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EFFECTS OF A CONVENTIONAL CHELATOR-MODIFIED ANTI-INTEGRIN PEPTIDE ON GLIOBLASTOMA CELL PROLIFERATION AND MIGRATION

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A B S T R A C T

Introduction/Justification: Glioblastoma (GB) is the most aggressive brain tumor, with high morbidity and mortality rates. The overall survival of GB patients is only 14 months, not improved by the traditional or latest therapeutic options, as surgical resection, temozolomide chemoradiation or gefitinib. FAPESP-founded "Cancer Innovation Center with Emphasis on Metals and Theranostics" (CancerThera) is dedicated to the development of new metallopharmaceuticals and radiopharmaceuticals for tumor diagnosis and treatment. Among these developments, an anti-integrin peptide modified by a conventional spacer (C6) and chelator (DOTA) was evaluated as a potential treatment of GB. Overexpressed in GB, integrins are transmembrane proteins that play essential roles in cell proliferation and migration. Therefore, integrin inhibition may be a potential targeted therapy for GB patients. **Objectives:** The study aimed to evaluate the effects of the DOTA-C6-anti-integrin peptide on GB cell lines proliferation and migration, as an initial step for GB theranostic development. **Materials and Methods:** The anti-proliferative activity of the DOTA-C6-anti-integrin peptide (0.01 nM - 100 μM) was evaluated in human GB (U87, U118, and U251), murine GB (GL261), and non-tumoral cell lines (HaCaT), by considering the cell amounts at baseline and 48h after exposure (two untreated control groups). Cells were fixed with 50% trichloroacetic acid and stained with sulforhodamine B. Spectrophotometric absorbance was performed at 540nm in a microplate reader. Cell migration was assessed in U118, U251, and Gl261 cells treated with the DOTA-C6-anti-integrin peptide (1 μM - 100 μM) using the wound-healing assay. Wound cells were photographed immediately (0h) and after 24h. Images were analyzed by the ImageJ software (National Institutes of Health). For statistical analysis, samples did assume normal distribution in Shapiro-Wilk's test, thus we used t test to compare the groups using SPSS 21 software (SPSS Incorporation). Resultados: At the tested concentration range, the DOTA-C6-anti-integrin peptide did not affect proliferation of GB and HaCaT cell lines. In U118 cells, we observed that treatment with the DOTA-C6-anti-integrin peptide had no effect on cell migration at any of the tested concentrations. In contrast, in U251 cells, the treatment significantly inhibited migration compared to untreated cells at a concentration of 100 μM ($p = 0.03$). In Gl261 cells, the treatment significantly inhibited migration compared to untreated cells at concentrations of 1 μM and 0.1 μM ($p = 0.04$). **Conclusion:** Despite the lack of anti-proliferative effect, the DOTA-C6-anti-integrin peptide inhibited migration in GB cell lines, U251 and Gl261. An invasive pattern being a GB hallmark, our data suggests that the DOTA-C6-anti-integrin peptide may aid in developing a GB theranostic agent. **Acknowledgements:** The study was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq #429463/2018-9), Fundação de Apoio ao Ensino e à Pesquisa do Estado de São Paulo (FAPESP #2023/09738-4, FAPESP #2023/012810-9, Cancer Theranostics Innovation Center (CancerThera), CEPID FAPESP #2021/10265-8), and International Atomic Energy Agency (IAEA) technical cooperation projects for development of

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Keywords: Anti-integrin peptide, Cell migration, Glioblastoma.

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COMPETITION RESPONSE OF PSMA-I&T RADIOLABELED WITH LUTETIUM-177 TO LNCAP, PC-3 AND RWPE-1 CELLS

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A B S T R A C T

Introduction/Justification: 177Lu-PSMA-I&T stands out as a promisor radiopharmaceutical for therapy of prostate cancer based on the specific bind of Glu-urea-Lys pharmacophoric group prostate-specific membrane antigen (PSMA), anchored in the epithelial prostate cell membrane, overexpressed in prostate cancer and increased in metastatic castration-resistant prostate cancer (mCRPC). To study the affinity of 177Lu-PSMA-I&T to target receptor, in vitro competition assay is frequently evaluated. **Objectives:** The purpose of this study was to compare the binding of 177Lu-PSMA-I&T in competition assay to three cell lines. LNCaP and PC-3 are the most used in vitro cell lines studies of prostate cancer research and LNCaP cells are known to have a mutated androgen receptor (AR) (T877A), PC3 is negative for AR expression, and RWPE-1 is frequently used as non-cancerous control. **Materials and Methods:** Radiochemical purity (%RP) of radiolabeling 177Lu-PSMA-I&T was determined by High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) with results of > 95% of main peak and < 3% of free 177Lu, respectively. In vitro assays were performed with LNCaP (ATCC® CRL- 1740, American Type Culture Collection), RWPE-1 and PC-3 (LIM55, FMUSP) cell lines, cultivated in RMPl 1640 medium (Life Technologies, MD, USA) plus 10% v/v Fetal Bovine Serum (FBS) with 100 UI/mL of penicillin and 300 μg/mL of streptomycin. 6-well plates were used, and to each well 2×105 cells. For the total binding, cell incubation medium was removed and replaced with 1 mL of 177Lu-PSMA-I&T (2.22 MBq (60 μCi), approximately 0.076 nmol of peptide, diluted with RMPl 1640 medium/10% v/v FBS) and 1 mL of RMPl 1640 medium, per well. The plates were incubated for 1 h at 37 °C. Cells were washed two times with 1 mL of 0.1 M PBS pH 7.4, followed by an incubation step of 5 minute at room temperature with 1mL ice-cold glycine buffer (0.05 M glycine pH 2.8) and lysed with 2 mL of 1 M sodium hydroxide and incubation step of 10 minutes at room temperature. The same procedure was repeated replacing 1 mL of RMPl 1640 medium with 1mL of competitor (PSMA I&T, molar excess of 7.6 nmol in RMPl 1640 medium). To have the same geometry, the tubes were filled to the same volume (1mL) at each step. An automatic gamma counter with NaI (TI) crystal (D5002 Cobra II, Packard) was used to measure the radioactivity (as cpm) at each tube, and the concentration of 177Lu-PSMA-I&T bonded to the cells was determined in fmol. The assays were performed in quintuplicate for each cell. **Results:** The binding of 177Lu-PSMA-I&T to LNCaP cells showed 1309.3 $\pm$ 176.8 fmol without competitor and 928.5 $\pm$ 84.7 fmol in the presence of competitor, with significant difference (P= 0.0152, GraphPad Prism®). PC-3 cell line showed 28.8 $\pm$ 15.2 fmol without competitor and 25.3 $\pm$ 6.2 fmol with competitor, showing no significant variation (P = 0.6599). The results of binding with RPWE-1 cell line showed 74.3 $\pm$ 6.2 fmol without competitor and 37.9 $\pm$ 7.7 fmol with competitor, a significant difference ($P \leq 0.0001$). **Conclusion:** These results demonstrated the affinity of 177Lu-PSMA-I&T for binding receptors in LNCaP cells and low uptake by PC-3 cells due to the lack of expression of specific receptors. RWPE-1 cell line is positive for AR/PSA mRNA/protein and sensitive to androgens. However, it expresses low levels of PSMA, which likely explains the reduced binding of the radiopharmaceutical.

Keywords: Binding, Lutetium-177, PSMA-I&T, Radiopharmaceutical;.

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SYNTHETIC QUINAZOLINONES AS NEW ANTILEUKEMIC AGENTS

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A B S T R A C T

Introduction/Justification: Acute leukemias are aggressive malignancies characterized by the uncontrolled proliferation of hematopoietic progenitor cells in the bone marrow, leading to impaired production of normal blood cells. Nitrogen heterocycles have attracted the attention of researchers from various fields, with an extensive list of different biological activities. Among the heterocycles, quinazolines stand out, which have been widely investigated for the development of new drugs. **Objectives:** Evaluation of the anticancer activity of quinazolinones against acute leukemic cell lines. **Materials and Methods:** The quinazolinones (A1-A20) were synthesized in the Laboratory of Synthesis of Natural Products and Drugs (Institute of Chemistry, Unicamp). In total 2×104 cells of T cell acute lymphoblastic leukemia (T-ALL), Jurkat, and acute promyelocytic leukemia (APL), NB4, per well were seeded in a 96-well plate in the appropriate medium in the presence of vehicle or different concentrations of compounds (ranged