal fragmentation test and DCFDA cytoplasmic ROS generation test.

The authors express their sincere gratitude to Dr. Mayukh Bose and the staff of the "Binfosol Private Limited" company for their very important contribution to experimental research and discussion of the results.

ადამიანისა და ცხოველთა ფიზიოლოგია

ტუტე ლითონების ხსნარებზე დაფუძნებული ანტისიმსივნური პრეპარატების შემუშავება და მწვავე ტოქსიკურობის ტესტირება არაწვრილუჯრედოვანი ფილტვის კიბოს სამკურნალოდ

ნ. მითაგვარია[*,**,§], ა. ჭირაქაძე[**,#,θ], გ. ჩუბინიძე[**], ნ. დვალი[**], თ. ჩიჩუა[**], ნ. ხუსკივაძე[**], მ. დევდარიანი[§], ლ. გუმბერიძე[§], ნ. კოსტიუჩიკი[**]

[*]აკადემიის წევრი, საქართველოს მეცნიერებათა ეროვნული აკადემია, ივ. ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი, საქართველო
[**]კავკასიის საერთაშორისო უნივერსიტეტი, ქიმიოთერაპიული პრეპარატების სინთეზისა და კვლევის ცენტრი, თბილისი, საქართველო
[§]ივანე ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თავის ტვინის სისხლის მიმოქცევისა და მეტაბოლიზმის განყოფილება, თბილისი, საქართველო
[#]საქართველოს ტექნიკური უნივერსიტეტი, ვლადიმერ ჭავჭანიძის კიბერნეტიკის ინსტიტუტი, გამთვლითი ტექნიკის ელემენტების და ნანომასალების განყოფილება, თბილისი, საქართველო
[θ]ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, ელეფთერ ანდრონიკაშვილის ფიზიკის ინსტიტუტი, კონდენსირებულ გარემოთა ფიზიკის განყოფილება, თბილისი, საქართველო

ავთვისებიანი სიმსივნეების მრავალკომპონენტიანი ძლიერად ლოკალიზებული კომბინირებული თერაპიის კონცეფცია პირველად წინა ათწლეულის ბოლოს იქნა შემოთავაზებული და მეცნიერულად დასაბუთებული. გემციტაბინი, ცისპლატინი და კარბოპლატინი ფილტვის არაწვრილუჯრედოვანი კიბოს (NSCLC) მკურნალობის ყველაზე ეფექტურ საშუალებათა ჯგუფს მიეკუთვნება, თუმცა იმავდროულად, ეს პრეპარატები პაციენტისთვის ყოველთვის არ არის საკმარისად ეფექტური და უსაფრთხო. ცეზიუმი (Cs) და რუბიდიუმი (Rb) არის ბუნებაში

სტაბილური ფორმით არსებული ყველაზე მძიმე ორი ტუტე ლითონი. მათი მარილები (ქლორიდები და კარბონატები) განიხილება როგორც კიბოს სამკურნალო, სავარაუდოდ, მაღალი ეფექტურობის ნაერთები. უკვე ჩატარებული კვლევების წინაღმდეგობრივი მონაცემების გათვალისწინებით, განსაკუთრებით საყურადღებოა ცეზიუმის და რუბიდიუმის პრეპარატების მწვავე და ქრონიკული ტოქსიკურობის და მათი სიმსივნის საწინააღმდეგო სხვა საშუალებებთან ურთიერთქმედების შესწავლა. მეტად საინტერესოა აგრეთვე ცეზიუმის და რუბიდიუმის ბიოკინეტიკის შესწავლა როგორც ფრინველთა ემბრიონებსა და ლაბორატორიულ ცხოველებში, ასევე ადამიანის ორგანიზმში.

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Received March, 2023