UDC 546.719: 54.024: 57.084:616-006.699 doi: https://doi.org/10.15407/ubj94.03.092

NEW FORMULATION AND ACTIVITY OF RHENIUM-PLATINUM ANTITUMOR SYSTEM

N. I. SHTEMENKO[1,2]✉, D. E. KYTOVA[1], O. V. BERZENINA[1], O. I. HRABOVSKA[1,3], A. V. SHTEMENKO[1]

[1]Ukrainian State University of Chemical Technology, Dnipro, Ukraine;
[2]Oles Honchar Dnipro National University, Ukraine;
[3]SI "Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine", Dnipro, Ukraine;
✉e-mail: n.shtemenko@ukr.net

Received: 16 November 2021; **Revised:** 10 January 2022; **Accepted:** 29 September 2022

Two-component Rhenium-Platinum system (Re-Pt system) is based on administration of a cluster dirhenium(III) compound and cisplatin to tumor bearing animals followed by a significant antitumor effect and decreased toxic effect of cisplatin on normal cells. The aim of this work was to obtain solid lipid nanoparticles (SLN) from surface lipids (waxes) of Chelidonium majus L. (Papaveraceae) leaves and to estimate whether capsulation of dirhenium(III) as a component of the Re-Pt system into SLN will affect its antitumor activity and red blood cells (RBC) morphology in a rat model of Guerin's carcinoma growth. Fourier-transform infrared spectroscopy, gas-liquid chromatography, microscopy, light scattering were used in the research. Solid lipid nanoparticles were obtained, characterized, loaded with cluster dirhenium(III) and being introduced together with cisplatin to rats with Guerin's carcinoma resulted in RBC morphology preservation and a significant decrease in tumor weight. It was concluded that the lipid coating of the rhenium cluster compound did not reduce the antitumor effect of the Re-Pt system and protected RBC from toxic cisplatin influence. A new formulation of the Re-Pt system is proposed.

K e y w o r d s: solid lipid nanoparticles, surface lipids, rhenium cluster compound, rhenium-platinum antitumor system, carcinoma.

Rhenium-platinum antitumor system (Re-Pt system) is a two-component system (based on a dirhenium(III) cluster compound and cisplatin (cisPt)) first presented by us in the journal Anticancer Research [1]. It showed not only significant antitumor efficacy of the system, but also decreasing toxic effects of cisPt in experimental animals. Dichlorotetra-μ- isobutyratodirhenium(III) (I) and other dirhenium compounds were studied by us in different biochemical experiments in liposomal forms, as summarized in the review [2]. Lipid coating in liposomes loaded by dirhenium(III) clusters used in these trials *in vitro* and *in vivo* was phosphatidylcholine. Together with the powerful synergistic or additive anticancer activity of I with cisplatin, the application of substances containing both metals, platinum and rhenium, eradicated toxic effects of cisplatin being applied alone, such as hemato-, hepato- and nephrotoxicity. In these experiments, the dirhenium(III) clusters showed themselves as powerful antiradical and antioxidant reagents due to the unsaturation of the quadruple bonds in their structures. Liposomal technique elaborated for the dirhenium(III) clusters [3] was necessary to prevent hydrolytic processes of the compounds [4]. Solid lipid nanoparticles (SLN) were defined as solid particles, where the drug is confined to a cavity surrounded by a solid lipid layer. SLN present the basis of on oral administration that is one of the preferred ways for drug delivery. Biodegradable, biocompatible SLN are used for drug incorporation and delivery in treatment of tumors [5, 6]. *Chelidonium majus* L. (Papaveraceae) (CM) has a long history of application in Chinese herbal medicine and in European countries and is known to contain secondary metabolites that exhibit a spectrum of biological activity, including antitumor properties [7-10]. Taking into account all the above, the aims of the present work were to elaborate preparation and to characterize SLN from surface lipids (waxes) of

CM leaves containing dirhenium(III) compound I; to investigate the obtained SLN in the model of tumor growth together with cisplatin in order to explore whether the capsulation of one component of the Re-Pt system in SLN forms would influence the antitumor and red blood cell-supporting activity of the Re-Pt antitumor system.

Materials and Methods

Reagents. Cisplatin (CisPt) was purchased from Ebewe, Austria; dichlorotetra-μ-isobutyratodirhenium(III) (I) was synthesized according to the procedure described in [11]. Fresh leaves of *Chelidonium majus* L. (Papaveraceae) (CM) collected in the Botanic garden of Dnipro National University were used for the extraction of surface lipids. Cells of Guerin's carcinoma (T8) were supplied by the R. E. Kavetskiy Institute of Experimental Pathology, Oncology and Radiology, NAS of Ukraine (Kyiv, Ukraine). Wistar rats weighing 100-120 g obtained from the vivarium of Dnipro Agricultural University were used in experiments with tumor growth.

SLN preparation and analysis. Plant surface lipids from leaves of CM were extracted by hot chloroform, analyzed by GC-MS of their methyl esters, and SLN were prepared according to [3, 12]. In short: the dried surface lipids extracts in chloroform were placed in a round-bottom flask and dried under mild conditions using a rotary evaporator to obtain a lipid film. Solution of **I** in chloroform was added with final ratio of weight lipid: rhenium compound of 8:1. The solvent was removed to obtain the **I** – lipid film. Physiological saline then was added to the film and stirred for 10 min to obtain a suspension that was treated by ultrasound for 10 min on Ultrasonic Perkin Elmer 3200 R to obtain a suspension of SLN loaded with I. Encapsulation efficiency (EE) of SLN was calculated as liposomes according to [3] by measuring the amount of free, not capsulated I by UV-vis spectrophotometry. Concentration of free I was determined in supernatant after centrifugation of an aliquot of SLN suspension at 20 000 g for 4 min at 20°C. EE was calculated as a percentage of capsulated and initial drug concentrations and reached 96,6%. FTIR ATR spectra (Fourier Transform Infrared Spectroscopy Attenuated Total Reflection) of SLN, I and lipids were measured on the IR spectrophotometer Thermo Nicolet Nexus 870 FTIR, USA. Surface morphology of the nanoparticles was estimated by transmission electron microscopy (TEM) using a JEM- 1011 microscope (Japan) with an electron kinetic energy of 100 keV. Particle size (mean volume diameter) and zeta potentials of all formulations were measured by dynamic light scattering using a Zeta Sizer Nano S (Malvern, UK 4 mW He-Ne laser, $\lambda 0 = 633$ nm, $\theta = 173°$) at 25ºC. Each sample was diluted with distilled water in order to obtain the appropriate concentration of SLN and each sample was measured in triplicate.

Animal model. Animal model was described in [13]. Animal experiments were planned and permitted by the Ministry of Education and Science of Ukraine. Tumor transplantation was performed by subcutaneous injection of 20% Guerin's carcinoma (T8) cell suspension in the thigh area of Wistar rats. Control group of tumor-bearing animals was not subjected to any treatment. A single intraperitoneal administration of cisPt at a dose of 8 mg/kg was made on the 9th day after tumor inoculation (group (T8 + cisPt)). The intraperitoneal administration of formulations of **I** in solutions (sol), liposome (l) and SLN forms (np) in dose 7 μM/kg (groups (T8 + [**I**]sol + cisPt), (T8 + [**I**]l + cisPt), (T8 + [**I**]np + cisPt)) started on the 3rd day after inoculation of tumor cells and was repeated every 2 days until day 21. The number of animals in each group was 15. Volumes of tumors were estimated *in vivo* every day in all experiments and groups. On day 21, animals were sacrificed under chloroform narcosis according to the rules of the Ethics Committee and the tumors were excised and weighed. Morphological forms of red blood cells (RBC) in blood of animals were counted according to commonly accepted methods.

Descriptive statistics were used for statistical analysis of the obtained material: comparisons of average values of variables were carried out using parametric methods (Student's *t*-test) on the normal distribution of data features expressed on the interval scale. The Shapiro-Wilk method was used to check that the type of feature distribution was correct for the law of normal distribution. The differences obtained by the method of Paired Comparisons were considered valid at $P < 0.05$.

Results and Discussion

Surface lipids of CM were composed of two major components – hydrocarbons (60%) and fatty acids (30%) (Fig. 1). The components of the surface lipids were mainly long-chained, the lipid fraction was solid ($T_{melt.}$ = 65–70°C).

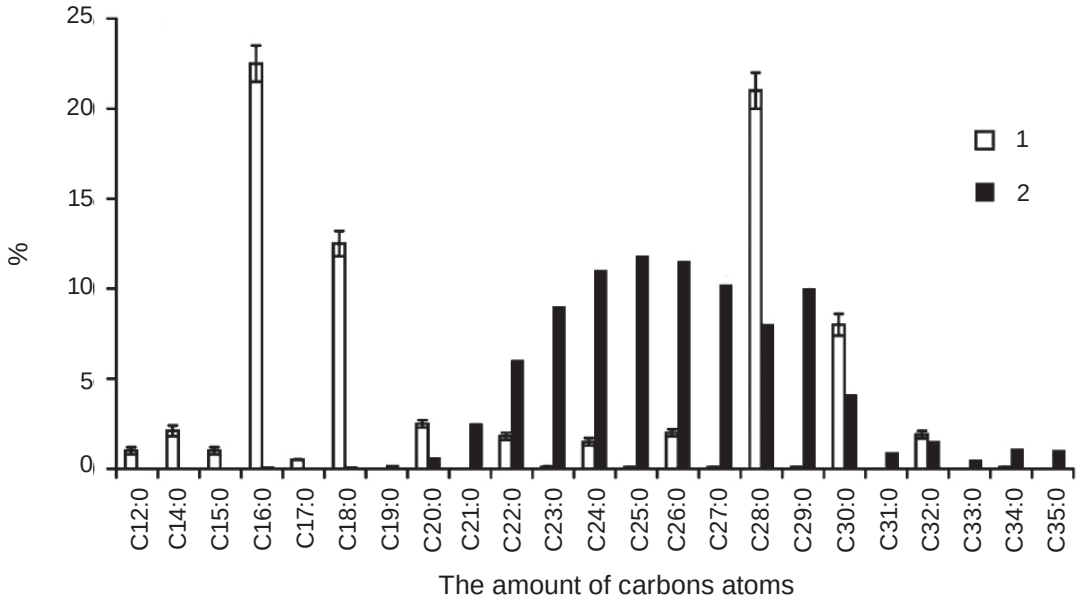

*Fig. 1. Composition of fatty acids (**1**) and hydrocarbons (**2**) of leaves of Chelidonium majus L. (in % to total) determined by GC MS method*

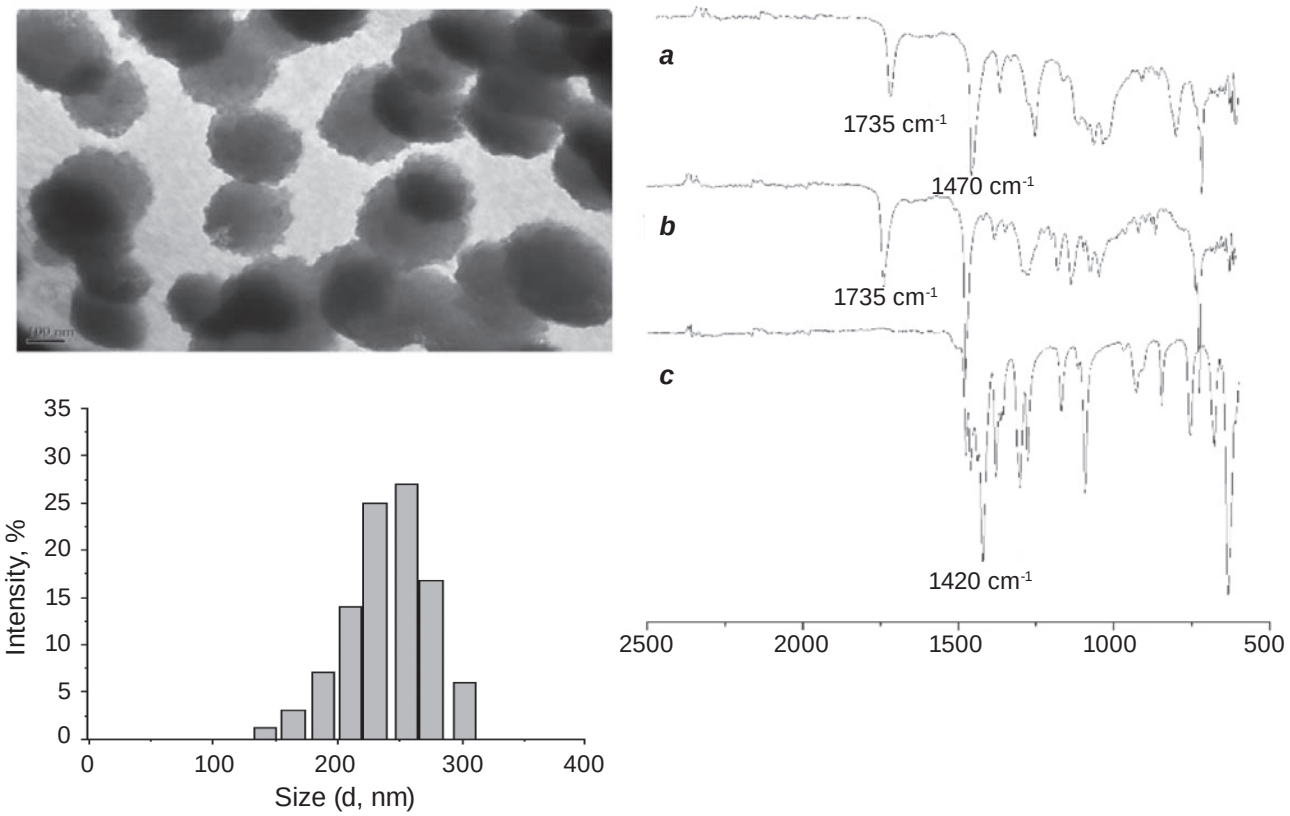

*Fig. 2. Morphology, size distribution of **I**/Chelidonium majus L. surface lipids SLN and IR-spectra of surface lipids from Chelidonium majus L. (**a**), SLN (**b**) and **I** (**c**)*

SLN were obtained. SLN TEM images (Fig. 2) showed that the SLN loaded with **I** had round shape. The average diameter of SLN obtained was 255 ± 15 nm (n = 3). The mean zeta potential of SLN was – the aims of this work were to obtain solid lipid nanoparticles (SLN) from surface lipids (waxes) of Chelidonium 27.05 ± 0.25 mV. FTIR ATR spectra (Fig. 2) of surface lipids (*a*) and SLN (*b*) are very similar, but different from the spectrum of **I** (*c*). Spectra (*a*) and (*b*) had characteristic stretching bands in the area 1735 and 1470 cm^{-1} that belong to the free COOH group of solid fatty acids and are typical for surface lipids of plants [14]. This was confirmed by a progression of the bands in the area 1380–1040 cm^{-1}. In contrast to (*a*) and (*b*), the spectrum of **I** (*c*) had no signals representing free carboxylic groups of fatty acids in the surface lipids, but vibrations of the coordinated carboxylic groups with conjugated C – O bonds (1420 cm^{-1}) were observed [15]. These data allowed us to conclude a dominating covering of molecules of **I** by the lipid layer, i.e. I is inside the lipid capsule.

Introduction of the SLN showed antitumor and RBC supporting activity (Fig. 3, Table). Administration of the system, where both components were in solutions (group T8 + [**I**]sol +cisPt), did not show good results, the tumor growth was not effectively inhibited; we still observed an increasing tumor growth on the last days of the experiment. The encapsulation of **I** into a liposome and introduction of these liposomal forms together with cisPt (group T8 + [**I**]l + cisPt) led to very good results and reached 99.76% inhibition of tumor growth. Introduction of a newly obtained SLN under the same conditions led up to 99.52% inhibition of the tumor weights, that was lower but may be considered also as effective antitumor activity.

SLN formulations are typically made from fatty acids (e.g., palmitic acid, stearic acid), fatty alcohols, mono-, di- and triglycerides, vegetable oils [15]. There are some examples, where natural lipids called waxes are used as a lipid component of SLN formulation, but mainly for the food sector [16]. To our knowledge, just a few studies have been reported

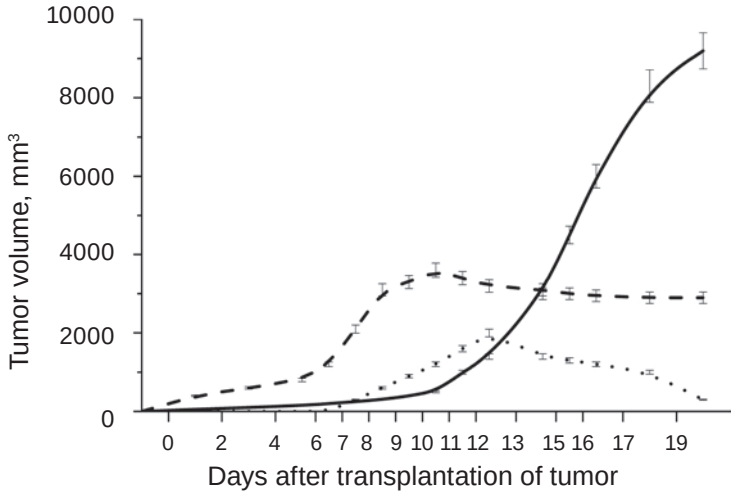

Fig. 3. Kinetics of the tumor growth under influence of the Re-Pt antitumor system: ── *T8+[**I**]sol+ cisPt; ;* ····· *T8+[I]l + cisPt;* - - - *T8+[**I**]np + cisPt*

T a b l e. Weights of tumors and morphological forms of RBC in blood of experimental animals

Groups	Weight of tumors, g	Discocytes, %	Damaged RBC, %
Control	–	65 ± 6	12 ± 2
T8	45.0 ± 2.0	9 ± 2	59 ± 5
T8+[**I**]sol + cisPt	24.0 ± 2.0	50 ± 5	37 ± 4
T8+[**I**]l + cisPt	0.11 ± 0.01	65 ± 6	12 ± 2
T8+[**I**]np + cisPt	0.21 ± 0.01	60 ± 5	10 ± 1

using plant lipids (waxes) for SLN preparation loaded with chemotherapeutic agents. Nevertheless, they demonstrated excellent drug encapsulation efficiencies while maintaining the plasma drug concentration during a prolonged period [17, 18]. In this vein, stable SLNs from surface lipids of CM were made first and their antitumor properties were expectable. CM contains as major secondary metabolites isoquinoline alkaloids, such as sanguinarine, chelidonine, chelerythrine, berberine coptisine, several flavonoids and phenolic acids, etc. CM extracts and purified compounds exhibited antiviral, antitumor and antimicrobial properties both in vitro and *in vivo* [7, 8, 10]. Some isoquinolines were shown to be telomeric G-quadruplex stabilizers and potent telomerase inhibitors [9]. The results obtained by us together with known properties of CM extracts open an opportunity to use SLNs and green chemistry (extracts) together in oncological practice. The essential antitumor efficacy was shown previously for the Re-Pt antitumor system including a range of the dirhenium(III) compounds introduced in liposomal forms together with cisPt [2]. In this work, we have observed an effective reduction of the tumor sizes in another lipid encapsulation of the dirhenium(III) compound. Comparison of these results with those obtained earlier definitely supports the idea of ability of dirhenium compounds to realize their anticancer and other biochemical potential only in conditions of protection from hydrolysis. We did not observe dramatic differences between nanopreparations with different kinds of lipid components. Obtained results demonstrated the possibility to encapsulate partly the Re-Pt system into a solid lipid coating and to enhance the antitumor properties of the system, when compared with the experiments with solutions. We consider these results of great importance, as SLN loaded with I were active in the model of tumor growth and opened a nice perspective to work with solid preparations. The development of the tumor was followed by a significant morphological shift in RBC population (Table) from the major group of discocytes to the side of damaged RBC. The introduction of **I** as in conventional liposomes and in SLNs in these experiments led also to practically normal morphological picture of RBC. Earlier it was shown that the introduction of **I** to tumor-bearing animals led to significant support of the system of RBC [19] that was explained by a possible influence of **I** on erythropoietin production and protection of erythrocytes from hemolytic destruction *in vitro* [14, 20] that took place due to the powerful antioxidant activity of the quadruple bond. Thus, it means that the properties of the rhenium cluster compounds were retained by lipid coatings as in liposomes as in nanoparticles that resulted in significant abilities of the introduced substances to support RBC (to protect from toxic influence of cisPt) in blood of tumor-bearing animals. An the whole, encapsulation of the Re-Pt system into solid lipid coating did not dramatically lower the effect of the Re-Pt system in the model of tumor growth, resulting in a significant decrease of tumor weight and actively supporting RBC in tumor-bearing animals.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

Funding. The authors wish to thank the Ministry of Education and Science of Ukraine (grants 0107U000528, 0111U000111 and 0122U001220), the National Science Foundation (NSF) the Interdisciplinary Nanotoxicity Center (grant HRD-0833178), for financial support and personally Prof. Dr. D. Leszczynska (Jackson State University, USA) for assistance in experimental work.

НОВА ФОРМА ВВЕДЕННЯ ТА АКТИВНІСТЬ РЕНІЙ-ПЛАТИНОВОЇ ПРОТИПУХЛИННОЇ СИСТЕМИ

Н. І. Штеменко[1,2]✉, Д. Є. Китова[1], О. В. Берзеніна[1], О. І. Грабовська[2,3], А. В. Штеменко[1]

[1]Український державний хіміко-технологічний університет, Дніпро, Україна;
[2]Дніпровський національний університет імені Олеся Гончара, Дніпро, Україна;
[3]ДУ «Інститут гастроентерології НАМН України», Дніпро, Україна;
✉e-mail: n.shtemenko@ukr.net

Двокомпонентна реній-платинова система (система Re-Pt) заснована на введенні кластерної сполуки диренію(III) і цисплатину тваринам із пухлинами з подальшим значним протипухлинним ефектом і зниженням токсичної дії цисплатину на нормальні клітини. Метою цієї роботи було отримати тверді ліпідні наночастинки (SLN) з поверхневих ліпідів (восків) листя *Chelidonium majus* L. (Papaveraceae) та оцінити, чи буде капсуляція диренію(III) як компонента

системи Re-Pt у SLN впливати на його протипухлинну активність і морфологію еритроцитів щурів з карциномою Герена. У дослідженнях використовувалися Фур'є-ІЧ-спектроскопія, газорідинна хроматографія, мікроскопія, світлорозсіювання. Були отримані тверді ліпідні наностатки, охарактеризовані, навантажені кластерним диренієм (III) і введені разом з цисплатином щурам із карциномою Герена, що призвело до збереження морфології еритроцитів і значного зменшення ваги пухлини. Зроблено висновок, що ліпідне покриття кластерної сполуки ренію не знижує протипухлинну дію системи Re-Pt і захищає еритроцити від токсичного впливу цисплатину. Запропоновано нову форму введення системи Re-Pt.

Ключові слова: тверді ліпідні наночастинки, поверхневі ліпіди, ренієва кластерна сполука, реній-платинова протипухлинна система, карцинома.

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