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MECHANISMS OF MORPHOLOGY DEATH OF TUMOR CELL BY EFFECT NON-RADIOACTIVE AND RADIOACTIVE ^{195m}Pt-CISPLATIN

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The photonuclear technology of preparation radioactive cisplatin ^{195m}Pt was been developed by the bremsstrahlung from the electron accelerators. In the novel technology, the production of ^{195m}Pt from reaction ^{197}Au(γ,np)^{195m}Pt with a high specific activity >1 Ci/mg^{-1} was realized. The Ehrlich adenocarcinoma cells were been used as a neoplastic model. There are two groups: 1. tumor cells with non-radioactive cisplatin; 2. tumor cells with radioactive cisplatin (0.17 pg ^{195m}Pt-cisplatin). The number of morphology death cells as a result of radioactive cisplatin action was more than after action of non-radioactive cisplatin. The different mechanisms of the morphology death cell (necrosis for non-radioactive and apoptosis for radioactive) were detected.

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INTRODUCTION

The development of technology for the production of radioactive cisplatin with high specific anticancer activity is currently being pursued by cancer researchers [1 - 3]. An important feature of ^{195m}Pt use is the availability of certified radiopharmaceuticals: cisplatin, oxiplatin, heptaplatin etc. Consequently, the mechanism of ^{195m}Pt – base radiopharmaceuticals uses in the medical practice is rather simple.

Some time ago we reached the specific activities of 250…1000 Bq/mg by irradiating the cisplatin solution in the electron accelerator with radiation energy of 23…25 MeV. For this the nuclear reactions ^{196}Pt(γ,n)^{195m}Pt, ^{194}Pt(γ,n)^{193m}Pt, ^{195}Pt(γ,γ`)^{195m}Pt were used.

Besides using certified solution cisplatin, we using and crystalline cisplatin too which gives us the possibility to increase the specific activity of radioactive cisplatin to 2 kBq/mg. However, such specific activities do not satisfy in full measure the nuclear medicine needs. In this connection, the novel technology of gold extraction with high specific activity isotope ^{195m}Pt was developed [4 - 7]. Cisplatin of high specific activity was obtained in the high-current electron accelerator using the nuclear reaction ^{197}Au(γ,np)^{195m}Pt with a threshold of 13.7 MeV. The cross-section of the reaction ^{197}Au(γ,np)^{195m}Pt slowly increases to 100 MeV and according to the estimation at 30 MeV energy has the value of 2 mbn [8]. The out-put of ^{195m}Pt isomer was near 30%. Therefore the ^{197}Au(γ,np)^{195m}Pt reaction cross-section for the 30 MeV gamma-quanta was about 0.6 mbn.

The first aim of the present research consists of significantly enhance date relatively specifications of photonuclear production of radioactive cisplatin (^{195m}Pt) in electron linac accelerator at KIPT and the second aim – to study the mechanisms of death tumor cell by effect non-radioactive and radioactive cisplatin.

1. MATERIALS AND METHODS

The samples of solution cisplatin produced by the Mili Healthcare Limited Company (Great Britain) and cisplatin crystal Sigma Aldrich, Pt (USA), placed in the polyethylene ampules and in the aluminum container, were irradiated under the bremsstrahlung from the electron accelerators "EPOS" and "KUT-20" with the energy of 26 and 34 MeV respectively [9, 10]. In this case the platinum isotopes from the nuclear reactions ^{196}Pt(γ,n)^{195m}Pt, ^{195}Pt(γ,γ`)^{195m}Pt, ^{194}Pt(γ,n)^{193m}Pt, ^{192}Pt(γ,n)^{191}Pt, ^{198}Pt(γ,n) ^{197}Pt were realized. The energy of platinum isotope recoil is several keV that leads to the atom escaping from the cisplatin compound and to the formation of $PtCl_4$ and $PtCl_6$ molecules. The platinum atom does not undergo a shift due to the ^{195}Pt(γ,γ`)^{195m}Pt reaction and the radioactive cisplatin molecule retains its structure.

There are the following of platinum isotopes: 0.0127% for platinum-190, 0.78% for platinum-192, 32.9% for platinum-194, 33.8% for platinum-195, 25.2% for platinum-196, and 7.19%, for platinum-198.

Under irradiation with bremsstrahlung in the (γ,n) reactions the following isotopes are formed: of platinum-195 ($T_{1/2}$=4.02 days), platinum-193 ($T_{1/2}$ = 4.38 days), platinum-191 ($T_{1/2}$ = 3 days), platinum-197 ($T_{1/2}$ = 18.3 h).

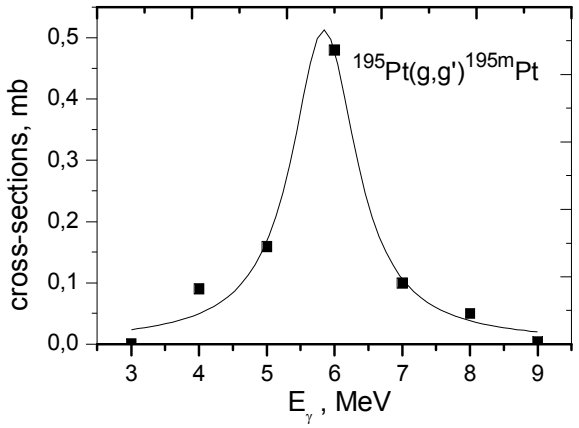

Fig. 1. Cross section reaction $^{195}Pt(\gamma,\gamma')^{195m}Pt$

The production of platinum-195m in the (γ,γ`) reaction consists of the recoil nucleus gains insignificant energy. The platinum-195m yield in the ^{196}Pt(γ,n)^{195m}Pt reaction was calculated with the help of the program complex PENELOPE [4].

The specific activity of platinum-197 increases with the beam energy. The platinum-195 yield in the ^{195}Pt(γ,γ`)^{195m}Pt reaction is given in Fig. 1 [11]. It is seen

that the cross-section reaction maximum is realized at gamma-quantum energy of 6 MeV. The cross-section of the $^{195}Pt(\gamma,\gamma`)^{195m}Pt$ reaction is less by a factor of 1000 than for the $^{196}Pt(\gamma,n)^{195m}Pt$ reaction.

Below the nuclear reaction and γ-spectrum of the Au by different conditions of radiation samples of Au are shown (Figs. 2, 3).

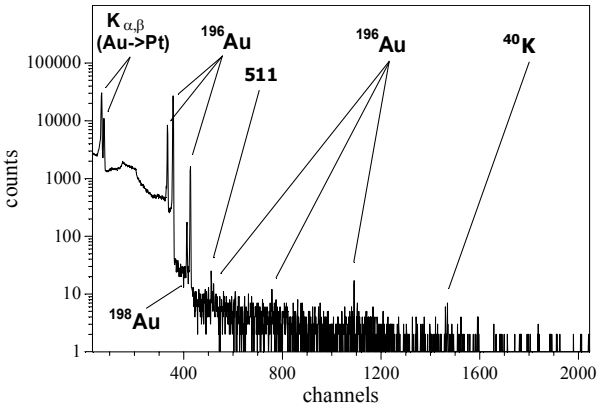

Fig. 2. The γ-spectrum of the Au solution before extraction (irradiation during 5 hours in the linear accelerator "KUT–20" at E_e=34 MeV, I=30 μA

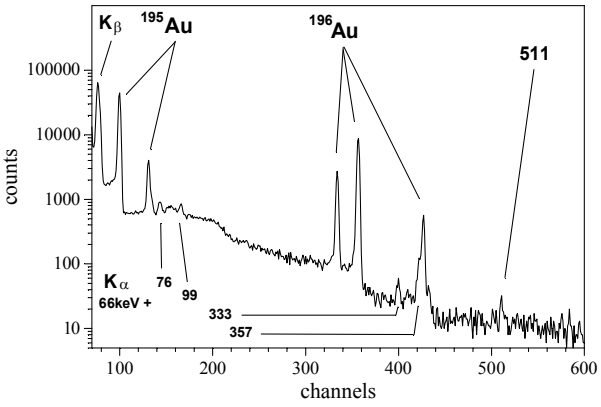

Fig. 3. The fragment of the γ-spectra of the Au sample after irradiation in the linear accelerator and after extraction during 4 h (irradiation during 5 h in the linear accelerator "KUT-20" at E_e=30...34 MeV, I=30 μA)

The specific activity range of production of radioactive cisplatin was 250…11000 Bk/ml, radiation dose – 1.5…5 MGy.

But we must be produced ^{195m}Pt with more high specific activity therefore the procedure of radioactive cisplatin synthesis was realized [5].

Synthesis of radioactive cisplatin [12, 13] (Figs. 4, 5).

The peculiarity of the radioactive cisplatin synthesis in our case is a small quantity of ^{195m}Pt (25 ng). The solution of platinum in the hydrochloride acid was repeatedly (3 - 4 times) evaporated on the water bath to obtain a yellow precipitate. The dry precipitate $H_2[PtCl_6]\cdot 6\,H_2O$ was subjected to treatment with boiling water and was evaporated again on the water bath. Then the fresh 25% solution of potassium chloride was added to the tenfold-water solution of platinum hydrochloric acid up to the complete precipitation.

$$H_2[PtCl_6] + 2KCl = K_2[PtCl_6] = 2HCl. \quad (1)$$

The yellow crystalline precipitation was obtained. After cooling the precipitate was washed during 1.5…2 h, with the diluted potassium chloride solution and alcohol. The compound is crystallized in the form of yellow octahedrons. The obtained precipitate in the 6-7 fold quantity of water was slowly heated to boiling. In the process, as the quantity was small, water was added to the required volume. A 5% excess of lemon salt (0.39 g $K_2C_2O_4\cdot H_2O$ per 1 g $K_2[PtCl_6]$ was added to the boiling mixture.

$$K_2[PtCl_6] + K_2C_2O_4 = K_2[PtCl_4] + 2KCl + 2CO_2\ . \quad (2)$$

Boiling of the solution for 1 h does not result in the formation of red-crimson precipitate. Therefore, once more portion of $K_2C_2O_4$ was added. After boiling for 1 h the red-crimson precipitate was formed. The obtained solution of potassium chloroplatinite $K_2[PtCl_4]$ with the addition of a necessary quantity of 20% solution of ammonium acetate (CH_3COONH_4) and potassium chloride was boiled for 1.5 h with water replenishment. After cooling the precipitate of Pejrone salt of canary color was precipitated.

$$K_2[PtCl_4] + 2CH_3COONH_4 = (NH_3Cl)2Pt + 2CH_3COOK + 2HCl. \quad (3)$$

Measurement of gamma-radiation spectra was performed by means of a Ge(Li)-detector with an energy resolution of 2.9 keV on the line of 1333 keV. The gamma-spectrum of cisplatin of 3 cm^3 volume, irradiated with the bremsstrahlung produced gamma-quanta at the energy of 26 MeV is shown in Fig. 2. Besides the platinum isotopes, the spectra contain the lines of iodine and sodium. The activity of cisplatin was from 1.5 to 5 MGy.

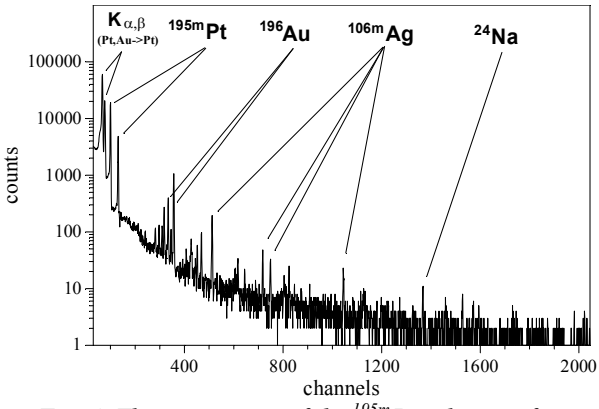

Fig. 4. The γ-spectrum of the ^{195m}Pt solution after the final extraction cycle (irradiation during 5 h in the linear accelerator "KUT-20" at E_e=30...34 MeV, I=30 μA)

The integrity of a complex molecule of irradiated cisplatin was studied using the technique of infra-red (IR) spectroscopy [14]. The vibrational absorption spectra in the IR region were 1obtained for the physiological solution (0.9 NaCl), non-radioactive and radioactive cisplatin (Fig. 6). There are not observed significant changes in the IR-spectra of non-radioactive and radioactive cisplatin. It can be noticed that the absorption band, corresponding to 1420 cm^{-1} non-radioactive cisplatin, is more distinct than in radioactive cisplatin. The

doublet of lines, corresponding to absorption bands 1190, 1170 cm^{-1} in radioactive cisplatin is more distinct than in non-radioactive ones.

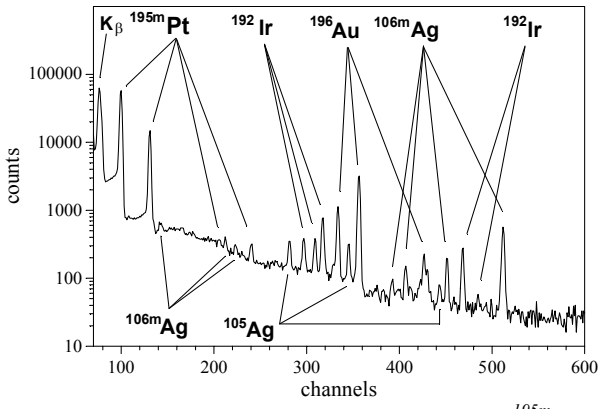

Fig. 5. The fragment of the γ-spectrum of the ^{195m}Pt solution after the final extraction cycle (irradiation during 5 h in the linear accelerator "KUT-20" at E_e=30…34 MeV, I=30 μA)

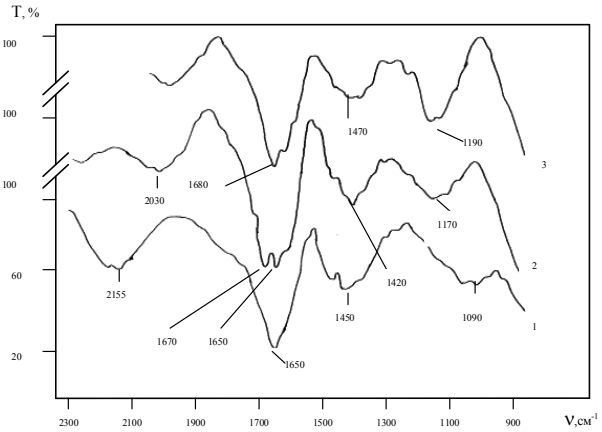

Fig. 6. IR spectra (1 – physiological solution; 2 – non-radioactive cisplatin; 3 – radioactive cisplatin)

By the method of infrared spectroscopy, it is shown that there no significant differences in the IR-spectra of non-radioactive and radioactive cisplatin (see Fig. 6).

IR-spectroscopy is one of the effective methods for the determination of chemical components and their binds.

2. RESULTS AND DISCUSSION

The preclinical investigation was carried out "in vitro" with using of a suspension of 5-7-10 days old Ehrlich adenocarcinoma cells ($2 \cdot 10^6$ cells, concentration 1.8 cells ml/1, vitality 98%). These cells were used as a neoplastic model. The suspension of tumor cells was separated into two groups: 1. Tumor cells with non-radioactive cisplatin; 2. Tumor cells with radioactive cisplatin (0.17 pg ^{195m}Pt-cisplatin).

The ideal variant is to use isotopes that are decaying with Auger electron emission. Then the specific efficiency of electron energy use will be by a factor of 100…300 higher than in the case of malignant tumor irradiation by the external irradiation with the use of isotopes having significantly higher energies of particles (gam-ma-radiation, electrons). Promising candidates for the Auger radiator use are isotopes ^{195m}Pt, ^{125}I, ^{123}I, ^{111}In. The amount of energy deposited per decay in a 5-nm sphere is correspondingly much higher for ^{195m}Pt (2000 eV) than for ^{125}I (1000 eV), ^{123}I (550 eV), and ^{111}In (450 eV). It is obvious that the best isotope for radiotherapy is platinum-195m [15, 16].

There are two types of death for cells: necrosis and apoptosis [17]. The morphological transformation of Ehrlich adenocarcinoma cells by effect non-radioactive and radioactive cisplatin was assessed with the used microscope "MBB-1".

The morphological death of Ehrlich adenocarcinoma cells by effect non-radioactive cisplatin associated with type necrosis: the destruction of membrane unite and cell, nuclear change, cytoplasm swelling. The morphological death of Ehrlich adenocarcinoma cells by effect radioactive cisplatin characterized as type apoptosis: decrease of volume cell and corrugation, hydrolysis, cell fragmentation, and formation of apoptosis bodies.

To obtain quantitative results of morphological changes provoked by the action of non-radioactive and radioactive cisplatin we carried out the count of the apoptotic index (AI). The apoptotic index is the ratio of the number of apoptotic cells to the total number of cells X 100%.

Apoptotic and mitotic indexes of Ehrlich adenocarcinoma cells with non-radioactive and radioactive cisplatin after incubation during 8 h

Tumor cell smear	Average number of tumor cells in the field of the microscope	Apoptotic index, %	Mitotic index, %
Tumor cells incubated with non-radioactive cisplatin	120	8	0
Tumor cells incubated with radioactive cisplatin	80	12	0

The count of the mitosis (division) index was carried out by staining the cell smear with lacmoid. The procedure of smear preparation consisted of the fixation for 9 min. The fixer was prepared by mixing 1 g glacial acetic acid with 3 g perfectly pure alcohol. The staining action was continued during 40…42 min. The reagent for cell staining was prepared by dissolving 1 g lacmoid in the 45 ml glacial acetic acid with the addition of 55 ml distilled water. Then the boiling was carried out for 30 min. By counting the mitotic index in the Goryaev chamber – the total number of dividing cells was taken as 100%. The count of the mitotic index in the Ehrlich adenocarcinoma cells incubated with non-radioactive and radioactive cisplatin did not reveal distinct differences in the level of this index during the first hours. However, it should be noted, that the number of cells in the metaphase which incubated with non-radioactive cisplatin was 70 and 95% for cells that incubated with radioactive cisplatin. The cells in the interphase practically were not observed.

From the table, it is seen that the apoptotic index prevails over the mitotic index for two groups of the tumor cells.

CONCLUSIONS

The photonuclear technology of preparation radioactive cisplatin ^{195m}Pt was developed using the bremsstrahlung from the electron accelerators "EPOS" and "KUT-20" with the energy of 26 and 34 MeV respectively.

The novel technology the production of ^{195m}Pt from reaction ^{197}Au(γ,np)^{195}Au → ^{195m}Pt was realized.

The procedure of radioactive cisplatin ^{195m}Pt synthesis with a high specific activity >1 Ci/mg^{-1} was realized.

The effect of non-radioactive and radioactive cisplatin on morphology transformation of tumor cells in the dynamics of the experiment was observed: the giant tumor cells were larger by the effect of non-radioactive cis-platin than after action of radioactive cisplatin. The number of morphology death cells as a result of radioactive cisplatin action was more than after action of non-radioactive cisplatin. The appearance of apoptotic cells after the action of radioactive cisplatin was observed after 6-8 h of incubation, while in the case appearance of the necrosis cells after-action non-radioactive cisplatin – in 12-16 h. In the case of the effect of radioactive cisplatin, the cells in necrosis state were not registered. The values of apoptotic and mitotic indices in tumor cells that were incubated with non-radioactive and radioactive cisplatin, apparently, evidence on the different mechanisms of the morphology death cell.

The most promising result is the fact that the number of cells dies and the number of apoptotic cells is higher in the case of radioactive cisplatin action.

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МЕХАНИЗМЫ МОРФОЛОГИЧЕСКОЙ ГИБЕЛИ ОПУХОЛЕВЫХ КЛЕТОК ПРИ ДЕЙСТВИИ НЕРАДИОАКТИВНОГО И РАДИОАКТИВНОГО ^{195m}Pt-ЦИСПЛАТИНА

Н.П. Дикий, Ю.В. Ляшко, Е.П. Медведева, Д.В. Медведев

Фотоядерная технология получения радиоактивного цисплатина ^{195m}Pt была разработана при использовании тормозного излучения на электронном ускорителе. Была реализована новая технология получения ^{195m}Pt из реакции ^{197}Au(γ,n)^{196}Au → ^{195m}Pt с высокой удельной активностью >1 Ci/mg^{-1}. В качестве опухолевой модели использованы асцитные клетки аденокарциномы Эрлиха, которые были распределены по двум исследуемым группам: 1 – клетки с нерадиоактивным цисплатином; 2 – клетки с радиоактивным цисплатином (0,17 пг·мл$^{-1}$). Морфологически погибших опухолевых клеток было больше при действии радиоактивного цисплатина. Механизм гибели опухолевых клеток при действии нерадиоактивного и радиоактивного цисплатина был разным (некроз и апоптоз соответственно).

МЕХАНІЗМИ МОРФОЛОГІЧНОЇ ЗАГИБЕЛІ ПУХЛИННИХ КЛІТИН ПРИ ДІЇ НЕРАДІОАКТИВНОГО ТА РАДІОАКТИВНОГО ^{195m}Pt-ЦИСПЛАТИНУ

М.П. Дикий, Ю.В. Ляшко, О.П. Медведєва, Д.В. Медведєв

Фотоядерна технологія одержання радіоактивного цисплатину ^{195m}Pt була розроблена на лінійному прискорювачі електронів з використанням гальмівного випромінювання. Була реалізована нова технологія одержання ^{195m}Pt з реакції ^{197}Au(γ,n)^{196}Au → ^{195m}Pt з високою питомою активністю >1 Ci/mg^{-1}. За пухлинну модель взяті асцитні клітини аденокарциноми Ерліха, які були розподілені на дві групи: 1 – клітини з нерадіоактивним цисплатином; 2 – клітини з радіоактивним цисплатином (у дозі 0,17 пг·мл$^{-1}$). При дії радіоактивного цисплатина морфологічно загиблих клітин було більше. Механізм загибелі пухлинних клітин при дії нерадіоактивного та радіоактивного цисплатину був різним (некроз та апоптоз відповідно).