ფლუორესცენციის (LIF) სპექტროსკოპია. დამზადდა სპეციალური ზონდი, რომლის საშუალებითაც შესაძლებელი გახდა დიფერენციული სპექტრების გადაღება და შესაბამისად ფლუორესცენციის სპექტრალური ხაზის ფორმების შედარება.

გაიზომა ადამიანის თითის ბალიშისა და ფრჩხილის LIF ინტენსივობის *in vivo* სპექტრები, რომლებიც გამოყენებული იყო ადამიანის ჯანმრთელობის მდგომარეობის დასადგენად, მის დასახასიათებლად ამ მდგომარეობის შემდგომი მონიტორინგისათვის რეალური დროის რეჟიმში. სხვადასხვა მოხალისეების სპექტრების დამუშავებით დადგინდა, რომ ფიზიოლოგიურად ჯანმრთელი და პათოლოგიის მქონე (ამ შემთხვევაში – დიაბეტის 1-ლი და მე-2 ფორმა) მოხალისეების კანის ფლუორესცენციის სპექტრები საგრძნობლად განსხვავდება ერთმანეთისგან. მეტიც, აღნიშნული სპექტრების ანალიზი იძლევა პათოლოგიის ხარისხის შეფასების შესაძლებლობას.

აღმოჩნდა, რომ სამი ექსპერიმენტულად ჩაწერილი ფლუორესცენციის სპექტრიდან ნებისმიერი ერთი წარმოადგენს დანარჩენი ორის სუპერპოზიციას. ადამიანის კანის ფლუორესცენციის მრავალშრიანი მოდელის თეორიულმა ანალიზმა აჩვენა, რომ აღნიშნული პრინციპი ყოველთვის სამართლიანია, როდესაც ფლუორესცენციაში მონაწილეობენ ერთი და იგივე ქრომოფორები.

"AMPHICEZINE": NEW APPROACHES TO FIGHTING CANCER PRELIMINARY THEORETICAL AND EXPERIMENTAL (*IN VITRO*) MESSAGE

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Fibrin content is increased in human tumor tissue, which, being the result of overall reactions of so-called "Machabeli Syndrome" present in oncologic patients, occupies the central place in the process of tumor metastasis [8-11]. The decrease of membrane potential of tumor cells allows them to sharply limit the penetration of foreign bodies into them to only a few components. This significantly hinders the effectiveness of anti-tumor medications.

Tumor cells secrete enzyme Hyaluronidase, which supports their break-off from primary site and attachment to the walls of other organ's blood or lymph vessels [4].

The emergence of metastasis is closely related to the interrelation of tumor cells and endothelium of blood and lymph vessels at physical-chemical level. Along with the increase of malignancy degree of tumor cells comes increase in their negative electronic charge, which due to its well-known life-giving qualities, increases their vital capacity. Significantly, it is the carcinogenic substances that support the increase of negative charge of cells [6-8].

Herewith, it seems interesting to use existing differences between electrostatic potentials of normal homologous cells and cancer cells to fight against cancer.

Our proposal is as follows: the use of new perspective class of respective drugs ("Amphicezine") for inhibition of metastasis of malignant cells – negative multi-charged long-chain organic ions. The proposed drugs are substances with macromolecule having polar-distributed qualities, one side charged (polar), another –hydrophobic, non-polar [3,6].

Hydrophilic qualities of macromolecules are preconditioned by number of functional groups, which in various biological environments are dissociated by producing long-chained organic anions [1,2,5], which interact simultaneously with lipophilic, as well as hydrophilic structures, that defines their biological activity. The proposed organic anions are characterized by heparin-like and fibrinolytic activities, which hampers the adhesion of tumor cells that break off from the primary site onto the endothelium. While regulating the penetration processes of the cell membrane in addition they support anti-tumor drug transportation to through cell. The above mentioned organic anions are not complexions by direct meaning, but separate fragments of their molecules could perform the role of chelating agents and take part in blocking of carcinogenic ions of heavy metals (chrome, cadmium etc.). Besides, the usage of these organic anions as carriers of cesium and rubidium ions to penetrate through tumor cells gives us opportunity to alkalinize internal environment of these cells. This process, in its turn, causes full destruction of these cells. Furthermore, it is significant that cesium and rubidium cations do not harm normal cells.

Taking into account the potential of the new class of synthetic inhibitors for tumor metastasis chemotherapy and radiotherapy strategies for malignant tumors are altered: it becomes possible to decrease the treatment-prophylaxis dosage or even not to use them at all. It is worth mentioning that the new class of proposed drugs considerably differs from traditional chemo-drugs, which are characterized by high cytotoxicity in relation to the normal cells [12].

Obviously, the authors well understand how complex and wacked pathology is the cancer, but we do hope that we are on the right path to find the "Achilles heel" of the cancer metastasis process. It is remarkable, that the usage of electric charge for treatment-prophylaxis goals in experimental and even in clinical oncology is not mentioned in worldwide scientific research literature as of today.

It is well known, that various new approaches in the treatment of malignant tumors, which gave good results in lab experiments (in vitro), have been unsuccessful in clinical settings. In our opinion, one of the reasons of above mentioned is, that it is not taken into account, that each proposed medication (in our case - metastasis inhibitor) should target and affect cancer cells only and should not damage the normal ones. We have all reasons to consider that the new approach discussed in our article will give us opportunity to solve this problem.

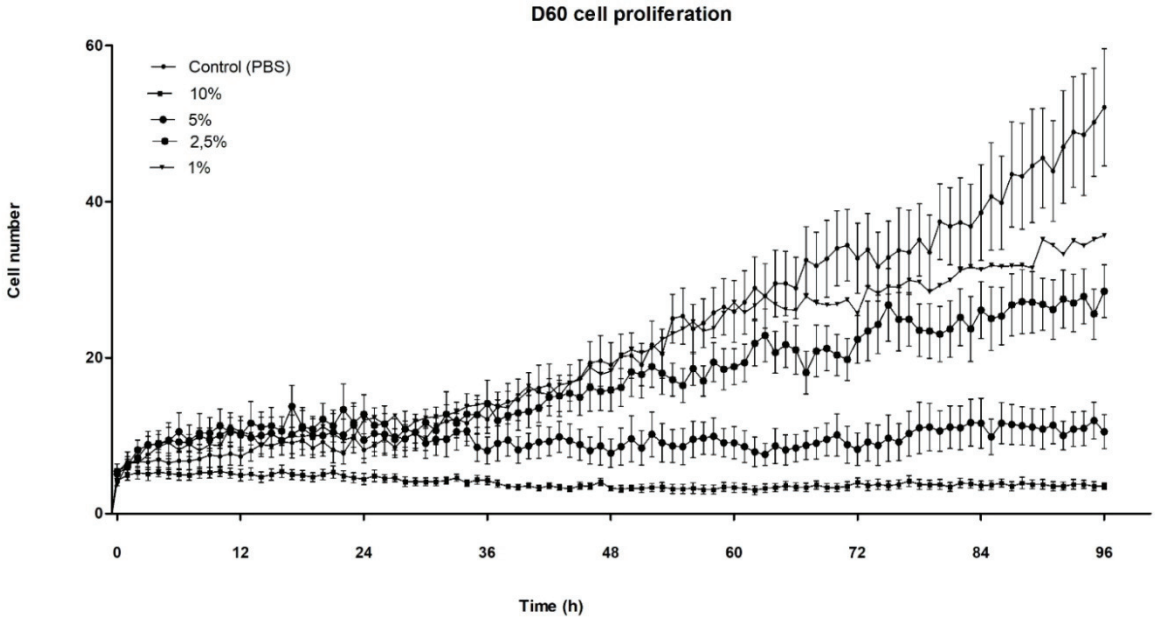

Fig. Effect of Amphicezine on D60 p4 cell culture viable cell count

Strategic objective – creation of fundamentally new inhibitors of metastasing of malignant tumors due to the radical surgeries carried out on them. Specific tactical objective – revealing the possible cytolytic and citostatic effectiveness of the drug "Amphicezine" created by us on atypical fibroblastic cells.

Material and methods. • "Amphicezine";
• Cell line D60 p4 (primary dermal atypical fibroblasts);
• FBS superior (Millipore cat. no. S0615);
• DMEM (Merck cat. no. FG 144)
• Penicillin-Streptomycin 10,000 U/mL (Millipore cat. no. 15140 - 122);
• Trypsin - EDTA (Thermo Fisher Scientific, cat. no. 25050 – 014)
• 10 x PBS (Thermo Fisher Scientific, cat.no. 70013016);
• Costar 24 well plate (Sigma – Aldrich, cat. no. CLS3527, cell cultivation area 1.9 cm^2);
• 75 cm^2 tissue culture flasks (Sarstedt cat. no. 83.3911);
• 15 ml tubes (Sarstedt cat. no. 62.554.002).

Thawing:

1. D60 p4 cell vial was removed from -80 °C freezer and immediately transferred to 37 °C incubator. When completely thawed, the content of the vial was transferred to a 15ml tube and 14 ml warm cell culture medium (DMEM+20% FBS+ 1% penicillin/streptomycin) was slowly added.
2. Cells were centrifuged at 300 × g for 5 minutes. The supernatant was discarded, and cells were resuspended in 1 ml cell culture medium.
3. Cells were seeded onto 75 cm^2 cell culture flasks with total of 10 ml of cell culture medium (DMEM+20% FBS+ 1% penicillin/streptomycin). Growth conditions: 37 ºC, 5% CO_2.

Cultivation:

1. Cells where cultivated till confluent monolayer (>90%) was microscopically observed.
2. Cell culture medium was removed, and cells were washed once with 5ml of 1xPBS.
3. 5 ml of pre-warmed Trypsin – EDTA solution was added to the flask with a gentle rock of the flask to get complete coverage of the cell layer. Cells were incubated for 5 min, 37 ºC, 5% CO_2.
4. Cell detachment reaction was stopped by adding 2 volumes (twice the volume used for the dissociation reagent) of pre-warmed DMEM+20% FBS+ 1% penicillin/streptomycin.
5. Cells were centrifuged at 300 × g for 5 minutes.
6. Cells were resuspended in 1 ml of pre-warmed complete growth medium, and the total number of cells was determined using a hemocytometer.

Treatment of cells:

10,000 cells were seeded (DMEM+20% FBS+ 1% penicillin/streptomycin) per 1 cm^2 in a 24 well plate. After 24h cultivation (to ensure complete cell attachment to plate surface) cell medium was removed and new (DMEM+10% FBS+ 1% penicillin/streptomycin) was added.

Four concentrations of "Amphicezine" were added: 1%, 2.5 %, 5% and 10% (v/v) of the total volume of the medium. An equal volume of DMEM with PBS (as diluent) medium was added to the control cells. Each test group was run in triplicate (n=3).

Cell proliferation was monitored for 96 hours with a live cell imaging system (Cell-IQ®). Phase contrast microscopy images after 0, 3, 6, 12, 24, 48, 72 and 96 hours of cell culturing were collected.

The inhibitory effect of "Amphicezine" was calculated at the end of culturing time (96h), using the formula: Inhibition (%) =100-(100xA/B), where A stands for cell number with "Amphicezine" at the end of culturing time, and B is control (control with PBS) the cell number at the end of the culturing time.

Results and discussion. Effect of "Amphicezine" on D60 p4 line viable cell count. The results of "Amphicezine" treatment of D60 p4 cell line didn't shown a cytolytic/cytotoxic effect for dermal atypical fibroblast cells. Control cells and cells added 2.5% and 1% "Amphicezine" continued to proliferate, while cells treated with 5% and 10% "Amphicezine" didn't change cell count.

The cell growth curves indicate viable cell count increase for control and 2.5%, 1% "Amphicezine" treated cells. 93.5% inhibition on D60 p4 were shown by 10% "Amphicezine" and 79.8% inhibition were shown on 5% "Amphicezine" treated cells. Cells treated with 2.5% and 1% "Amphicezine" showed similar proliferation profile as the control (PBS) sample.

The maximal inhibition of cell growth by 10% "Amphicezine" compared to Control (PBS) was 93.5% and by 5% "Amphicezine" it was 79.8%.

The maximal inhibition of cell growth by 2.5% "Amphicezine" compared to Control (PBS) was 45.3% and by 1% "Amphicezine" it was 31.3%.

Conclusions. The effect of "Amphicezine" on viable D60 p4 cell line cell count was measured using live cell imaging as a measure of cell proliferation. Maximal inhibition on D60 p4 cells were shown by 10% "Amphicezine" (93.5%). The results show that the growth curves for viable D60 p4 cell culture didn't indicate a cytolytic/cytotoxic effect for all samples (concentrations) treated with "Amphicezine". Cells treated with 2.5% and 1% "Amphicezine" showed similar proliferation profile as the control (PBS) sample.

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SUMMARY

"AMPHICEZINE": NEW APPROACHES TO FIGHTING CANCER PRELIMINARY THEORETICAL AND EXPERIMENTAL (*IN VITRO*) MESSAGE

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Objectives - strategic objective – creation of fundamentally new inhibitors of metastasing of malignant tumors due to the radical surgeries carried out on them. Specific tactical objective – revealing the possible cytolytic and citostatic effectiveness of the drug "Amphicezine" created by us on atypical fibroblastic cells.

The effect of "Amphicezine" on viable D60 p4 line cell count was measured using live cell imaging as a measure of cell proliferation.

The results of "Amphicezine" treatment of D60 p4 cell line didn't shown a cytolytic/cytotoxic effect for dermal atypical fibroblast cells. Control cells and cells added 2.5% and 1% "Amphicezine" continued to proliferate, while cells treated with 5% and 10% "Amphicezine" didn't change cell count. The cell growth curves indicate viable cell count increase for control and 2.5%, 1% "Amphicezine" treated cells. 93.5% inhibition on D60 p4 were shown by 10% "Amphicezine" and 79.8% inhibition were shown on 5% "Amphicezine" treated cells. Cells treated with 2.5% and 1% "Amphicezine" showed similar proliferation profile as the control sample.

The effect of "Amphicezine" on viable D60 p4 cell line cell count was measured using live cell imaging as a measure of cell proliferation. Maximal inhibition on D60 p4 cells were shown by 10% "Amphicezine" (93.5%). The results show that the growth curves for viable D60 p4 cell culture didn't indicate a cytolytic / cytotoxic effect for all samples (concentrations) treated with "Amphicezine". Cells treated with 2.5% and 1% "Amphicezine" showed similar proliferation profile as the control (PBS) sample.

Keywords: "Amphicezine", inhibitors of metastasis, D60 p4 line cell.

РЕЗЮМЕ

„АМФИЦЕЗИН": НОВЫЕ ПОДХОДЫ В БОРЬБЕ С РАКОМ. ПРЕДВАРИТЕЛЬНОЕ ТЕОРЕТИЧЕСКОЕ И ЭКСПЕРИМЕНТАЛЬНОЕ (IN VITRO) СООБЩЕНИЕ

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Цель исследования - создание принципиально нового ингибитора послеоперационного метастазирования злокачественных опухолей. Конкретная тактическая задача - выявление возможной цитолитической и цитостатической активности созданного авторами препарата „Амфицезин" на атипичных фибробластных клетках.

Влияние „Амфицезина" на количество жизнеспособных клеток линии D60 p4 измеряли с использованием визуализации живых клеток в качестве меры клеточной пролиферации.

Контрольные клетки и клетки под воздействием 2,5% и 1% „Амфицезина" продолжали пролиферировать, в то время как клетки, обработанные 5% и 10% „Амфицезином", не меняли число клеток. Кривые роста клеток показывают увеличение числа жизнеспособных клеток в контроле и клеток, обработанных 2,5% и 1% „Амфицезином".

93,5% ингибирования на D60 p4 было показано 10% „Амфицезином" и 79,8% ингибирования - на клетках, обработанных 5% „Амфицезином". Клетки, обработанные 2,5% и 1% „Амфицезином", показали такой же профиль пролиферации, как и контрольный образец.

Влияние „Амфицезина" на количество жизнеспособ-

ной линии клеток D60 p4 измеряли с использованием визуализации живых клеток в качестве меры клеточной пролиферации. Максимальное ингибирование клеток D60 p4 показано 10% „Амфицезином“ (93,5%). Результаты показали, что кривые роста жизнеспособной культуры клеток D60 p4 цитолитического/цитотоксического эффекта для всех образцов, обработанных „Амфицезином“, не выявили.

რეზიუმე

“ამფიცეზინი“: ახალი მიდგომები კიბოს წინააღმდეგ ბრძოლაში. წინასწარი თეორიული და ექსპერიმენტული (in vitro) შეტყობინება

ი. ნადირაძე, ნ. ჩიგოგიძე

საქართველო-ისრაელის ერთობლივი კლინიკა “გიდმედი”; საქართველოს ტექნიკური უნივერსიტეტი, თბილისი, საქართველო

კვლევის მიზანს წარმოადგენდა ავთვისებიანი სიმსივნეების პოსტოპერაციული მეტასტაზირების პრინციპულად ახალი ინჰიბიტორის შექმნა. სპეციფიკური ტაქტიკური მიზანი - ავტორების მიერ შექმნილი პრეპარატ “ამფიცეზინის“ შესაძლო ციტოლიზური და ციტოსტატიკური ეფექტურობის გამოვლენა ატიპიურ ფიბრობლასტურ უჯრედებზე.

“ამფიცეზინის“ გავლენა სიცოცხლისუნარიანი D60 p4 ხაზის უჯრედების რაოდენობაზე განისაზღვრა ცოცხალი უჯრედების ვიზუალიზაციის გამოყენებით, როგორც უჯრედების გამრავლების შეფასების საშუალება.

საკონტროლო და 2.5% და 1% “ამფიცეზინ“-დამატებული უჯრედები განაგრძობენ გამრავლებას, ხოლო 5% და 10% “ამფიცეზინით“ დამუშავებულმა უჯრედებმა არ შეცვალეს უჯრედების რაოდენობა. უჯრედების ზრდის დიაგრამები მიუთითებენ სიცოცხლისუნარიანი უჯრედების რაოდენობის ზრდაზე კონტროლში და 2.5%, 1% “ამფიცეზინით“ დამუშავებისას.

D60 p4 93.5% ინჰიბირება მიღებულია 10% “ამფიცეზინით“ და 79.8% - 5% “ამფიცეზინით“ დამუშავებულ უჯრედებში.

“ამფიცეზინის“ გავლენა სიცოცხლისუნარიანი D60 p4 ხაზის უჯრედების რაოდენობაზე იზომება ცოცხალი უჯრედების ვიზუალიზაციის გამოყენებით, როგორც უჯრედების გამრავლების შეფასების საშუალება. მაქსიმალური ინჰიბირება D60 p4 უჯრედებზე გამოავლინა 10% “ამფიცეზინმა“ (93.5%). 2.5% და 1% “ამფიცეზინით“ დამუშავებულმა უჯრედებმა აჩვენეს პროფილის ისეთივე ზრდა, როგორც საკონტროლო ნიმუშმა.

SUPPORTIVE PHARMACOTHERAPY FOR SYSTEMIC AUTOIMMUNE DISEASES WITH HYPERIMMUNOCOMPLEX SYNDROME (EXPERIMENTAL RESEARCH)

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Systemic autoimmune diseases are pathological processes that are characterized by the development of a stable humoral or cellular response directed against their own antigens, which leads to the defeat of even the whole organism [29].

Systemic autoimmune diseases are protracted (chronic) due to the constant presence of antigen in the body, because it is a normal component of cells [9,19]. The etiology of systemic autoimmune diseases is still unclear [3,22].

It is believed that one of the reasons for the development of systemic connective tissue diseases and autoimmune pathologies is the increase in the activity of immunotropic infections and the state of the body's humoral immune response [8,15].

One of the important indicators that characterize the state of the body's humoral immune response is the level of immune complexes that are formed by the direct connection of exogenous and endogenous antigens with antibodies [11].

Any immune response in the human body is accompanied by the formation of immune complexes "antigen + antibody", which are phagocytosed and eliminated from the body [5, 10].

In systemic autoimmune diseases, immune complexes are represented by autoantibodies, autoantigens and other components that can be deposited in the walls of blood vessels, tissues of organs and systems, causing the formation of the syndrome of immune-dependent pathology [8,9,23].

Immune complexes activate the complement system with the formation of circulating immune complexes that are capable of developing aggressive reactions, act as a pathogenic factor in the development of inflammatory and autoimmune processes, vascular lesions [4,10,14,30].

In systemic autoimmune diseases, elevated levels of circulating immune complexes can be maintained for a long time, which adversely affects the reactivity of the organism [44,45,42].

It is well known that the suppression of the excitatory ability of phagocytes or lack of its activation is a favorable factor in the formation of hyperimmunocomplex syndrome [7].

Important tasks of pharmacotherapy of systemic autoimmune diseases include the study of immune-dependent complexes on the background of hyperimmunocomplex syndrome.

Among the systemic autoimmune diseases are systemic lupus erythematosus, systemic vasculitis, psoriasis, rheumatoid arthritis [6,12,13,19-21].

Thus, the correction of phagocytic-dependent processes may be a promising direction in the pharmacotherapy of hyperimmunocomplexemia.