BJRS
BRAZILIAN JOURNAL OF RADIATION SCIENCES

doi.org/10.15392/2319-0612.2025.2614
2025, 13(1) | 01-15 | e2614
Submitted: 2024-08-19
Accepted: 2024-11-21

Additional radiation effect on cell viability using neutron irradiated Cisplatin and Carboplatin: preliminary results

Leal [a*], A. S..; Silva [a], J. B.; Morais [a], A. O. G.; Schirmer [a], B. G. A.

[a] Centro de Desenvolvimento da Tecnologia Nuclear (CDTN), Av. Antônio Carlos 6.627, Campus da UFMG, Pampulha, 31270-901, Belo Horizonte, Minas Gerais, Brazil.

*Correspondence: asleal@cdtn.br

Abstract: The objective of this work was to investigate the effect on cell viability of radiolabeled complexes of cisplatin and carboplatin in cancer cells of the 4T1 and MDA-MB-231 lines from mice with mammary carcinoma. The complexes were labeled in the CDTN's TRIGA Mark I nuclear research reactor after 3 hours of irradiation. The analyzes were carried out after decay times of 1h and 24h. The results showed a more effective action of cisplatin compared to carboplatin in both cell lines and greater resistance in MDA-MB-231 cells. The additional effect of radiation was more evident in conditions of low resistance. Increasing the final activity of labeled compounds can amplify this effect. Investigations in this regard are underway.

Keywords: Cisplatin, carboplatin, radiochemotherapy antitumoral effects

SBPR

BJRS
BRAZILIAN JOURNAL OF RADIATION SCIENCES

doi.org/10.15392/2319-0612.2025.2614
2025, 13(1) | 01-15 | e2614
Submitted: 2024-08-19
Accepted: 2024-11-21

Efeito adicional da radiação na viabilidade celular usando Cisplatina e Carboplatina irradiadas com nêutrons: resultados preliminares

Resumo: O objetivo deste trabalho foi investigar o efeito na viabilidade celular de complexos radiomarcados de cisplatina e carboplatina em células cancerígenas das linhagens 4T1 e MDA-MB-231 de camundongos com carcinoma mamário. Os complexos foram marcados no reator nuclear de pesquisas TRIGA Mark I do CDTN em 3h de irradiação. As análises foram feitas após tempos e 1h e 24h de decaimento. Os resultados mostraram ação mais efetiva da cisplatina em comparação a carboplatina nas duas linhagens de células, e resistência maior das células tipo MDA-MB-231. O efeito adicional da radiação se mostrou mais evidente nas condições de baixa resistência. Um aumento da atividade final dos compostos marcados pode ampliar esse efeito. Investigações nesse sentido estão em andamento.

Palavras-chave: Cisplatina, carboplatina, radioquimioterapia, efeitos antitumorais

SBPR

1. INTRODUCTION

The platinum complexes cisplatin: cis-diamminedichloroplatinum (II), $(NH_3)_2PtCl_2$ and carboplatin: cis-diammine (cyclobutane-1,1-dicarboxylate-O,O')platinum(II), $C_6H_{12}N_2O_4Pt$, are two of the most important chemotherapeutic agents used in the treatment of a wide variety of solid tumors and its interaction with DNA is pointed out as the main mechanism of cytotoxic action [1, 2]. But, in spite of their strong anticancer potency, chemotherapy with platinum complexes is associated with very serious side effects, such as: nephrotoxicity, ototoxicity, nausea, neuropathy and allergy [3].

In recent years, a multifactorial strategy for cancer treatment has been acknowledged and different treatment modalities are often combined to optimize treatment efficacy. One of the approaches is the utilization of the new drugs able to induce simultaneous low ionizing radiation and chemotherapy effects.

Radiolabeled chemotherapeutics can be an exciting strategy for the therapy of cancer, as lethal cytotoxic radiation dose delivered to the cancerous lesions allows to minimize the amount of chemotherapy drugs and the serious side effects to the patients. Radiolabeling the drugs with suitable therapeutic radionuclides have been achieved successfully and these radiolabeled agents have shown considerable promise in the pre-clinical studies. The synergy of two different mechanisms may reduce the chemotherapy drug dose, frequently associated with severe or undesirable side effects providing significant benefits for the patients [4-8].

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, the synergetic radio chemotherapy effect against cells of glioma was demonstrated with of application of labeled cisplatin. The use of internal radio chemotherapy with low irradiation dose rate and enhanced selectivity to the target tissues may become a new and promising alternative of treatment for some unresectable malignant tumors [9-11]. In another work, a comparative investigation of the antitumor effects using the radioactive and the non-radioactive complex of tetracycline with Pt (II), [$PtCl_2(C_{22}H_{24}N_2O_8)$], demonstrated an antitumor activity against the K562 cells until six times higher for the radioactive complex [10]. This result confirms the additional effect in the inhibition of tumor cell growth and the potential of synergy of the radio and chemotherapy effects for therapy in the future [12-14].

One of the most promising strategies is to label the drugs with Auger electron emitters. Auger radiation delivered into the DNA of tumor cells can be a very attractive systemic radiation therapy goal. Natural platinum activation produces some radioisotopes with suitable half-life and energy, both gamma and beta emitters [15]. Our previous dosimetric studies of platin radiolabeled complexes, as a preliminary step for the feasibility assessment of clinical use, showed a good agreement with experimental data [16,17].

This work describes the preparation of the radiolabeled cisplatin and carboplatin obtained through the direct irradiation in the TRIGA Mark 1 research reactor of the CDTN to investigate their possible potential enhancement of cytotoxicity in vitro in cancer cells line 4T1 mice mammary carcinoma and MDA-MB-231 cells (ATCC®), compared to the original, the non-labeled molecules. The preliminary results obtained suggests that this strategy may be promising as antitumor therapeutic in the future. Additional investigations into the efficiency of this strategy are ongoing.

2. MATERIALS AND METHODS

Samples of 5.0mg of cisplatin and carboplatin were irradiated by 3h in the TRIGA´s CDTN research reactor in an average thermal and epithermal neutrons flux of 6.6 x 10^{11} and 4.4 x 10^{10} $cm^{-2}.s^{-1}$, respectively, at 100kW. The gamma spectra were obtained using an HPGe detector with 15% nominal efficiency and 1.85 keV FWHM for the 1332 keV peak of ^{60}Co and samples were measured at 5.0 cm from the detector. The software used for data acquisition and spectrum analysis was GENIE 2000 (CANBERRA).

Radiochemical identity of cisplatin and carboplatin irradiated were determined by scanning the TLC SG plate with a suitable collimated radiation detector (Minigita star beta detector, Raytest®). The TLC stationary phase was silica gel and the mobile phase was acetone: 0,1M HCl 7:3. The determination of chemical purity was performed using high performance liquid chromatography (HPLC), Shimadzu, and variable wavelength UV detector. Separations were made using LUNA 5µM NH2 250 x 4,6 MM column. The flow rate was 1.0 $mL.min^{-1}$, the sample volume was 20 µL and the solvent system was composed of methanol/ethyl acetate/N, N-dimethylformamide/water 4:4:1:1 and the flow rate were of 1.0 mL min−1. Samples (20µL) were injected, and the eluate absorbance was monitored at 300 nm.

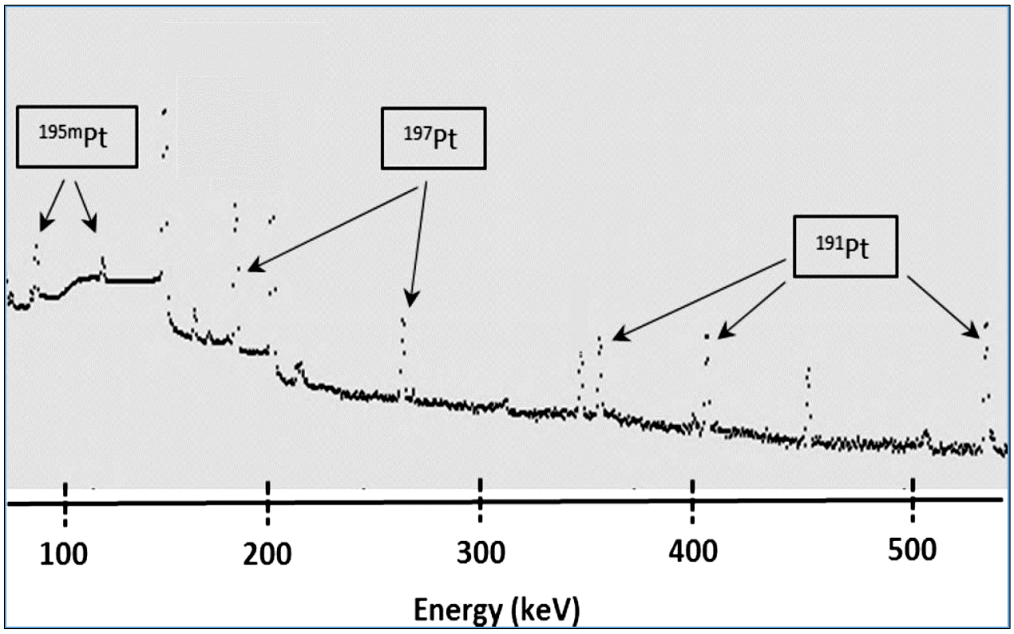
The *in-vitro* tests were performed after a decay time of 1h and 24 hours. The cell viability was evaluated by CCK-8 assay (Sigma-Aldrich). MDA-MB-231 and 4T1 cells were plated in the 96-well plates (5 × 10^3 cells per well) and incubated for 24 hours. Cisplatin and carboplatin (nonirradiated and at 1 and 24 hours after the irradiation) were introduced separately to cells with different test concentrations (20.0, 10.0, 5.0, 2.5 and 0.75 µmol/L) in culture medium. After 48 hours of incubation, 10 microliters of CCK-8 solution was added to each well and incubated for an additional 3 h at 37 °C. The optical density (OD) of each well at 450 nm was recorded on a Microplate Reader (Varioskan Flash, Thermo Fisher Scientific, US). The cell viability (% of control) is expressed as the percentage of (ODtest − ODblank)/ (ODcontrol − ODblank), where ODtest is the optical density of the cells

exposed to treatment, ODcontrol is the optical density of the control sample and ODblank is the optical density of the wells without cells.

3. RESULTS AND DISCUSSIONS

Fig. 1 illustrates the gamma spectra and some photo peaks of platinum radioisotopes, after 3h of irradiation and 1h of decay time. The energies and half-lives are presented in Table 1. This time was chosen to obtain the minimum activity of Pt radioisotopes necessary according to our previous studies [9, 10, 14].

Figure 1. Gamma spectra of carboplatin after 3h of irradiation.



In Fig. 1, the photopeak of 194mPt, 197Pt and 191Pt can be seen in the range 100 to 550keV. β^- radiation appears in the decay of ^{197}Pt and ^{199}Pt with energies from 451 to 670keV. The activity of the cisplatin after irradiation is approximately 1.11MBq after 1h and 0.10MBq after 24h of decay. For carboplatin the values are 0.55 and 0.07MBq, respectively.

Table 1. Radionuclides Decay Data

Pt isotopes	Radionuclides produced	Half-life	Decay modes	γ energy in keV
^{190}Pt	^{191}Pt	2.96d	IT	359.0, 409.5, 439.9
^{192}Pt	^{193m}Pt	4.33d	EC	---
^{194}Pt	^{195m}Pt	4.02d	EC	98.8, 129.7
^{196}Pt	^{197}Pt	18.3h	β^-, IT	269.1, 191.4
^{198}Pt	^{199}Pt	30.8m	β^-, IT	317.1, 493.7, 542.9

The results of a TLC analysis of irradiated cisplatin indicates a retention factor (Rf value) of approximately 0.70, and the absence of any impurities or degradation products. The results of a High-Performance Liquid Chromatography (HPLC) analysis of irradiated cisplatin and carboplatin, indicating the absence of any impurity products, corroborate the results of the TLC analysis. The radiochemical purity (> 90%) for both cisplatin and carboplatin confirm the results of previous studies, including our own, indicating that the irradiation process did not cause degradation of the platinum complex. samples [4,9,10,17]. It is the minimum radiopharmaceutical purity for in vitro studies that typically needs to be high to ensure reliable and reproducible results. Generally, a radiochemical purity of at least 90% is considered acceptable. This means that at least 90% of the radiopharmaceutical should be in the desired form, with the remaining percentage consisting of impurities [18-22]

Figs. 2 and 3 show the results of cell viability tests of radiolabeled and non-irradiated cisplatin (Fig.2) and carboplatin (Fig.3) in 4T1 mice mammary carcinoma cells. In relation to cisplatin, a very effective action of the non-irradiated drug is observed, with cell viability reduced to < 10% at a concentration of 20 μmol/L. These results are like those obtained with the labeled molecular after 1h of decay and decrease by half after 24 of decay.

Figure 2. Percentage of the 4T1 mice mammary carcinoma cells viability for non-irradiated and irradiated cisplatin, after 1 and 24 hours of decay. The cell viability (% of control) results were expressed as Mean ± SD of the average of 3 independent experiments (N=3); *p < 0.05 using Student's t-test.

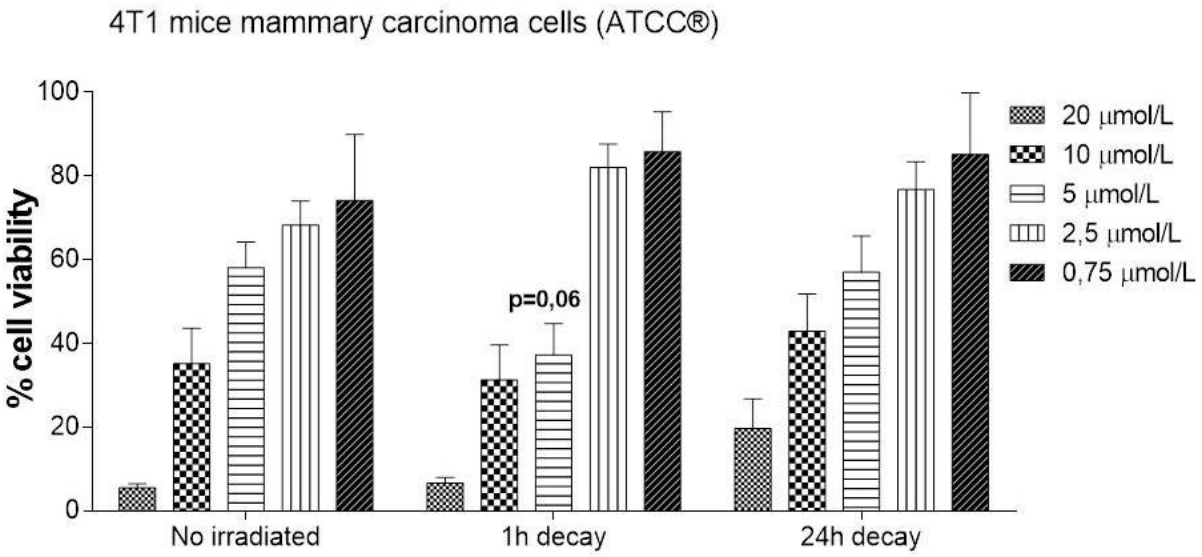
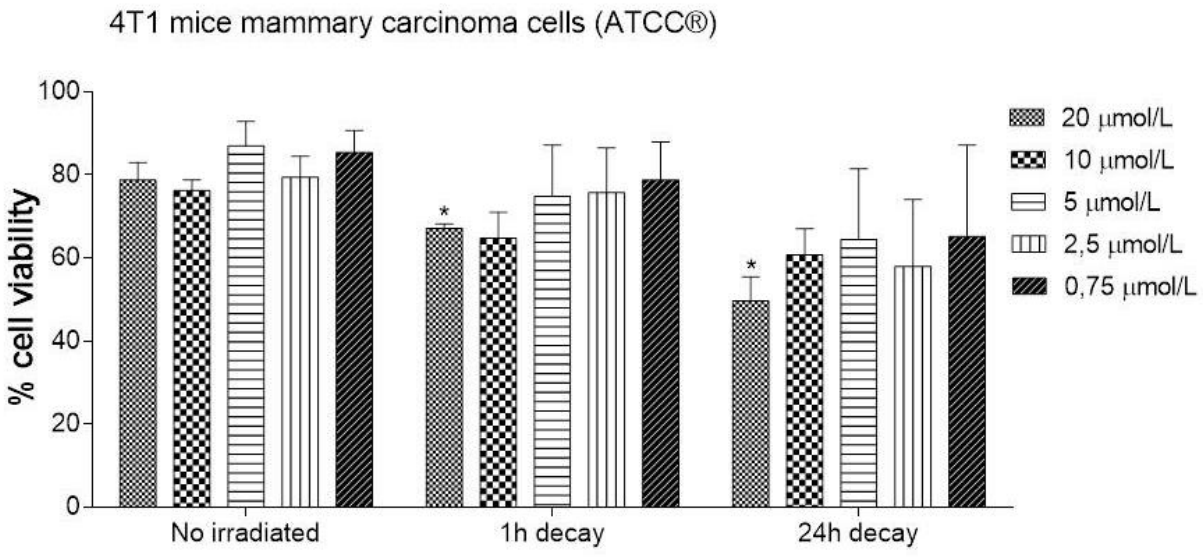


Figure 3. Percentage of the 4T1 mice mammary carcinoma cells viability for non-irradiated and irradiated carboplatin, after 1 and 24 hours of decay. The cell viability (% of control) results were expressed as Mean ± SD of the average of 3 independent experiments (N=3); *p < 0.05 using Student's t-test.



At a concentration of 5 µmol/L with a decrease in cell viability from 60 to 40% after 1 hour of decay and less markedly after 24 hours (Fig. 2).

Concerning the carboplatin, a more effective action of the irradiated drug is observed with 1h of decay when compared to the no irradiated treated cells. The cell viability of 4T1 cells drops from ~80% to ~50% after 24h of decay at a concentration of 20 μmol/L. This effect is also observed, although less evident, at concentrations of 5 μmol/L and 0.75 μmol/L, after 24h of decay (Fig. 3).

Souza et al. (2013) [23] demonstrated previously that carboplatin has been an efficient regulator of 4T1 growth and dissemination, and its action seems to be related to the induction of apoptosis and inhibition of angiogenesis and cell proliferation in vivo. Our results suggest that this effect can be enhanced after carboplatin irradiation, with in vivo experiments being the next step for this study.

Figs. 4 and 5 show the results of cell viability tests of radiolabeled and non-irradiated cisplatin (Fig.4) and carboplatin (Fig.5) in MDA-MB-231 cell lineage. In relation to cisplatin, it can be observed that the non-irradiated samples have only a very limited chemotherapeutic action. In all the concentrations tested, cell viability is higher than 70%. It can be observed only a low effective action of the irradiated drug, compared to the non-irradiated one, only in the concentrations of 20 μmol/L (~78 to 50% of cell viability) and 10 μmol/L (~70 to 50% of cell viability) for 1h of decay time. After 24h of decay time, any effect was observed (Fig. 4). In the case of carboplatin, Fig. 5 shows that the MDA-MB-231 cells are very resistant to this drug. Only a very limited action is observed in the (~80% of cell viability) in the concentration of 20 μmol/L. Any important additional effect was detected for the irradiated carboplatin.

Cisplatin is a chemotherapeutic agent that is often effective in treating various cancer cell lines, including MDA-MB-231 and 4T1, due to its mechanism of action, mainly due to mechanisms of DNA damage and cell cycle arrest, apoptosis and ROS generation [24-26]. Our results suggest that this effect can be enhanced after cisplatin irradiation, with in vivo experiments being the next step for this study.

Figure 4. Percentage of the MDA-MB-231 human breast adenocarcinoma cells viability for non-irradiated and irradiated cisplatin after 1 and 24 hours of decay. The cell viability (% of control) results were expressed as Mean ± SD of the average of 3 independent experiments (N=3); *p < 0.05 using Student's t-test.

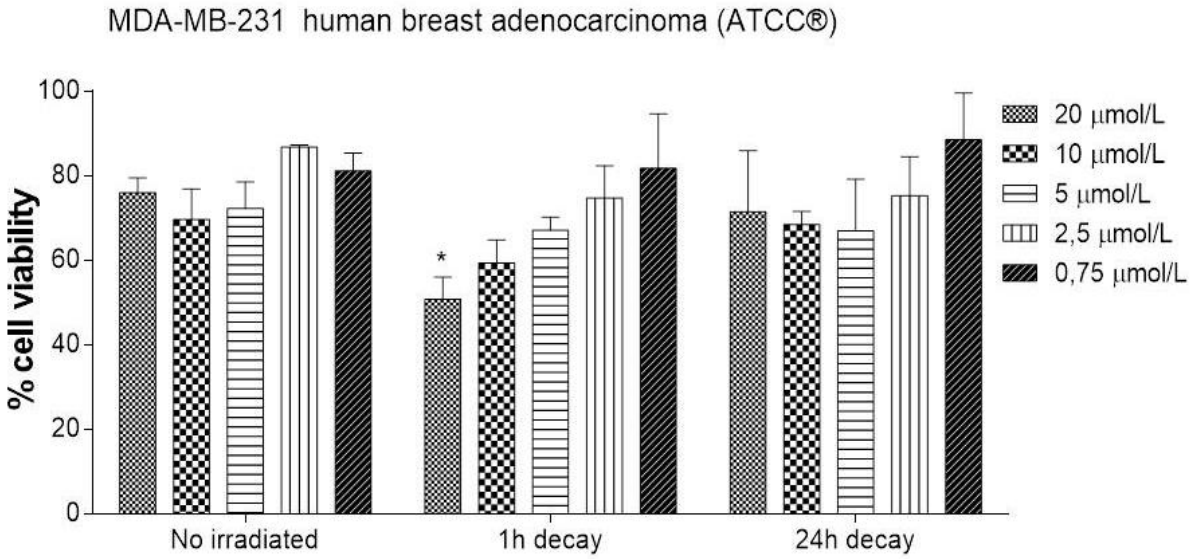
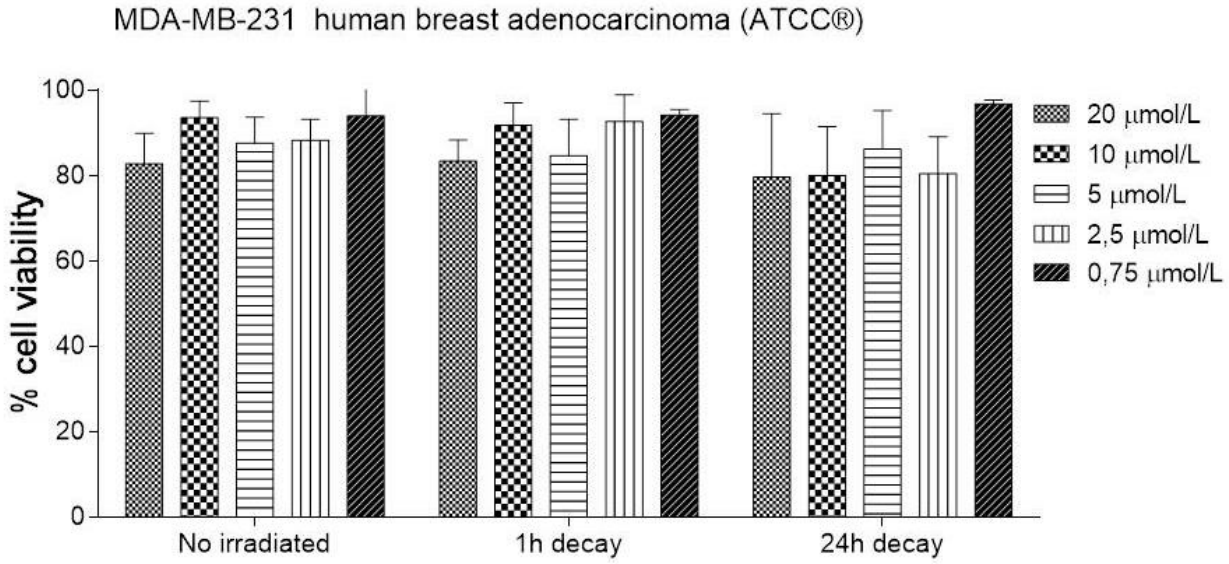


Figure 5. Percentage of the MDA-MB-231 human breast adenocarcinoma cells viability for non-irradiated and irradiated carboplatin after 1 and 24 hours of decay. The cell viability (% of control) results were expressed as Mean ± SD of the average of 3 independent experiments (N=3); *p < 0.05 using Student's t-test.



MDA-MB-231 cells are a widely used model of triple-negative breast cancer (TNBC), which is known for its aggressive behavior and poor prognosis. Several factors contribute to the observed resistance of MDA-MB-231 cells to carboplatin, a chemotherapy drug, such as presence of mutations that can lead to an impaired apoptotic response to DNA-damaging and presence of DNA repair mechanisms [27]. Some studies have already demonstrated that these cells are more resistant to carboplatin. Guiro et al [28] showed that this cell lineage is resistant even with the carboplatin optimization at a maximum carboplatin concentration of 220 μg/ml, these treated cells maintained higher percent viability of 80% as compared to percentage of viability for the non-treated cells.

4. CONCLUSIONS

The results obtained in cell viability tests of type 4T1 cells show much greater resistance of the cells to the action of carboplatin compared to cisplatin considering the labeled and irradiated molecule, except in the case of the concentration 0.75 μmol/L for 24h decay, in which cell viability drops from ~90% with cisplatin to ~70% with carboplatin. The results suggest that the decrease in cell viability with the increase in decay time in the case of carboplatin is related to the properties: concentration, half-life time, energy and type of radiation emitted from the different platinum radioisotopes formed, as Pt-191, Pt-193, Pt-195m, and Pt-199. The results suggest that radiation may have additional effect on chemotherapy, allowing a reduction in drug dose and side effects. Additional studies are underway to confirm this hypothesis.

ACKNOWLEDGMENT

The authors thank the team of the TRIGA´s CDTN reactor for their operational support.

FUNDING

The authors thank the CNPq and FAPEMIG for their financial support (PPM 00260-18).

CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

REFERENCES

[1] DIAS, G.H.M. Cisplatina e Análogos: Drogas Inorgânicas Anticancerígenas. **Química Nova** vol. 12, p. 57-59, 1989.

[2] SIDDIK, Z.H., JONNES, M., BOXALL, F.E., HARRAP, K.E., Comparative distribution and excretion of cisplatin and carboplatin in mice. **Cancer Chemother Pharmacol** vol. 21, p.19-24, 1988.

[3] AREBERG, J. Studies of radioactive cisplatin (Pt) for tumor imaging and therapy. PhD Thesis. Lund University, Malmo/Sweden, 2000.

[4] SOARES, M.A., MATTOS, J.L., PUJATTI, P.B, LEAL, A.S., SANTOS, W.G., SANTOS, R.G. Evaluation of the synergetic radiochemotherapy effects of the radio labelled cisplatin for the treatment of glioma. **J Radioana Nucl Chem.** vol. 292, p. 61-65,2011.

[5] NEWMAN, M.S., COLBERN, G.T., WORKING, P.K., Comparative pharmacokinetics, tissue distribution and therapeutic effectiveness of cisplatin encapsulated in long-circulating, pegylated liposomes (SPI-077) in tumor bearing mice. **Cancer Chemother Pharmacol,** vol. 43, p. 1-7, 1999.

[6] AREBERG, J., BJORKMAN, S., EINARSSON, L., FRANKENBERG, B., LUNDQVIST, H., MATTSSON, S., SORRGREN, K., SCHEIKE, O., WALLI, R. Gamma camera imaging of platinum in tumors and tissues of patients after administration of *™Pt-cisplatin. **Acta Oncologica**, vol. 38, p.221-228, 1999.

[7] CORNELISSEN, B., VALLIS, K.A. Targeting the Nucleus: An Overview of Auger-Electron Radionuclide Therapy. **Curr Drug Discov Technol**, vol. 7, p. 263-279, 2010.

[8] KAWAI, K., MAKI, H., EHRLICH, W. Synthesis of 195mPt Radiolabeled, cis—dichlorodiammineplatinum (II) of High Chemical and Radiochemical Purity Using High Performance Liquid Chromatography. **J Radioanal Nucl Chem** vol. 136, p. 67-74, 1989.

[9] LEAL, A.S., JUNIOR, A.D.C., ABRANTES, FM, MENEZES, M.A.B.C., FERRAZ, V., CRUZ, T.S., OLIVEIRA, M.C. Production of the radioactive antitumoral cisplatin. **Appl Rad Isot** vol. 64, p. 78-181, 2006.

[10] LEAL, A.S., MARZANO, I.M., PEREIRA-MAIA, E.C., JACIMOVIC, R. Investigation of the potential antitumor radioactive complex of platinum (II) with tetracycline. **J Radioanal Nucl Chem.** vol. 309, p.85-89, 2016.

[11] LEAL, A.S., MENDES, B.M., BERNARDES, F.D. Estudo preliminar da dose absorvida e efetiva da cisplatina radiomarcada com 19^{5m}Pt. **Braz. J Rad.** vol. 08-01, p.01-15, 2020.

[12] JAIN, A., DAS, T. Radiolabeled chemotherapeutics as a novel strategy for targeted cancer therapy: current insights and future perspectives. **J Radioanal Nucl Chem** vol. 333, p.1–15, 2024.

[13] BUCHEGGER, F., PERILLO-ADAMER, F., DUPERTUIS, Y.M., DELALOYE, A.B. Auger radiation targeted into DNA: a therapy perspective. **Eur J Nucl Med Mol Imaging** vol. 33, p. 1352-1363, 2006.

[14] REISSIG, F., RUNGE, R., NAUMANN, A., KOTZERKE, J. Cisplatin – A more efficient drug in combination with radionuclides? **Nuklearmedizin** vol. 61, pp 325-332, 2022.

[15] CORNELISSEN, B., VALLIS, K.A. Targeting the Nucleus: An Overview of Auger-Electron Radionuclide Therapy, **Curr Drug Discov Technol** vol. 4, p. 263-279, 2010.

[16] BUSSOLOTTI, G.R., NIKEZIC, K.D., LEAL, A.S., ZIVKOVIC, M., MENDES, B.M. Computational Internal dosimetry of natural cisplatin activated in neutron flux: the ^{191}Pt contribution. **Braz. J Rad** vol. 10-02A, p.01-17, 2022.

[17] JÚNIOR, A.D.C., ABRANTES, F.M., MENEZES, M.A.B.C., LEAL, A.S., OLIVEIRA, M.C. Use of Labelled Cisplatin Obtained by Direct Irradiation in a Preliminary Biodistribution Study. **Braz Arch Biol Technol** vol.48, p. 85-88, 2005.

[18] KOMAROV E., WALLEN Q. Quality Control of Radiopharmaceuticals. Bulletin International Atomic Energy Agency (IAEA), vol 15-2, 1973.

[19] European Directorate for Quality of Medicines and Healthcare (EDQM) of the Councik of Europe. Available at: https://www.ema.europa.eu. Accessed on 10 Nov 2024.

[20] Quality Control in Production Radiopharmaceuticals. International Atomic Energy Agency (IAEA) TEC-DOC 1856, 2018

[21] GILLINGS, N., HJELSTUEN, O., BALLINGER, J. *et al.* Guideline on current good radiopharmacy practice (cGRPP) for the small-scale preparation of radiopharmaceuticals. **EJNMMI radiopharm. chem. 6**, 8, 2021.

[22] MOLAVIPORDANJANI S., HOSSEINIMEHR, S.J. Fundamental Concepts of Radiopharmaceuticals Quality Controls. **Pharm Biomed Res** vol.4(3), p.1-8, 2018.

[23] SOUZA, C.M., OLIVEIRA, G.C., CAMPOS, C.B., LOPES, M.T.P., FERREIRA, M.D., ANDRADE, SP. Carboplatin delays mammary cancer 4T1 growth in mice. **Pathol. Res. Pract**. vol. 209(1), p. 24-29, 2013.

[24] GALLUZZI, L., et al. Molecular mechanisms of cisplatin resistance. **Oncogene,** vol. 31(15), p. 1869-1883, 2012.

[25] Dasari, S, Tchounwou, PB. "Cisplatin in cancer therapy: molecular mechanisms of action", *Eur J Pharmacol*. Vol. 740, pp. 364-378 (2014).

[26] MARULLO, R. et al. Cisplatin induces a mitochondrial-ROS response that contributes to cytotoxicity depending on mitochondrial redox status and bioenergetic functions. ***PLoS One***, vol. 8(11), e81162, 2013.

[27] YANG, X. et al. Functional consequences of BRCA1 missense variants, **Cancers** vol. 3(1), p. 75-88, 2011.

[28] GUIRO, K., PATEL, A.S., GRECO, S.J., RAMESHWAR, P., ARINZEH, T.L Investigating breast cancer cell behavior using tissue engineering scaffolds. **PLoS One,** vol. 10(4), e0118724, 2015.

LICENSE