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SIMPLE METHODS FOR SCREENING OF POTENTIAL ANTICANCER AGENTS AND OTHER REMEDIES

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The aim of this study was to elaborate quick, simple and low-cost methods for preliminary screening of a broad spectra of drugs and medical foods suggested to be used in clinical oncology. **Materials and methods.** The methods have been elaborated are based on originally modified carcinolysis reaction (I group) and on viscosimetry and spectrofotometry of the lysates of tumor tissue and tissue of potential side toxicity target organs prepared with certain detergent (II group). The methods were tested using the following experimental tumor models: Ehrlich carcinoma (ascitic and solid form), Lewis lung carcinoma, Ca755 mammary carcinoma, B16 melanoma, P388 and L1210 lymphatic leukemia; in rats – Guerin carcinoma (a standard strain and original cisplatin-resistant and doxorubicin-resistant substrains), Walker W-256 mammary carcinosarcoma. **Results.** In the carcinolysis methods, in each case, the sign (+ or -) of carcinolysis modulations and tumor growth retardation indexes were identical if the same food or drug was applied. At the same time, absolute value of carcinolysis activity did not correlate significantly with growth retardation index. The viscosimetry methods adequtely reflected a priori known therapeutic and toxic effects of the anticancer drugs. **Conclusion**. So, both groups of the methods show adequacy to the task of preliminary screening.

Key words: anticancer drugs, medical foods, non-clinical investigations, screening, drug sensitivity, side toxicity, in vivo methods.

INTRODUCTION

For today, non-clinical development of potential anticancer agents is a lengthy and expensive process. To save time and costs, different methods of pre-non-clinical selection (screening) are used, having a goal to perform full-scale experiments only with the most effective agents have been found in the screening tests. But, in contrast to non-clinical development itself, pre-non-clinical investigations are not standardized. Because of this, different laboratories apply different approaches and their data are sometimes non-comparable to one another. In addition, the most of these methods suppose *in vitro* tests (cell culture etc.) and so their data may be inadequate to *in vivo* effect of the same substances. At last, it is quite impossible to predict optimal doses and schedules of *in vivo* application on the base of these data.

In this connection, we are developing a complex of methods for hastening screening which enable to evaluate a broad spectra of substances and applying doses and schedules and to do it comparatively quickly and by comparatively low costs. For today, our institute is an owner of the following process patents of this group.

MATERIALS AND METHODS

To test potential drugs not suggesting direct cytotoxic effect on the tumor cells, as well as to test foods being designed for the cancer patients' clinical and usual nutrition, we apply the methods have been described in the patents [1-3]. These methods are based on an original modification of known carcinolysis reaction (non-immune cytolysis of the cancer cells by blood serum or plasma) [4]. This modification has essentially increased precision, repeatability, diagnostic and prognostic significance versus it's classic variant.

Carcinolysis reaction (CL-reaction) was performed accordingly to the patent [1], taking into account the following peculiarities discovered in our previous studies: 1) CL-reaction is mainly caused not by exogenous perforation of the plasmalemma but by programmed cell death (autophagia or apotosis) induced by the serum regulatory factors; 2) adequate cell reaction on the regulatory factors needs safe extracellular matrix; 3) concentration of the target cells in the reaction mix is an algebraic sum of two controversial processes: cytolysis itself and cell division, and so CL-reaction is a quasiperiodic process, its period depends on the spectra of the regulatory factors and may not be predicted in each real experiment.

Reaction was performed in 96-wells round-

bottomed serologic plates (unlike the classic method [4], where the hematologic melangeurs were used and thus injection of the stop solution was impossible). Working solutions (1:100) of serum of the intact animals (non-inbred mice or rats) or healthy animals of the same species, sex and age been administered during 7 days with the drug or food being tested [2, 3], as well as the working suspension (2×10^6 cells/ml) of the target cells (ascite Ehrlich carcinoma) were prepared in saline. Unlike the classic method, target cells were not washed off the ascitic fluid and thus native extracellular matrix was saved. In each well, 100 mcl of serum working solution and then 100 mcl of working target cells suspension were instilled. Unlike the classic method, 4 wells with identical reaction mix were made for each serum sample in each working line of wells. Then the plates were incubated under 37°C. After the incubation deadline (30 min — for the first well in each line, 60 min — for second, 90 min — for third и 120 min — for fourth), reaction was stopped (unlike the classic method) by injection of 10 mcl potassium dichromate saturated solution. Then the well content was carefully pipetted and cell concentration was calculated using haemocytometer or any automatic cell-counting device. When cell count in the fourth wells in all lines over, obtained amounts were summed in each line individually and these sums were used to calculate carcinolysis index (CI) of each experimental sample versus intact control with the following formula: (Σcontrol – Σexperiment) / Σcontrol. Ex facte, CI is positive when cell concentration decrease (cytolysis prevails) and negative when cell concentration increase (cell division prevails).

To test any potential drugs, especially ones suggesting direct cytotoxic effect as a key therapeutic factor, we have designed the methods described in the patents [5, 6]. All measurements we perform with tumor (or an organ being a potential target for the preparation's side toxicity) materials of non-treated (control) and treated (experiment) animals. Tumor tissue or tissue of a target organ (preliminary weighted) we subject to lysis with an ionic detergent. As a detergent, for example, saturated NaCl solution in preliminary prepared saturated urea solution is handy. In these tissue lysates, we measure DNA content and lysate's viscosity. Then, we calculate coefficient of tumor sensitivity to the tested preparation (SC) or coefficient of side toxicity (TC) of tested preparation as to the certain organ using the following formula:

$$SC(TC) = \left(\frac{m\Delta t C_{DNA}}{V}\right)_{Control} : \left(\frac{m\Delta t C_{DNA}}{V}\right)_{Experiment}$$

where m — mass of the tissue sample; V — obtained lysate's volume; Δt — duration difference between equal volume of a lysate and pure lytic solution passing through a viscosimeter; C_{DNA} - DNA concentration in a lysate or any value being in direct proportion to DNA concentration, for example, lysate extinction under 260 nm. The calculation result we interpret by the following way: if SC>1 (TC>1), it shows therapeutic sensitivity of tested tumor to tested preparation (side toxicity of tested preparation as to tested organ), and this sensitivity (toxicity) is the higher the higher is SC (TC) value; if SC<1 (TC<1), this shows tested preparation promotes cell survival in tested tumor (organ). This method enables to evaluate therapeutic sensitivity of many kinds of tumors and side toxicity as to different organs much quicker, easier and more cheaply than generally and, in addition, with a standard method for all cases. Thus, comparison of all results becomes quantitative.

Experimental tumors. The strains of the experimental transplantable tumors been tested in our experiments are listed in the descriptions of the separate experiments. All strains were transplanted by the commonly used methods. Antitumor effect of tested agents was evaluated by average tumor mass and/or dynamics of average tumor volume for the solid tumors, by number and volume of metastatic lesions in lungs for mouse Lewis lung carcinoma and B16 mouse melanoma, by ascites cellularity for ascitic tumor strains.

Laboratory animals. All animals were bred in the vivarium of R.E.Kavetsky IEPOR. Species (mice or rats), line and sex of the animals was in agreement with the common standards for corresponding tumor strains. Initial age of the animals was near 2 months in all experiments.

Statistical treatment of the data was performed using Student's t-criterion and, where it was necessary, exact Fisher's method.

RESULTS AND DISCUSSION

The method based on the CL-reaction was tested in healthy and tumor-bearing mice and rats administered with the following classes of foods and drugs [3, 7]: different composites made on the base of green tea extract (GTE), red wine lees (W) and lemon peel (L); soybean curd-like thermally treated (CLT) and rough (CLR) products; bilberry paste; non-cytotoxic immunomodulators.

The following tumor strains were used: in mice – Ehrlich carcinoma (ascitic and solid form), Lewis lung carcinoma, Ca755 mammary carcinoma, B16 melanoma, P388 and L1210 lymphatic leukemia; in rats – Guerin carcinoma (a standard strain and original cisplatin-resistant and doxorubicin-resistant substrains), Walker W-256 mammary carcinosarcoma.

In the healthy animals, CL-index of treated animals versus intact ones varied within the limits of (-19%) – (+55%) (Table 1). In the tumor-bearing animals, index of tumor growth retardation (TGR-index) achieved with the tested agents varied within the limits of (-85%) – (+100%) (Table 2).

Table 1

Effect of the tested agents on carcinolysis activity of the healthy animals' blood serum

Tested agent	Species and strain of animals	Carcinolysis index versus intact animals, %
GTE	Mice, non-inbred	28*
Nano-GTE	Mice, non-inbred	20*
Nano-GTEW	Mice, non-inbred	21*
Nano-GTEWL	Mice, non-inbred	16*
CLT	Rats, non-inbred	31*
CLR	Rats, non-inbred	-9*
Blackberry paste	Rats, non-inbred	19*
Blackberry paste	Mice, C57Bl/6	55*
Non-cytotoxic immunomodulator	Rats, non-inbred	22*
Non-cytotoxic immunosuppressor	Rats, non-inbred	-19*

Note: * - difference versus intact animals is significant (P<0,05).

Table 2

Antitumor effect of tested agents

Tested agent	Species and strain of animals, tumor strain	Tumor growth retardation index, %
GTE	Non-inbred rats, Cisplatin-resistant Guerin carcinoma	22*
Nano-GTE	Non-inbred mice, sarcoma 180, solid	23*
Nano-GTE	Non-inbred mice, Ehrlich carcinoma, solid	21*
Nano-GTE	CDF1 mice, P388 ascite	9
Nano-GTE	CDF1 mice, L1210 ascite	10
Nano-GTE	C57Bl/6 male mice, LLC (metastasis volume)	54*
Nano-GTE	Non-inbred rats, Guerin carcinoma	28*
Nano-GTE	Non-inbred rats, Cisplatin-resistant Guerin carcinoma	34*
Nano-GTEW	CDF1 mice, P388 ascite	29*
Nano-GTEW	Non-inbred rats, Guerin carcinoma	26*
Nano-GTEW	C57Bl/6 female mice, Ca755	28*
Nano-GTEW	C57Bl/6 male mice, LLC (metastasis volume)	67*
Nano-GTEW	C57Bl/6 female mice, LLC (metastasis volume)	100*
Nano-GTEWL	C57Bl/6 female mice, Ca755	27*
CLT	Non-inbred rats, Walker carcinosarcoma	48*
CLT	Non-inbred rats, Guerin carcinoma	21*
CLR	Non-inbred rats, Walker carcinosarcoma	-26*
CLR	Non-inbred rats, Guerin carcinoma	-2
Blackberry paste	Non-inbred rats, Guerin carcinoma	49*
Blackberry paste	Non-inbred mice, Ehrlich carcinoma, ascite	13**
Blackberry paste	C57Bl/6 mice, LLC (primary tumor)	49*
Non-cytotoxic immunomodulator	Non-inbred rats, Walker carcinosarcoma	49*
Non-cytotoxic immunosuppressor	Non-inbred rats, Walker carcinosarcoma	-85*

Note: * - difference versus control is significant (P<0,05); ** - difference versus control is less significant (0,05<P<0,1).

In each case, the sign (+ or -) of these effects was identical if the same food or drug was applied. At the same time, absolute value of CL- and TGR indexes did not correlate significantly, if essentially different agents were tested.

In contrary to this, if the tested agents were obtained by quantitative variations of components content in the same products, quite different picture was obtained (Fig. 1).

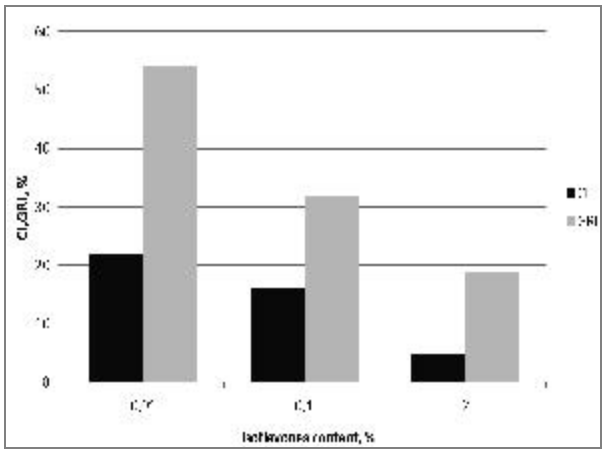

Fig. 1. Correlation between serum carcinolysis index (CI) in healthy rats and tumor growth retardation index (GRI) in Walker carcinosarcoma bearing rats both fed with soybean protein isolate (90% of protein in dry mass) with different content of soybean isoflavones.

Thus, our CL-test was found to be suitable for primary screening of foods and non-cytotoxic drugs but not for replacement of standard oncologic experiments.

The method of lysates viscosimetry was tested in tumor-bearing rats with Guerin carcinoma (a standard strain and original cisplatin-resistant substrain with >80% resistance).

SC was evaluated as to each strain, comparing tumor tissue after cisplatin application (experiment) with tumor tissue of non-treated animals (control). There was obtained the following (M±m): SC(standard strain) = 2.29±0.21; SC(resistant strain) = 1.10±0.18 ($P<0.05$). As it is seen, SC value adequately reflects real drug-sensitivity of a tumor.

TC was evaluated in heart and renal tissue of the rats with a standard Guerin carcinoma. As it is commonly known, cisplatin is nephrotoxic but not cardiotoxic substance, in contrary to doxorubicin which is high cardiotoxic and low nephrotoxic. Renal and heart tissues of cisplatin (doxorubicin) treated animals were compared with the analogous tissues of non-treated animals. There was obtained the following (M±m).

For cisplatin: TC(cardiac) = 0.56±0.13; TC(renal) = 1.67±0.12 ($P<0.01$). As it is seen, TC level adequately shows significant renal toxicity of cisplatin and cisplatin-induced compensatory reactions in cardiac muscle.

For doxorubicin, an opposite situation was shown: TC(cardiac) = 1.75±0.16; TC(renal) = 1.09±0.19 ($P<0.05$).

Other possible applications of CL- and viscosimetric methods. Seeing the results have been presented above, naturally appears an idea to use our methods in different fields of clinical oncology, namely: individual diagnosis, individualized therapy and therapy monitoring. But, most probable, these methods are practically useless in these fields.

Especially, CL-reaction in our modification, as we think, may be applied to diagnosis at the initial stage of a patient checkup, but only as a kind of a non-specific cancer test, with all precautions which are common for all tests of this group (taking into account all concomitant chronic and recent acute diseases, consumed drugs, alcohol and some foods, etc.).

Direct application of CL-reaction to the individualized therapy is impossible. It may be realized only indirectly, during the therapy monitoring. But this way is accompanied by a few of fundamental difficulties, and the main of these are the followings: a) side toxicity of the anticancer drugs can disturb CL-activity; b) we can verify therapeutic activity only post factum and only for thous therapeutic agents which have been also applied. That's why, we not regard CL-reaction as a prospective method to individualize the anticancer therapy.

Therapy monitoring may be executed only during the interval between the therapy seances, when all anticancer drugs an toxic metabolites would be eliminated out of an organism. But even under these conditions CL-reaction can not add any essential information to one obtained by the standard diagnostic methods, such as X-ray tomography, ultrasonic methods, nuclear-magnetic-resonance tomography.

The methods of lysates viscosimetry are a priori useless for a diagnosis. Their applicability to individualized therapy and therapy monitoring is very doubtful due to the same difficulties that were described above for CL-method.

CONCLUSIONS

Thus, the data of our studies permit to suggest that the methods described above are able to form a standardized complex for quick, simple and reasonably priced pre-non-clinical screening of wide groups of anticancer drugs and medical foods and as well as their applying doses and schedules.

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ПРОСТІ МЕТОДИ СКРИНІНГУ ПОТЕНЦИАЛЬНИХ ПРОТИПУХЛИННИХ АГЕНТІВ ТА ІНШИХ ЛІКУВАЛЬНИХ ЗАСОБІВ

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Мета: розробити швидкі, прості та маловитратні методи для попереднього скринінгу широкого спектру лікарських препаратів та продуктів лікувального харчування, передбачуваних для застосування у клінічній онкології. **Матеріали та методи.** Розроблені методи засновані на оригінальній модифікації реакції канцеролізу (I група) та на віскозиметричному й спектрофотометричному дослідженні лізатів пухлинної тканини і тканин потенційних органів-мішеней побічної токсичності, приготованих за допомогою детергентів (II група). Методи були апробовані на таких експериментальних пухлинних моделях: на мишах — карцинома Ерліха (асцитна та солідна форми), карцинома легень Льюіс, карцинома Са755 молочної залози, меланома В16, лімфолейкози Р388 та L1210; на щурах – карцинома Герена (стандартний штам та оригінальні субштами, резистентні до цисплатину і доксорубіцину), карциносаркома Уокер W-256 молочної залози. **Результати.** При застосуванні методів, заснованих на реакції канцеролізу, в усіх випадках, знаки (+ або -) модуляції канцеролітичної активності та індексу гальмування пухлинного росту були однаковими при застосуванні одного й того самого продукту або препарату. Водночас, абсолютне значення канцеролітичної активності не корелювало з індексом гальмування пухлинного росту. Віскозиметричні методи адекватно відображали a priori відомий терапевтичний і токсичний ефект протипухлинних препаратів. **Висновок.** Таким чином, обидві групи методів показали свою адекватність задачі попереднього скринінгу.

Ключові слова: протипухлинні препарати, продукти лікувального харчування, доклінічні дослідження, скринінг, лікарська чутливість, побічна токсичність, дослідження in vivo.

ПРОСЫЕ МЕТОДЫ СКРИНИНГА ПОТЕНЦИАЛЬНЫХ ПРОТИВООПУХОЛЕВЫХ АГЕНТОВ И ДРУГИХ ЛЕЧЕБНЫХ СРЕДСТВ

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Цель: разработать быстрые, простые и малозатратные методы для предварительного скрининга широкого спектра лекарственных препаратов и продуктов лечебного питания, предполагаемых для применения в клинической онкологии. **Материалы и методы.** Разработанные методы основаны на оригинальной модификации реакции канцеролиза (I группа) и на вискозиметрическом и спектрофотометрическом исследовании лизатов опухолевой ткани и тканей потенциальных органов-мишеней побочной токсичности, приготованих за допомогою детергентів (II група). Методы были апробированы на следующих экспериментальных опухолевых моделях: на мышах — карцинома Эрлиха (асцитная и солидная формы), карцинома легког Льюис, карцинома Са755 молочной железы, меланома В16, лимфолейкозы Р388 и L1210; на крысах – карцинома Герена (стандартный штамм и оригинальные субштаммы, резистентные к цисплатину и доксорубицину), карциносаркома Уокер W-256 молочной железы. **Результаты.** При использовании методов, основанных на реакции канцеролиза, во всех случаях, знаки (+ або -) модуляции канцеролитической активности и индекса торможения опухолевого роста были идентичны при применении одного и того же продукта или препарата. Вместе с тем, абсолютное значение канцеролитической активности не коррелировало с индексом торможения опухолевого роста. Вискозиметрические методы адекватно отражали a priori известный терапевтический и токсический эффект противоопухолевых препаратов. **Вывод.** Таким образом, обе группы методов показали свою адекватность задаче предварительного скрининга.

Ключевые слова: противоопухолевые препараты, продукты лечебного питания, доклинические исследования, скрининг, лекарственная чувствительность, побочная токсичность, исследования in vivo.