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Investigation of the potential antitumor radioactive complex of platinum(II) with tetracycline

A. S. Leal[1] · I. M. Marzano[2] · E. C. Pereira-Maia[2] · R. Jacimovic[3]

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Abstract The proposal of this work was to investigate the effect of the radioactive complex of platinum(II) with tetracycline, $[PtCl_2(C_{22}H_{24}N_2O_8)]^*$, or [Tc–Pt(II)]*, on K562 cells—blood human cells of leukemia—and verify if the internal radio-chemotherapy would be able to produce additional effects compared with the non-labelled complex, $[PtCl_2(C_{22}H_{24}N_2O_8)]$, or [Tc–Pt(II)]. The concentration required to inhibit 50 % of cellular growth, (IC_{50}), was 2.5 ± 0.2 μM for [Tc–Pt(II)]* and 14.5 ± 0.9 μM for the non-labelled molecule [Tc–Pt(II)]. This result suggest that the [Tc–Pt(II)]* could be a potent radiosensitizer evoking a supra additive effect. Treatment using the internal radio-chemotherapy may be a useful alternative to reduce the drug concentration required for effective inhibition of the tumor growth.

Keywords Complex of platinum(II) · Tetracycline · Cisplatin · Antitumor effects

✉ A. S. Leal
asleal@cdtn.br

1 Centre for Development of Nuclear Technology (CDTN), Brazilian Commission for Nuclear Energy (CNEN), UFMG, Av. Antonio Carlos 6627, Belo Horizonte, Minas Gerais CEP 31270-901, Brazil

2 Department of Chemistry, Federal University of Minas Gerais (UFMG), Antonio Carlos 6627, Belo Horizonte, Minas Gerais CEP 31270-901, Brazil

3 Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia

Introduction

cis-Diamminedichloroplatinum(II), $[(NH_3)_2PtCl_2]$, cisplatin, or CDDP, is one of the most important chemotherapeutic agents used in the treatment of a wide variety of solid tumors [1, 2] and its interaction with DNA is pointed out as the main mechanism of cytotoxic action [3, 4]. Despite the important contribution of cisplatin in cancer therapy, its use presents limitations such as development of resistance and side effects, which has stimulated the search for novel compounds [5–8].

In last years, new strategies for cancer treatment has been investigated in order to enhance the therapy efficiency. One of the approaches is the utilization of the new drugs able to induce simultaneous low ionizing radiation and chemotherapy effects. The synergy of two different mechanism may reduce the chemotherapy drug dose, frequently associated with severe or undesirable side effects providing significant benefits for the patients. Initial investigations have showed positive results in this direction [9–18].

Considering that the efficiency of the treatment by radiotherapy depends on the tumor radiosensitivity, new strategies to enhance it using ionizing radiation may be positive. One of the approaches to obtain the enhanced radiosensitivity of the tumor cells is the simultaneous application of chemotherapeutic agents that alter DNA sensitivity to the radiation.

In a previous work of our group, this synergetic radio-chemotherapy effect against cells of glioma was demonstrated with of application the radioactive or labelled cisplatin, (CDDP*), compared to CDDP, the non-labelled molecule [12]. The use of internal radio-chemotherapy with low irradiation dose rate and enhanced selectivity to the target tissues has may become a new and promising alternative of treatment for some unresectable malignant tumors [15–18].

Another example that demonstrates the enhanced effect of the drug using radioactive isotope was the in vitro investigation of the radioactive and the non-radioactive ^{159}Gd-Gadodiamide against Erlich tumor cells. The cytotoxicity of labelled ^{159}Gd-Gadodiamide proved to be 95 times higher compared to the non-labelled ^{159}Gd [19].

The antitumor properties of the platinum(II) compounds and the favorable characteristics of tetracycline led us to synthesize Pt(II) compounds of tetracycline, Fig. 1, and to investigate their antimicrobial and antitumor effects [20, 21]. Tetracycline is an antibiotic of large spectrum but its use has been limited due to the emergence of bacterial resistance. The chemical structure of tetracycline is particularly complex, presenting several potential metal-binding sites [22]. The description of the synthesis and full characterization of the complex [$PtCl_2(C_{22}H_{24}N_2O_8)$] can be found at [20].

This work describes the preparation of the radiolabelled, [Tc–Pt(II)]*, aiming to investigate the possible enhancement of its cytotoxicity potential in vitro K562 cells compared to the original, the non-labelled molecule, [Tc–Pt(II)]*. The results obtained suggests that the [Tc–Pt(II)]* may be a new strategy in the antitumor therapeutic in the future.

Materials and methods

Irradiation and characterization of the complex

The samples of the [Tc–Pt(II)]* with 1.0–2.0 mg each were obtained in a similar way of the CDDP* using the TRIGA IPR-R1 research reactor of the CDTN, with thermal neutron flux of 6.4×10^{11} cm^{-2} s^{-1} [23]. The gamma radiation counting system used was CANBERRA hyper pure germanium detector, (HPGe), nominal efficiency of 50 % and a full-width at half maximum resolution, (FWHM) of 1.75 at 1332 keV. The software Genie-2000 (CANBERRA) was used to obtaining the gamma spectra and calculation of the specific activity. The samples were counted after the irradiation for 2 h at 5 cm from the detector. The dead time was lower than 3 %. More details about the reactor and gamma spectra analysis can be found at [24].

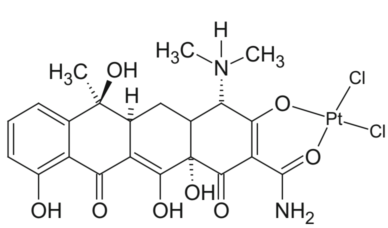

Fig. 1 Chemical structure of the tetracycline-platinum(II) complex

The samples were obtained using two different times of irradiation, 2 and 3 h, and four different times of decay: 1, 3 h and 3, 7 days, see Table 1. These times were chosen considering the following aspects: For the sample 1, the time of irradiation of 2 h was the minimum necessary to obtain some activity of Pt radioisotopes according our previous studies [9, 23]. The time of decay of 1 h was the lowest required operational time, due to the transport of the [Tc–Pt(II)]*. For the samples, 2, 3 and 4, the time of irradiation of 3 h was used to obtain a higher specific activity of the Pt isotopes but without the risk of disrupting the molecule, that can occurs of higher time of irradiation are used [23]. The decay time of 3 h for sample 3, was used in order to get more operational flexibility between the end irradiation and the inoculation of the cells. The decay time of 3 and 7 days were used, as an initial guess to investigate the influence of the emitted radiation from the different isotopes in the result of the cytotoxicity activity of the [(Tc–Pt(II)]*.

The Fig. 2 illustrates the gamma spectra of the [(Tc–Pt(II)]* of the sample 2 and the some photopeaks of some Pt radioisotopes. Their energy and half-live are presented in the Table 2 [23, 25, 26].

The final specific activity for [Tc–Pt(II)]* was approximately 60.0 Bq mg^{-1} after 24 h of decay. A higher

Table 1 Time of irradiation and decay for the [Tc–Pt(II)]* samples

Sample	Time of irradiation	Time of decay
Control	–	–
1	2 h	1 h
2	3 h	3 h
3	3 h	3 days
4	3 h	7 days

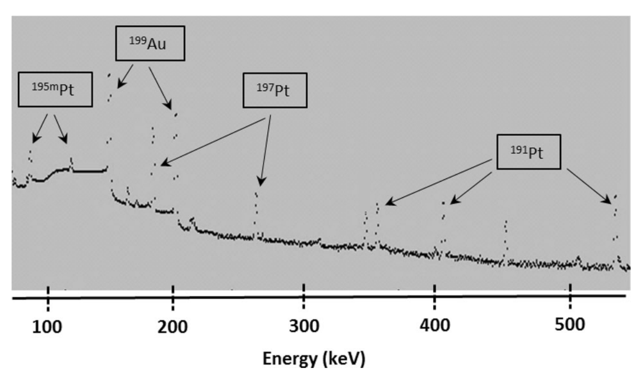

Fig. 2 Gamma spectra of the [Tc–Pt(II)]* after 2 h of irradiation

Table 2 Pt radionuclides and the decay modes produced by the [Tc–Pt(II)] irradiation

Stable nuclide	Nuclide produced	Half-life	Daughter nuclide	γ energy (keV)
^{190}Pt	^{191}Pt	2.96 days	^{191}Ir	359.6, 409.5, 538.9
^{192}Pt	^{193m}Pt	4.33 days	^{193}Ir	–
^{194}Pt	^{195m}Pt	4.02 days	^{195}Pt	99.8, 129.7
^{196}Pt	^{197}Pt	18.3 h	^{197}Au	279.1, 191.4
^{198}Pt	^{199}Pt	30.8 min	^{199}Au	317.1, 493.7, 542.9

specific activity of [Tc–Pt(II)]* can be obtained using higher times of irradiation. However, in this case, the sample must be irradiated inside a cadmium capsule to avoid the disruption of the molecule due to the Szilard–Chalmers effect that occurs during long irradiation times or higher neutron flux [23]. In this work, it was decided to irradiate without the cadmium capsules.

Cell line and culture

The K562 cell line was purchased from the Rio de Janeiro Cell Bank (number CR083 of the RJCB collection). This cell line was established from pleural effusion of a 53-year-old female with chronic myelogenous leukemia in terminal blast crisis. Cells were cultured in RPMI 1640 (Sigma Chemical Co.) medium supplemented with 10 % fetal calf serum (CULTILAB, São Paulo, Brazil) at 37 °C in a humidified 5 % CO_2 atmosphere. Cultures grow exponentially from 10^5 cells mL^{-1} to about 8×10^5 cells mL^{-1} in 3 days. Cell viability was checked by Trypan Blue exclusion. The cell number was determined by Coulter counter analysis. All the experiments with cells were performed outside the CDTN, in the Laboratories of Inorganic Chemistry of the Department of Chemistry of UFMG.

For cytotoxicity assessment, 1×10^5 cells mL^{-1} were cultured for 72 h in the absence and the presence of various concentrations of the tested compounds. The sensitivity to drug was evaluated by the concentration that inhibits cell growth by 50 %, IC_{50}. The experiments were performed in five different conditions of irradiation and decay times, see Table 2. The stock solutions were prepared in dimethyl sulfoxide, (DMSO), and diluted in cell culture medium in a maximum concentration of 0.1 %.

Statistics

The experiments were performed in triplicate with a good agreement between the results, expressed as the mean ± standard deviation. The following values of concentrations, in (/μM): 0.3, 0.6, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 15.0 and 20.0, were used for determining the IC_{50}.

Results and discussion

The radiochemical purity of [Tc–Pt(II)]*, for the sample 2 was determined using the UV–Vis spectroscopy, see Fig. 3. The result confirms that the irradiation did not affect the integrity of the molecule.

The IC_{50} values determined after the different times of irradiation and decay are indicated in the Table 3.

The [Tc–Pt(II)]* complex was much more active in all the four different experimental conditions, compared with the activity of [Tc–Pt(II)], the non-irradiated complex. The

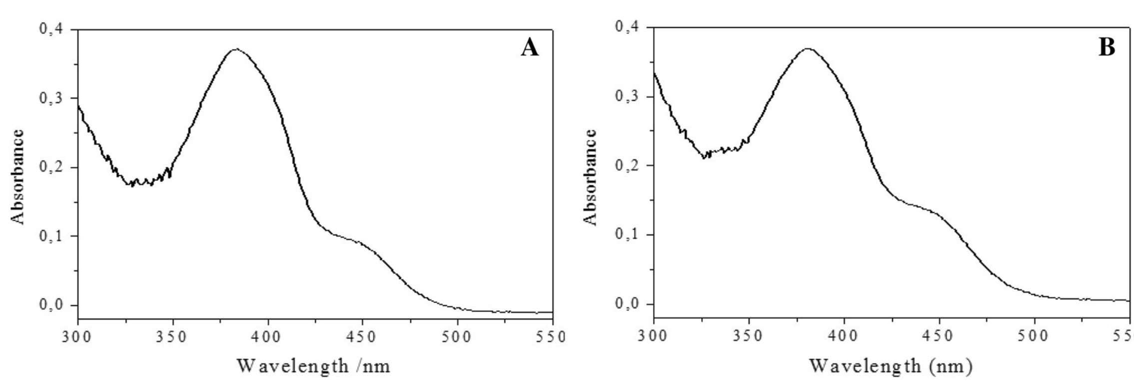

Fig. 3 UV–Vis spectra of the non-irradiated $[PtCl_2(C_{22}H_{24}N_2O_8)]$ (**a**) and after 3 h of irradiation (**b**). Complex concentration = 3×10^{-5} mol L^{-1}

Table 3 Values of IC_{50} for different times of irradiation and decay

Sample	IC_{50} value (μM)	Improving factor
Control	14.5 (5)	–
1	6.9 (3)	2
2	2.5 (1)	6
3	2.7 (1)	5
4	5.3 (2)	3

The improving factor represents the value of IC_{50} compared to the control

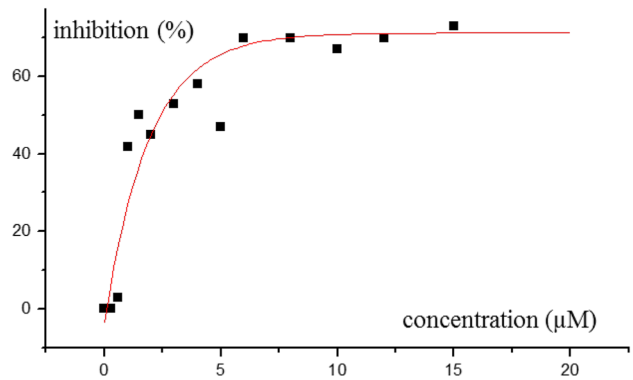

Fig. 4 Dose-response curves for sample 2. The obtained IC_{50} was 2.5 μM, six times more effective than the control

best result was obtained with sample 2, with an improvement factor of 6.

Sample 2 was irradiated for 3 h and, subsequently, left to decay for 3 h, before its addition to the cell culture. The IC_{50} value obtained under these conditions was 2.5 μM, which represents an increase of six times in the cytotoxic activity compared to the non-activated complex, [Tc–Pt(II)]*, see Fig. 4. The counting of the cells and determination of the IC_{50} occurred 72 h after the addition of the complexes to cells.

A detailed investigation of the influence of each Pt radioisotope and a description of the mechanisms involved in the enhancement of the cytotoxicity is out of the scope of this work. This is a much more complex discussion due to the several parameters involved, as the half-life, energies and abundance of all the gamma rays of each isotope and still the role of secondary radiation of daughter nuclides. This investigation is necessary to confirm the potential therapeutic of the [Tc–Pt(II)]*, for a better comprehension of the mechanism of radiation-cell interaction and the role of each Pt radioisotopes. In this case, a higher number of samples must be irradiated using different times of decay. Planning of work in this direction is already under way.

In the last few years, platinum-based radio chemotherapy has become standard treatment for patients with advanced, surgically unrespectable non-small-cell lung cancer (NSCLC). The combined-modality approaches show good prognosis for the treatment of unrespectable tumors. The results presented here reinforce the literature [8, 11, 13] about the additional tumor cells killing action of platinum compounds. The synergetic radio and chemotherapeutic effect was demonstrated with different preparations of [Tc–Pt(II)]* using the radioisotopes ^{191}Pt, ^{195m}Pt or natural platinum with a required radiochemical purity. The possibility of using natural platinum is very important aspect considering the high price of enriched Pt isotopes.

Conclusions

The radiolabeled complex of tetracycline with Pt(II), $[PtCl_2(C_{22}H_{24}N_2O_8)]$, was obtained through the direct irradiation in the TRIGA Mark 1, 100 kW research reactor, of this molecule. The UV–Vis spectra the molecule did not show any modification of the molecule after the irradiation. A comparative investigation of the antitumor effects using the radioactive and the non-radioactive complex demonstrated an antitumor activity against the K562 cells until six times higher for the radioactive complex. This result confirms the additional effect in the inhibition of tumor cell growth. This synergy of the radio and chemotherapy effects and can be a potential of therapy in the future.

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References

1. Rosenberg B, Van Camp L, Trosco JE, Mansour VH (1969) Platinum compounds: a new class of potent antitumour agents. Nature 222:385–386
2. Matthey J (1990) Drugs of the future. Bristol-Myers Squibb, New York
3. Pisters KM, Le Chevalier TJ (2005) Adjuvant chemotherapy in completely resected non-small-cell lung cancer. J Clin Oncol 23:3270–3278
4. Jamieson ER, Lippard SJ (1999) Structure, recognition, and processing of cisplatin-DNA adducts. Chem Rev 99:2467–2498
5. Silva H, Almeida M, César ET, Silveira JN, Garnier-Suillerot A, Paula FCS, Pereira-Maia EC, Fontes APJ (2008) Impact of the carbon chain length of novel platinum complexes. J Inorg Biochem Inorg Biochem 102:767–772
6. Sykes AG (1988) Reactions of complexes of platinum metals with bio-molecules. Platin Met Rev 32:170–178
7. Pasini A, Zunino F (1987) New cisplatin analogues—on the way to better antitumor agents. Angew Chem Int Ed Engl 26:615–624

8. Farrell N, Qu Y, Hacker MP (1990) Cytotoxicity and antitumor activity of bis(platinum)complexes. A novel class of platinum complexes active in cell lines resistant to both cisplatin and 1,2-diaminocyclohexane complexes. J Med Chem 33:2179–2184
9. Junior ADC, Abrantes FM, Menezes MA, Leal AS, Oliveira MC (2005) Braz Arch Biol Technol 48:85–88
10. Wolinskya JB, Colsonb YL, Grinstaff MW (2012) Local drug delivery strategies for cancer treatment: gels, nanoparticles, polymeric films, rods, and wafers. J Control Release 159:14–26
11. Duan X, He C, Kron SJ, Lin W (2016) WIRES nanomed nanobiotechnol. doi:10.1002/wnan.1390
12. Soares MA, Mattos JL, Pujatti PB, Leal AS, Santos WG, Santos RG (2011) Evaluation of the synergetic radio-chemotherapy effects of the radio labelled cisplatin for the treatment of glioma. J Radioanal Nucl Chem. doi:10.1007/s10967-011-1414-2
13. Júnior AD, Mota LG, Nunan EA, Wainstein AJ, Wainstein APD, Leal AS, Cardoso VN, De Oliveira MC (2007) Tissue distribution evaluation of stealth pH-sensitive liposomal cisplatin versus free cisplatin in Ehrlich tumor-bearing mice. Life Sci 80:659–664
14. Bodnar NE, Dikiy MP, Medvedeva EP (2014) Photonuclear production and antitumor effect of radioactive cisplatin (^{195m}Pt). J Radioanal Nucl Chem 305:133–138
15. Rozy K, Piyali C, Chadha VD (2014) Radiolabeling of cisplatin and its biodistribution in an experimental model of lung carcinogenesis. J Environ Pathol Toxicol Oncol 33:11–17
16. Wheller RH, Spencer S (1995) Cisplatin plus radiation therapy. J Infus Chemother 5:61–66
17. Chatal JF, Hoefnagel CA (1999) Radionuclide therapy. Lancet 354:931–935
18. Soares DCF, Menezes MABC, Santos RG, Ramaldes GA (2010) 159Gd: preparation and preliminary evaluation as a potential antitumoral radionuclide. J Radioanal Nucl Chem 284:315–320
19. Soares DCF, Oliveira MC, Santos RG, Andrade MS, Vilela JMC, Cardoso VM, Ramaldes GA (2011) Liposomes radiolabeled with ^{159}Gd-DTPA-BMA: preparation, physicochemical characterization, release profile and in vitro cytotoxic evaluation. Eur J Pharm Sci 42:462–469
20. Chartone-Souza E, Loyola TL, Bucciarelli-Rodriguez M, Menezes MABC, Nicolas AR, Pereira-Maia EC (2005) Synthesis and characterization of a tetracycline–platinum(II) complex active against resistant bacteria. J Inorg Biochem 99:1001–1008
21. Silva PP, de Paula FCS, Guerra W, Silveira JN, Botelho FV, Vieira Leda Q, Bortolotto T, Fischer FL, Bussi G, Terenzi H, Pereira-Maia EC (2010) Platinum(II) compounds of tetracyclines as potential anticancer agents: cytotoxicity, uptake and interactions with DNA. J Braz Chem Soc 7:1237–1246
22. Pereira-Maia EC, Silva PP, Almeida WB, Santos HF, Marcial BL, Ruggiero R, Guerra W (2010) Tetraciclinas e glicilciclinas: uma visão geral. Quim Nova 33:700–706
23. Leal AS, Júnior ADC, Abrantes FM, Menezes MABC, Ferraz V, Cruz TS, Cardoso VN, Oliveira MC (2006) Production of the radioactive antitumoral cisplatin. Appl Rad Isot 64:178–181
24. Leal AS, Sepe FP, Gomes TCB (2014) J Radional Nucl Chem 300:645–651
25. TEC DOC-564 (1990) Practical aspects of operating a neutron activation analysis laboratory, IAEA, Vienna, Austria
26. Lederer CM, Shirley VS (1978) Table of isotopes. Wiley Interscience Pub, New York