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Selective Toxicity Testing of Gemcitabine, DMSO, Rubidium and Cesium Salts and Saline Solution Compositions in A549 and NHDF Cell Lines

Archil Chirakadze[*,,§], Giorgi Chubinidze[*], Mayiukh Bose[§§], Labanyamoyee Hatui[§§], Nugzar Dvali[*], Nana Khuskivadze[*], Sudip Bhattacharyya[§§], Rabina Pradhan[§§], Marina Devdariani[#], Lali Gumberidze[#], Lena Davlianidze[#], Nino Kostiuchik[*]**

[*] *Center of Synthesis and Research of Chemotherapeutic Drugs, Caucasus International University, Tbilisi, Georgia*
[**] *Department of Condensed Matter Physics, Elevter Andronikashvili Institute of Physics, Ivane Javakhishvili Tbilisi State University, Tbilisi, Georgia*
[§] *Department of Computing Engineering Elements and Nanomaterials, Vladimir Chavchanidze Institute of Cybernetics, Georgian Technical University, Tbilisi, Georgia*
[§§] *Bio-testng Laboratory, Company "Binfosol Private Limited", Kolkata, West Bengal, India*
[#] *Department of Cerebral Circulation and Metabolism, Ivane Beritashvili Center of Experimental Biomedicine, Tbilisi, Georgia*

(Presented by Academy Member Nodar Mitagvaria)

Developing of new methodologies for cancer therapy is a modern critical and urgent task. Essential aspects of cancer therapy are necrosis (direct killing of the cell) and inducing apoptosis, a programmed cell death process, specifically in cancer cells. The proapoptotic action of cancer therapy aims to trigger the natural self-destruction mechanism of cancer cells, thereby inhibiting their uncontrolled growth and promoting their elimination. The purpose of the present research was to measure the apoptosis inducing capacity and assess the selectivity of soluble compositions of cesium and rubidium chlorides and carbonates to non-small cell lung cancer A549 and NHDF (Normal Human Dermal Fibroblasts) cell cultures in comparison with gemcitabine (which has a high efficiency and high capacityof necrosis and apoptosis induction for the treatment of lung cancer). In addition, the effect of adding DMSO (dimethylsulfoxide) to gemcitabine has been evaluated. Rubidium chloride and gemcitabine plus DMSO revealed the highest selectivity to cancer cells. *© 2023 Bull. Georg. Natl. Acad. Sci.*

anticancer efficacy, rubidium, cesium, chlorides, carbonates, selectivity, A549 cells, NHDF cells, necrosis, apoptosis

Development of new methodologies for cancer therapy is a critical and urgent task [1-11]. The study of acute toxicity of solutions of rubidium and cesium chlorides and carbonates to chicken embryos using the ovoscopic method carried out at the Caucasus International University revealed that

in a wide range of concentrations these substances are non- or slightly toxic to normal chick embryos and healthy white rats. One of the essential aspects of cancer therapy is inducing apoptosis, a programmed cell death process, specifically in cancer cells. The proapoptotic action of cancer therapy aims to trigger the natural self-destruction mechanism in cancer cells, thereby inhibiting their uncontrolled growth and promoting their elimination. When discussing cancer therapy and induction of apoptosis in cancer cells, the focus primarily lies on molecularly targeted agents specifically developed for that purpose such as CDK, EGFR, HER2 and PARP inhibitors [3]. Less attention is paid to metal salts molecules like rubidium and cesium chlorides and carbonates that exhibit various interesting biological and therapeutic properties [5, 9, 11]. Results of different studies devoted to the use of cesium and rubidium salts in oncological practice are quite controversial [1-3, 8] and still there are no unified, systematic and reliable data on the acute and chronic toxicity, biokinetics and effects of these substances on tumor cells. Rubidium salts and cesium salts are generally considered to have low acute toxicity [4, 6]. Cesiuum and rubidium chlorides and carbonates have low acute oral toxicity in animal studies, while their inhalation toxicity is generally low. Cesium chloride and cesium carbonate are also considered to have low acute toxicity to human, although ingestion of cesium salts can cause gastrointestinal disturbances. Extremely high doses of cesium chloride have been associated with cardiac arrhythmias in animals. In terms of specific characteristics of these substances, both cesium chloride and rubidium chloride have low acute toxicity but can affect the cardiovascular system at high doses. Rubidium chloride has been studied for potential therapeutic uses in depressive disorders, while cesium chloride has been investigated for its potential anticancer effects. Cesium carbonate and rubidium carbonate are generally considered to have low acute toxicity, but specific toxicity data for rubidium carbonate is limited. It is worth noting that while acute toxicity data suggests low toxicity, long-term exposure to high concentrations of these salts may have different effects and could lead to adverse health effects including sudden heart arrest. The potential therapeutic applications of cesium rubidium and carbonate salts solutions were previously studied in [12]. Acute toxicity and cell proliferation inhibition capacity of cesium chloride (CsCl), cesium carbonate (Cs_2CO_3), rubidium chloride (RbCl) and rubidium carbonate (Rb_2CO_3), gemcitabine plus DMSO (dimethyl sulfoxide) and gemcitabine standard (0.9%) saline solutions were tested using visible light ovoscopy method and the long-term monitoring of behavioral and physiological parameters of white rats in the elevated branched maze. Interestingly, all tested solutions showed a high anticancer efficacy, while RbCl revealed a higher efficacy compared to CsCl, Rb_2Co_3 and Cs_2Co_3. The obtained results clearly showed that in the range of studied concentrations, the ratio of apoptotic efficacy of the drugs to both the cancerous and healthy cells do not increase monotonously with concentration, which causes a reasonable interest in a detailed study of the dose/concentration dependence of this ratio in order to find its optimal values. Higher acute efficacy of rubidium salts in comparison to cesium salts and chlorides in comparison to carbonates is very difficult to interprete in terms of the so called “high pH therapy concept” [13] and a comprehensive additional study of the distribution of all tested elements in the intra- and extra-cellular media of cancer and healthy cells of tumour and tumour microenvironment (TME) is urgently required [14]. It is also essential to study the ability of various drug compositionss to induce apoptosis in cancerous and healthy cells.

Materials and Methods

Chemicals and reagents. Chemicals and reagents were obtained from Merck (India), HiMedia (India), Invitrogen (India), SRL (India) and Sigma-Aldrich (USA). DCFDA (# D6883) was purchased

from Sigma-Aldrich (India). Fetal bovine serum (#16000044) was obtained from Gibco, USA and MEM sodium pyruvate, MEM non-essential amino acids L-glutamine and Gentamicin, were procured from Hi-Media, India. Cesium and rubidium chlorides and carbonates for test solutions were obtained from Acros Organics B.V.B.A, Belgium. Gemcitabine (Hospira Inc, UK) was mixed with dimethyl sulfoxide (Merck, India) and saline solutions to exclude their reaction with atmospheric gases. In total, 6 samples were prepared (Table). The initial molar concentration of all solutions of gemcitabine and of cesium and rubidium salts (144 mM) was significantly lower than the safety limits established by us by the method of the visible light ovoscopy [12].

Table. Composition of the four test and two control samples

№	Drug sample solution	Concentration of the active component in saline solution	Destination of the sample
1	Cesium chloride	150 mM	Test sample
2	Cesium carbonate	150 mM	Test sample
3	Rubidium chloride	150 mM	Test sample
4	Rubidium carbonate	150 mM	Test sample
5	Gemcitabine + DMSO	150 mM + 150 mM	Control sample
6	Gemcitabine	150 mM	Control sample

Cell cultures maintenance and Annexin V-FITC/PI staining for apoptosis assay. Cell cultures maintenance and apoptosis assay were identical to that described in [12]. Induction of apoptosis in treated cells was quantified via flow cytometric analysis of RbCl, CsCl, Rb2Co3, Cs2Co3, gemcitabine plus DMSO and gemcetabine solutions using the Annexin V-FITC apoptosis detection kit according to the manufacturers' protocol (BD Bioscience) [7].

Experimental Results and Discussion

RbCl, CsCl, Rb_2CO_3 and Cs_2CO_3 promoted apoptotic cell death. To better understand whether RbCl, CsCl, Rb_2CO_3 and Cs_2CO_3 mediated inhibition of A549 cells proliferation has a significant relationship with apoptotic cell death, we used Annexin V-FITC/PI flow cytometric assay to determine the mode of cell death. Based on the LC50 values given in [12], different concentrations of RbCl (25 μM, 50 μM and 100 μM) CsCl (40 μM, 80 μM and 160 μM), Rb_2CO_3 (112 μM, 224 μM and 448 μM), Cs_2CO_3 (135 μM, 270 μM and 540 μM) have been selected for Annexin V-FITC/PI flow cytometric assay. Flow cytometric data for A549 cell line showed an increased number of apoptotic cell population with the increase of RbCl, CsCl, Rb_2CO_3 and Cs_2CO_3 concentrations as shown in Fig. 1. RbCl (Fig. 1a) treatment of 25 μM, 50 μM and 100 μM for 48 hours showed a dose dependent increase in apoptotic A549 cell population 37.88%, 68.89% and 80.77% respectively from 0.70% in control setup. In contrast, RbCl treatment showed a limited dose dependent induction of apoptosis in NHDF cells with the apoptotic cell population 1.79%, 3.95% and 12.43% at 25 μM, 50 μM and 100 μM concentration respectively, compared to control 0.30%. CsCl (Fig. 1b) treatment of 40 μM, 80 μM & 160 μM for 48h showed a dose dependent increase in apoptotic A549 cell population to 49.83%, 51.83%, and 81.88% respectively from 3.38% in control setup. In contrast, CsCl treatment showed a limited dose dependent induction of apoptosis in NHDF cells with the apoptotic cell population 3.67%, 4.77%, 14.65% at 40 μM, 80 μM & 160 μM concentration respectively, compared to control 2.29 %. Rb_2CO_3 (Fig. 1c) treatment of 112 μM, 224 μM & 448 μM for 48 h showed a dose dependent increase in apoptotic A549 cell population 13.02%, 34%, 57.22% respectively from 6.35% in control setup. In contrast, Rb_2CO_3 treatment showed a limited dose dependent induction of apoptosis in NHDF cells with the apoptotic

cell population 10.66%, 26.89%, 34.03% at 112 µM, 224 µM and 448 µM concentration respectively, compared to control 1.98 %. Cs_2CO_3 (Fig. 1d) treatment of 135 µM, 270 µM and 540 µM for 48 h showed a dose dependent increase in apoptotic A549 cell population 30.18%, 44.2% and 70.51% respectively from 0.60% in control setup. Cs_2CO_3 treatment showed a limited dose dependent induction of apoptosis in NHDF cells with the apoptotic cell population 19.36%, 23.24% & 38.77% at 135 µM, 270 µM and 540 µM concentration respectively, compared to control 2.07%. At the same time, although gemcitabine plus DMSO (Fig. 1e) and gemcitabine (Fig. 1f) treated A549 cells reached to an around 80% apoptotic cell death population even at a lower concentrations of 9 µM and 16 µM respectively, they show a significantly greater cytotoxicity towards normal cell line NHDF and reach to an around 38% apoptotic cell death population even at an lower concentration of 9 µM and 16 µM respectively (Fig. 1e and 1f).

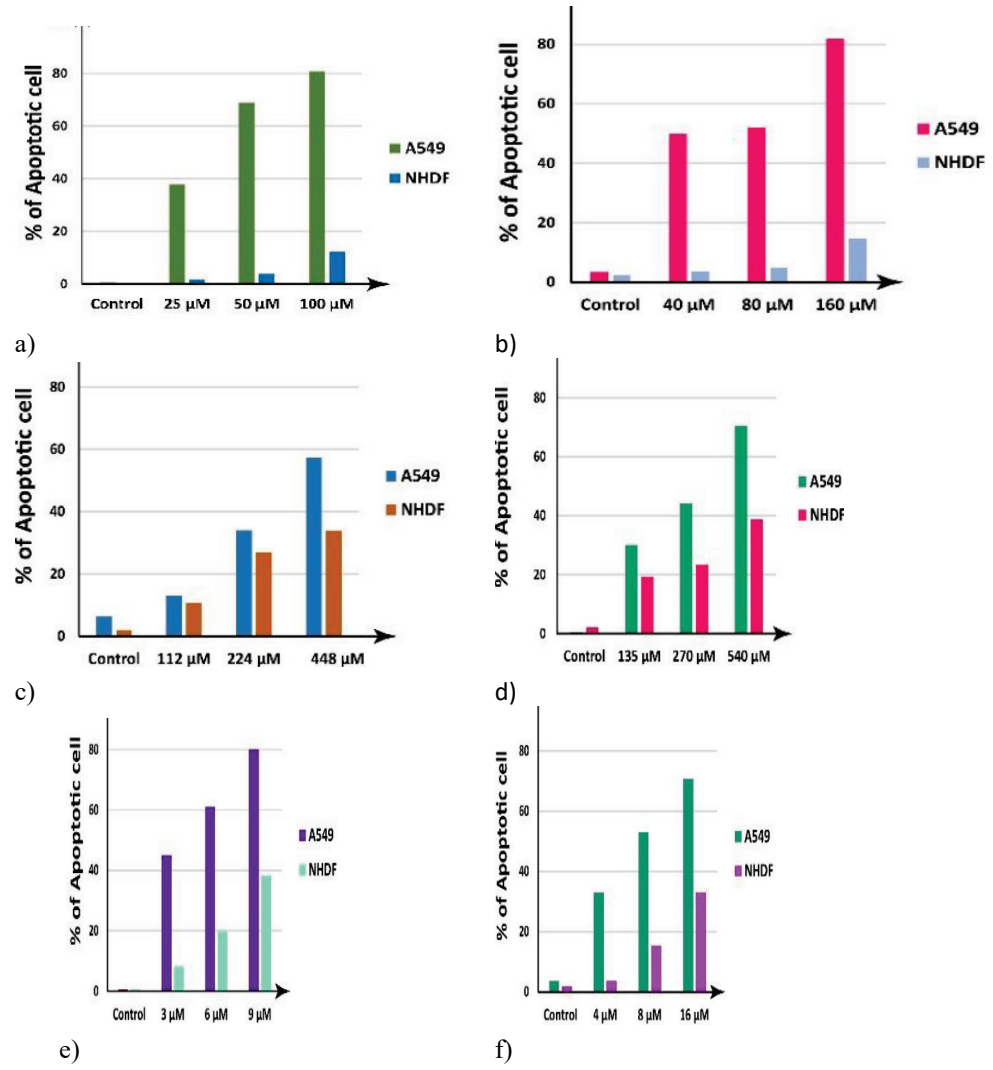

Fig. 1. Capacity of inducing apoptosis in A549 (let columns) and NHDF (right columns_cells: a) rubidium chloride; b) cesium chloride; c) rubidium carbonate d) cesium carbonate; e) gemcitabine plus DMSO; f) gemcitabine.

Thus, all results from Annexin V-FITC/PI flow cytometric assay clearly depict that RbCl, CsCl, Rb_2CO_3, and Cs_2CO_3 mediated inhibition of A549 cell proliferation is mainly due to the induction of apoptotic cell death. RbCl and CsCl show higher rate of apoptotic cell death of A549 cell line at lower concentration than Rb_2CO_3 and Cs_2CO_3. A549 cell treated with RbCl show around 80% apoptotic cell death population at a concentration 100 μM, while CsCl, Rb_2CO_3 and Cs_2CO_3 reach to same or close to 80% apoptotic cell death population at a concentration of around 160 μM, more than 448 μM and more than 540 μM. So, in comparison to gemcitabine. RbCl and CsCl induce a significantly less number of apoptotic cell deaths in the NHDF cell line.

Conclusion

The above results from Annexin V-FITC/PI flow cytometric assay justify our findings from MTT assay [12] and show RbCl being the most effective and safe among the four test compounds and that RbCl and CsCl can be selected as a potent highly selective anticancer therapeutic agent for further in-depth studies, showing a significantly higher selectivity to cancer cells in comparison with gemcitabine and having a significantly higher ratio of toxicity to A549 vs. NHDF cells than gemcitabine. The above results also show that DMSO can be suggested to serve as an effective synergising agent to the studied solutions and a detailed and comprehensive study of DMSO-containing compositions is needed. An useful characteristic of the studied solution is the ratio of apoptotic efficacies of the drugs to cancerous vs. healthy cells which do not increase monotonously with concentration, causing a reasonable interest in studying the dose/concentration dependence of this ratio in order to find its optimal values. It should be emphasized that that the results of Annexin V-FITC testing are even more promising than of the MTT testing. The next steps to increase the effectiveness of combined anticancer drugs based on rubidium and cesium salts can be a detailed study of combinations, containing nanoparticles of metals (iron, copper, zinc, gold, platinum, cesium, rubidium etc.), their oxides and ionophores, as well as the effect of high temperature (41-44^{0}C) on the cytotoxicity and apoptosis inducing capacity of the studied combinations. A special interest should be given to identification of synergistic compositions of the modalities aimed showing the maximum of ratio of cytotoxicity and apoptosis induction capacity to cancerous cells vs. healthy cells.

ადამიანისა და ცხოველთა ფიზიოლოგია

გემციტაბინის, DMSO, რუბიდიუმის და ცეზიუმის მარილების და ფიზიოლოგიური ხსნარის კომპოზიციების სელექციური ტოქსიკურობის ტესტირება A549 და NHDF უჯრედულ ხაზებში

ა. ჭირაქაძე[*,**,§], გ. ჩუბინიძე[*], მ. ბოსე[§§], ლ. ჰათუი[§§], ნ. დვალი[*], ნ. ხუსკივაძე[*], ს. ბჰათაჩარია[§§], რ. ფრადჰანი[§§], მ. დევდარიანი[#], ლ. გუმბერიძე[#], ლ. დავლიანიძე[#], ნ. კოსტიუჩიკი[*]

[*] *კავკასიის საერთაშორისო უნივერსიტეტი, ქიმიოთერაპიული პრეპარატების სინთეზისა და კვლევის ცენტრი, თბილისი, საქართველო*

[**] *ივანე ჯავახიშვილის სახ. თბილისის სახელმწიფო უნივერსიტეტი, ელეფთერ ანდრონიკაშვილის ფიზიკის ინსტიტუტი, კონდენსირებულ გარემოთა ფიზიკის განყოფილება, თბილისი, საქართველო*

[§] *საქართველოს ტექნიკური უნივერსიტეტი, ვლადიმერ ჭავჭანიძის სახელობის კიბერნეტიკის ინსტიტუტი, გამოთვლითი ტექნიკის ელემენტების და ნანომასალების განყოფილება, თბილისი, საქართველო*

[§§] *კომპანია „ბინფოსოლ ფრაივით ლიმითედ“, ბიო-ტესტირების ლაბორატორია, კოლკატა, დასავლეთ ბენგალი, ინდოეთი*

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

(წარმოდგენილია აკადემიის წევრის ნ. მითაგვარიას მიერ)

კიბოს მკურნალობის ანტისიმსივნური თერაპიის ახალი საშუალებებისა და მეთოდების შემუშავება თანამედროვეობის მწვავე და ფრიად აქტუალური ამოცანაა. კიბოს თერაპიის თანამედროვე მიდგომების უმეტესობის უმნიშვნელოვანესი ასპექტებია ნეკროზი (უჯრედის „პირდაპირი“ განადგურება) და აპოპტოზის ინდუცირება (უჯრედის დაპროგრამებული თვითგანადგურების მექანიზმის, უმეტესწილად სიმსივნურ უჯრედებში, ჩართვა). კიბოს თერაპიის პროაპოპტიკური მოქმედება ისწრაფვის „ჩართოს“ თვითგანადგურების პროცესი და მთლიანად შეწყვიტოს კიბოს უჯრედების ზრდა და გავრცელება. მოცემული კვლევის საგანია განისაზღვროს ფილტვის კიბოს A549 და ადამიანის ნორმალურ დერმალურ ფიბრობლასტურ NHDF უჯრედულ ხაზებში ცეზიუმის და რუბიდიუმის ქლორიდების სხნარების მიერ აპოპტოზის ინდუცირების პოტენციალი და შეფასდეს მათი კიბოს უჯრედების მიმართ სელექციურობა გემციტაბინთან შედარებით. ამასთანავე, შეფასდეს გემციტაბინის ხსნარში დიმეთილსულფოქსიდის (დმსო) დამატებით გამოწვეული ეფექტებიც. გამოკვლეულ ხსნარებს შორის რუბიდიუმის ქლორიდმა და გემციტაბინ-დიმეთილსულფოქსიდის კომბინაციამ გამოავლინა კიბოს უჯრედების მიმართ ყველაზე მაღალი სელექციურობა.

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