ANTINEOPLASTIC THERAPY BY RADIOACTIVE CISPLATIN IN EXPERIMENT

N.P. Dikiy*, A.N. Dovbnya, Y.V. Lyashko, E.P. Medvedeva, D.V. Medvedev, V.L. Uvarov

National Science Center "Kharkov Institute of Physics and Technology", 61108, Kharkov, Ukraine

(Received March 23, 2007)

The method of ^{195m}Pt production on linear electron accelerators and cyclotron of NSC KIPT are described. The method of ^{195m}Pt separation from the irradiated samples are developed. The methods of cisplatin synthesis with use of a radioactive isotope of ^{195m}Pt are realized. Results of biological tests of radioactive cisplatin on animals are described. Our preliminary results demonstrate the feasibility of radioactive cisplatin for treatment of cancer diseases.

PACS: 29.17.+w, 28.41.Kw

1. INTRODUCTION

Last years new methods find expanding application in the medical treatment of cancerous diseases. One of the most promising approaches is brachytherapy. Generally it is the implantation of miniature radioactive sources in the form of a thin wire, capsules or little seeds. Implants are introduced either after surgical operations for irradiation of residuary malignant cells or by means of catheters. The main isotopes used in brachytherapy are ^{103}Pd, ^{125}I, ^{192}Ir, ^{106}Ru, ^{198}Au [1]. There are two types of brachytherapy: with low levels and with high levels of irradiation intensity. In the case of the high-intensity brachytherapy a source is introduced directly into the carcinoma for the definite time to attain a therapeutic effect. Usually this type of brachytherapy is applied for the cure of the malignant tumor of the lacteal gland, lungs etc. The low-intensity brachytherapy, for example, use of isotopes of ^{103}Pd and ^{185}Ir for the therapy of early stages of adenoma cancer. The use of open sources of radioisotopes in the nuclear medicine is more effective. Several decades ago this method was applied for the therapy of various cancerous diseases. However, only during last years this method finds more extensive use. It is conditioned by the development of more effective methods of isotope transport into the malignant tumor. It should be notice also the use of labelled peptides and other receptor-specific molecules, isotopes for the palliative therapy of a pain in the case of osteal metastasizes, labeled antibody agents, e.g. copper-containing monocarbonyl porphine [2]. The radiopharmaceuticals under consideration possess a high tropic sensitivity to some tumors that can be effectively cured with the using of the monocarbonylporphine labeling with ^{67}Cu. A particular attention is given to the methods of therapy using the high-value losses of Auger electrons [3-5]. It is known that the average number of Auger and Coster-Kronig electrons for ^{111}In, ^{123}I, ^{125} is equal to about 8, 11 and 20 electrons, respectively, with the energy from 12 to 24 keV. Deceleration of these electrons leads to the energy release in a negligible volume (a few cubic nm) and, as a result, the local dose is 10^4 - 10^7 Gy. In this case the radio toxicity from Auger electrons is caused by 90% by indirect mechanisms [6]. The high specific losses of α-particles allow of effective use of ^{211}At and ^{212}Bi for treatment in medicine. For example, for α-particles of these isotopes the radiation effect on a cell reaches the value of 1200. Therefore, a high-efficiency radiation effect will be realized by means of introduction of these isotopes into the tumor cell [7]. Practically all the antineoplastic pharmaceuticals used in the oncology practice have a strong toxicity that menaces the patient life. This feature of antineoplastic pharmaceuticals stimulates the search for new modifications of pharmaceuticals. The use of radiations with high deceleration losses permits to realize a synergetic bystander effect too [8-11]. This effect, a high density of deceleration losses, cytotoxic effect and apoptosis can be reached by application of radioactive cisplatin. Therefore, today a necessity of carrying out the research on production and use of radioactive cisplatin is an urgent question. The combined influence of all the abovementioned factors will allow considerable decreasing of cisplatin dose and, consequently, decreasing of the toxic cisplatin influence in the course of medical treatment of cancer carriers. With the purely radioactive cisplatin the dose can be decreased by a factor of 10 and more. Note that the influence of radioactive cisplatin is possible in the form of transplatin and in other forms penetrating in the nucleus of a cell.

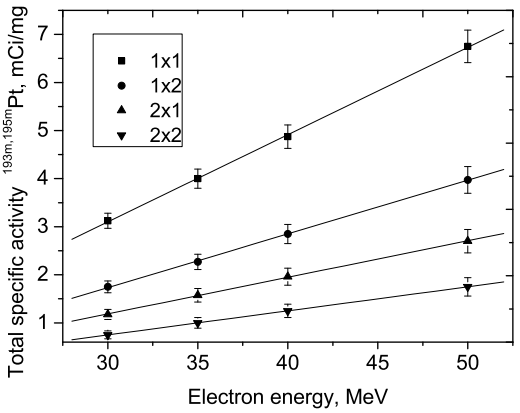
*Corresponding author. E-mail address: dikiy@kipt.kharkov.ua

The aim of work is to develop a method for production of radioactive cisplatin with the help of powerful electron accelerators and to test the obtained substance at biological objects.

2. PROCEDURE OF ^{195m}Pt EXTRACTION AND CISPLATIN SYNTHESIS

The use of a powerful accelerators of NSC KIPT for irradiation of platinum by electrons (200 μA, 50 MeV) permits to reach the total specific activity of ^{195m}Pt and ^{193m}Pt 7 mCi/mg. The ^{195m,193m}Pt yield in the reactions ^{196}Pt(γ,n)^{195m}Pt and ^{194}Pt(γ,n)^{193m}Pt was calculated with the help of the program PENELOPE (Fig.) [12]. Such a specific activity in many cases is insufficient for the effective application in medicine practice. The specific activities of ^{195m}Pt to 10 Ci/mg are realized by the ^{199}Hg(γ,α)^{195m}Pt reaction. The separation of ^{195m}Pt from mercury has been accomplished using the electrolysis. The yield of ^{195m}Pt in reaction ^{199}Hg(γ,α)^{195m}Pt at a current of 200 μA and energy 26 MeV from the Hg target having a natural isotope composition is about 30 mCi/day.

The cyclotron CV28 allows production of higher specific activity of ^{195m}Pt by the ^{192}Os(α,n)^{195m}Pt reaction. At a current of 20 μA and energy 28 MeV the yield of ^{195m}Pt from the Os target having a natural isotope composition is 1 mCi/day [13]. Such yield is smaller than yield of ^{195m}Pt obtained by the method with the use of the bremsstrahlung from electron accelerators.



Specific activity at saturation of different targets as a function of electron energy (beam current 200 μA, diameter of beam 5 mm). Symbols - cylindrical targets (diameter × height, cm)

We carried out the experiments of separation of platinum isotope of ^{195m}Pt which was obtained in the ^{197}Au(γ,np)^{195m}Pt reaction. The ^{195m}Pt specific activity of 1 Ci/mg are obtained by means of the ^{197}Au(γ,np)^{195m}Pt reaction for electron energy of 34 MeV and current of 200 μA. The sample of 1g of gold (99.9%) was used as a target. The technology of ^{195m}Pt separation from the gold target was developed. The procedure by this technology included the process of gold target dissolution by boiling (Au, HCl and HNO_3 1:3). After dissolving the metal target, the HNO_3 excess should be removed by gradual addition of 10% HCl solution in the amount sufficient to obtain the solution with an acid concentration of 3N.

For ^{195m}Pt extraction we used the extractor of Kucher-Shtoll type designed for extraction by light solvents. Then the round-bottomed flask was filled with the prepared solution and the ethyl acetate $CH_3COOC_2H_5$ was gradually added up to the total volume of 100 ml. The extraction process was carried out by slow boiling. The total extraction time was 5 hours. After that the platinum chloride was cooled and 30 ml of ethyl acetate were reextracted. This procedure was repeated three times. After reextraction the solution was poured into the separating funnel where it was held during 2−3 min. Then the solution was mixed by a magnetic stirrer during 5 min. To establish equilibrium the solution was kept in rest.

The radioactivity of platinum chloride solution was measured by the Ge(Li)-detector (50 cm^3) with the energy resolution of 2.2 keV for the line of 1333 keV. The spectrum contained 82.5% of Pt and 17.5% of Au. In addition to these elements there was ^{126}I in the spectra.

In the next procedure by this technology to the platinum-hydrochloride acid solution diluted in the 10 fold water amount we added a freshly prepared 25% potassium chloride solution for the full completion of precipitation. A yellow crystalline precipitate K_2PtCl_6 was deposited. The precipitate was filtered in 1.5−2 hours after deposition. The obtained precipitate of potassium chloroplatinite was flooded with water (10-fold weight amount) and heated to boiling. The boiling mixture was gradually filled up with little portions of 1% oxalic salt $K_2C_2O_4$. The solution was evaporated in the water-bath until the crystallization onset and then it was cooled at room temperature. The solution of potassium chloroplatinite $K_2[PtCl_4]$ was mixed with the 20% solution of ammonium acetate CH_3COONH_4 and heated to boiling. After cooling Peyrone's salt $(NH_3Cl)_2Pt$ was separated. The admixture of ^{196}Au activity in synthesized cisplatin was 1.4% relatively of the ^{195m}Pt activity.

3. BIOLOGICAL TESTS

The cisplatin molecule containing ^{195m}Pt could have an exceptional therapeutic use. Therefore, it is important to possess information and reliable evaluation of absorbed doses for different organs and tissues in order to calculate subsequently the effective doses for patients which will take ^{195m}Pt-cisplatin.

The experiment was made on animals (male white mice, weighing 18 g). Radioactive cisplatin in the dose of 13 kBq was introduced into animals intraperitoneally. The absorbed doses to animal from ^{195m}Pt (mGy/MBq) was measured in different organs (kidneys, liver, spleen, bowels, testicles, wall of urinary

bladder, skin). The organs from 10 animals to be studied were weighed and placed in an aluminums container which was installed on the Ge(Li)- detector for registration of the absorbed dose.

The results obtained demonstrated that the highest ^{195m}Pt-cisplatin absorption dose is registered in the liver (0.72±0.23), kidneys (0.40±0.07), testicles (0.55±0.15), spleen (0.35±0.05), wall of urinary bladder (0.25±0.02); skin (0.21±0.04) mGy/MBq. The experiments were carried out to study the influence of initial and radioactive cisplatin on a cell suspension of adenocarcenoma of Ehrlich. The cell concentration was $1.8 \cdot 10^6$ cells/ml. The cell viability was determined by the method of supravital staining. The introduced dose of the initial cisplatin was 7.5 μg/ml, of the radioactive cisplatin it was 0.017 pg/ml. Into every sample we introduced 0.03 ml of penicillin (180 units) and 0.03 mg of sulfanilamide (300 μg). The samples were held in the thermostat at a temperature of 37°C for incubation. The tumor cell size was measured by means of a polarization microscope (×200) and was 0.015 mm before introduction of cytostatic agents. After 12 hours of incubation with the initial cisplatin the cell size was 0.010−0.015 mm, with the radioactive cisplatin it was 0.005−0.007 mm.

A series of experiments were carried out to study the effect of untreated and radioactive cisplatin on the growth of a solid tumor in mousses. To estimate the effect of introduced radioactive drugs on the tumor growth we defined the rate of its growth inhibition (D%) calculated by the formula:

$$D = ((V_0 - V_1)/V_0) \cdot 100,$$

where V_0 is the tumor volume in the control animals, V_1 is the tumor volume in the animals which received untreated and radioactive cisplatin.

The studies were carried on male mousses of 18 g weight bred at the Kharkiv Academy of Pharmacology. A slurry of ascetic cells of the Ehrich's adenocarcinoma received from the Institute of Cryobiology and Cryomedicine was used as a tumor model. The slurry of cells (10^6 cells/ml) was inoculated into the right thigh region of mousses. There were three groups of animals: group I - animals-tumor carriers (10 mousses); group II - animals selected for introduction of untreated cisplatin (15 mousses); group III - animals-tumor carriers selected for introduction of radioactive cisplatin (15 mousses).

Every day the animals have been examined, weighted, fed by the ration; coops were daily cleaned. Since 11 day after tumor subinoculation every other day we introduced untreated cisplatin into the animals from group II (introperitonial introduction by the dose of 0.8 mg per kg of the animal's weight). In total 5 injections were made. The animals from group II received 0.5 ml of injection water by the same scheme. The animals from group III received only one dose (6.5 kBk per 15 g) of radioactive cisplatin.

In 21 day after tumor subinoculation we have measured the physiologic indices of animals (total weight (g), weights of the spleen (mg), thymus (mg), tumor (mg)), as well as, some hematological indices. We have noticed the decrease of the animals' weight and the tumor growth inhibition in the animals of group II and group III. The rate of tumor growth inhibition, as a result of radioactive cisplatin introduction, was 65% that is significantly higher than the rate of tumor growth inhibition caused by the action of untreated cisplatin (32.5%). The results obtained give hope for the advanced use of radioactive cisplatin in the oncological practice.

4. CONCLUSIONS

1. The method of production ^{195m}Pt with high specific activity by means of an irradiation of a gold target by bremsstrahlung of the electronic accelerator has been developed.

2. The method of separation of ^{195m}Pt by means of extraction of gold has been developed and realized.

3. Synthesis of radioactive cisplatin has been developed.

4. Test of radioactive cisplatin on experimental animals has been carried out. High efficiency of radioactive cisplatin for treatment of cancer tumors of animals has been received.

This work was supported by STCU, project No.1768.

REFERENCES

1. *Benefical uses and production of isotopes* OECD publications, ISBN 92-64-18417-1, No.516252000, 1992, 80 p.

2. A.A. Shishkin, A.G. Ivanov, A.V. Zaitsev et al. Copper-containing monocarbonylporphyrin - a prototype of new DNA-bonding cytotoxic compounds // *DAN.* 2005, v.403, No.6, p.833-836 (in Russian).

3. I.E. Feinendegen I.E. Biological damage from the Auger effect: Possibile benefits // *Radiat. Environ. Biophys.* 1975, v.12, p.85-99.

4. S.J. Adelstein, A.I. Kassis. Radiobiologic implications of the microscopic distribution of energy from radionuclides // *Nucl. Med. Biol.* 1987, v.14, p.165-169.

5. A.I. Kassis, S.J. Adelstein, C.K. Haycock et al. Lethality of Auger electrons from the decay of Br-77 in the DNA of mammalian cells // *Radiat. Res.* 1982, v.90, p.362-373.

6. M.A. Waliska, S.J. Adelstein, A.I. Kassis. Chemical modification of 5-[125I]iodo-2b-dcoxyuridine toxicity in mammalian cells in vitro // *Int. J. Radiat. Biol.* 2001, v.77, p.625-630.

7. E. Dadachova, R.W. Howell, R.A. Bryan et al. Cryptococcus neoformans and Histoplasma capsulatum to γ-radiation versus radioimmunotherapy with α- and β-emitting radioisotopes // *Rad.*

Susceptibility Hum. Pathogens. 2004, v.45, No.2, p.313-320.

8. X.Y. Xue, N.J. Butler, G.M. Makrigiorgos et al. Bystander effect produced by radiolabeled tumor cells in vivo // *Proc. Nat. Acad. Sci.* 2002, v.99, No.21, p.13765-13770.

9. E.J. Hall, S. Mitchell, D.J. Brenner How Many Bystander Effects Are There? // *Radiat. Res.* 2004, v.161, p.117-118.

10. H. Zhou, G. Randers-Pehrson, M. Suzuki et al. Genotoxic damage in non-irradiated cells: contribution from the bystander effect // *Radiat. Prot. Dosim.* 2002, v.99, p.227-232.

11. H. Zhou, M. Suzuki, C.R. Geard, T.K. Hei Effects of irradiated medium with or without cells on bystander cell responses // *Mutat. Res.* 2002, v.499, p.135-141.

12. N.P. Dikiy, A.N. Dovbnya, V.I. Nikifirov, V.L. Uvarov Peculiarities of photonuclear production of medical // *Proc. of 11th Conf. on Appl. of Charged Part. Accel. in Industry and Medicine.* 2005, StP., Russia, P.391-394 (in Russian).

13. *Progress report on nuclear data research in the Federal republic of Germany.* 2005, Forschungszentrum Juilich GmbH, Juilich, FRG, 82 p.

ПРОТИВООПУХОЛЕВАЯ ТЕРАПИЯ РАДИОАКТИВНЫМ ЦИСПЛАТИНОМ В ЭКСПЕРИМЕНТЕ

Н.П. Дикий, А.Н. Довбня, Ю.В. Ляшко, Е.П. Медведева, Д.В. Медведев, В.Л. Уваров

Рассматриваются способы производства ^{195m}Pt на линейных ускорителях электронов и циклотроне ННЦ ХФТИ. Разработаны методы выделения ^{195m}Pt из облученных образцов. Реализованы методы синтеза цисплатина с использованием радиоактивного изотопа ^{195m}Pt. Приведены результаты биологических испытаний радиоактивного цисплатина на животных. Наши предварительные результаты демонстрируют возможность использования радиоактивного цисплатина для лечения канцерогенных заболеваний.

ПРОТИПУХЛИННА ТЕРАПІЯ РАДІОАКТИВНИМ ЦИСПЛАТИНОМ В ЕКСПЕРИМЕНТІ

М.П. Дикий, А.М. Довбня, Ю.В. Ляшко, О.П. Медведєва, Д.В. Медведєв, В.Л. Уваров

Розглядаються способи виробництва ^{195m}Pt на лінійних прискорювачах електронів і циклотроні ННЦ ХФТІ. Розроблено методи виділення ^{195m}Pt з опромінених зразків. Реалізовано методи синтезу цисплатину з використанням радіоактивного ізотопу ^{195m}Pt. Наведено результати біологічних випробувань радіоактивного цисплатину на тваринах. Наші попередні результати демонструють можливість використання радіоактивного цисплатину для лікування канцерогенних захворювань.