synthesized employing the solid-phase peptide synthesis through the Fmoc/tBut strategy, characterized, and purified utilizing high-performance liquid chromatography (HPLC). By using the tricarbonyl method, it was possible to label the histidine residue of the peptide fragments with the organometallic aqua-ion [99mTc(H2O)3(CO)3]+, abbreviated as [99mTc]TcCO3, directly from reaction of [99mTc]TcO4- under 1 atm of CO for 30 min at 70°C. Subsequently, both peptides were labeled with approximately 148 MBq and incubated for 30 min at 85°C. The stability of the radiolabeled peptides in saline and serum was assessed at 1, 2, 3, and 4 h and evaluated using HPLC. The partition coefficient was determined for both radiopeptides. Studies to assess the percentage of binding to serum proteins were conducted at 60 min. The binding and internalization of radiolabeled peptides with tumorigenic cells derived from breast cancer (MCF-7) were assessed at 1, 2, and 4 h. **Results:** The HYIGSR and HIKVAV peptides were efficiently synthesized and characterized. The radiolabeling process with [99mTc]TcCO3 was optimized and the [99mTc]TcCO3-HYIGSR and [99mTc]TcCO3- HYIKVAV were successful obtained with radiochemical yields of 95.53% ± 1.19 and 95.13% ± 1.96 (n = 6), respectively. Notably, stability studies revealed that both radiopeptides exhibited stability within a four-hour timeframe when stored in either saline or serum. The [99mTc]TcCO3-peptides demonstrated hydrophilic properties, as indicated by Log P values of -2.12 ± 0.16 and -1.39 ± 0.19 (n = 3) for [99mTc]TcCO3-HYIGSR and [99mTc]TcCO3-HYIKVAV, respectively. Additionally, the binding percentage to serum proteins for [99mTc]TcCO3-HYIGSR and [99mTc]TcCO3-HYIKVAV was found to be 46.08% ± 3.75 and 24.87% ± 6.24 (n = 3) within 60 min, respectively. Furthermore, binding and internalization studies conducted with MCF-7 cells (n = 4) demonstrated a higher percentage of binding and internalization for [99mTc]TcCO3-HYIKVAV, with values of 9.20% ± 2.87 and 51.74% ± 8.00, respectively. In contrast, [99mTc]TcCO3-HYIGSR exhibited percentages of 2.96% ± 0.60 and 25.85% ± 3.33 for binding and internalization within a 1-hour period. **Conclusion:** Both peptides exhibited good radiochemical yields and demonstrated sustained stability over the course of the study. Both peptides showed hydrophilic characteristics and our findings specifically underscored the higher affinity of [99mTc]TcCO3-HYIKVAV towards human breast cancer cells. Nevertheless, it is imperative to note that further in vivo investigations are necessary.

Keywords: Breast cancer, Laminin-111, MCF-7 cells., Tricarbonyl-labeled peptides.

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ASSESSMENT OF IN VITRO STUDIES OF [131I]I-LABELED DEDEYFELV PEPTIDE AS PROSPECTIVE BIOMARKER FOR GLIOBLASTOMA

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Introduction/Justification: Glioblastomas (GBM) constitute the most prevalent malignant primary tumor in adults and rank as the third most frequent tumors in the central nervous system. The predominant alteration observed in GBM is associated with the tyrosine-kinase pathway, facilitating the connection of growth factors to receptors. Notably, in GBM the overexpression of the epidermal growth factor receptor (EGFr) has opened new treatment perspectives, including molecular targeted therapies, with peptides taking center stage. Recently, radiolabeled peptides with high affinity for EGFr have been employed as potential agents for molecular imaging or targeted radionuclide therapy as anti-tumor agents. **Objectives:** The aim of the study was to assess the interaction between [131I]I-DEDEYFELV peptide and human glioblastoma cells (U-87 MG), as well as with GBM tissue. Materiais e Métodos: The DEDEYFELV peptide was synthesized through solid-phase peptide synthesis using the Fmoc/tBut strategy. Peptide cleavage from the resin was performed using a mixture containing a high concentration of trifluoroacetic acid (reagent K). Subsequently, the peptides underwent a characterization and purification process employing high performance liquid chromatography (HPLC) and mass spectrometry. The U-87 MG cell line was cultured in supplemented DMEM F-12 medium at 37°C and 5% CO2 until reaching 90% confluence. The neoplastic tissue was surgically removed, histopathologically analyzed, and preserved at -80°C, with its homogenate prepared using PBS buffer (pH 7.4) at 10 mg of tissue/mL. The DEDEYFELV (20 nmol) was radiolabeled with the [131I]NaI radionuclide (18.5 MBq), using the chloramine-T method. The radiochemical yield of the [131I]I-DEDEYFELV was carried out by on Whatmann 3MM strips using 95% MeOH / 5% H2O as eluent. Binding and internalization studies of the [131I]I-DEDEYFELV with tumorigenic cells (U-87 MG) and neoplastic tissue homogenate were evaluated at 1 and 3 h of incubation and measured in an automatic gamma counter. **Results:** The DEDEYFELV peptide was efficiently synthesized and characterized, with yield of approximately 92%. Chromatographic analyzes obtained by HPLC confirmed that the entire synthesis, cleavage, and characterization process of peptides were performed efficiently, as evidenced by the presence of only a single peak corresponding to the synthesized peptide with molecular mass of 1158.18 g/mol. The radiolabeling process was successful obtained with radiochemical yield > 95%. Binding and internalization studies of the [131I]I-DEDEYFELV conducted with U-87 MG cells showed values of 15.90% ± 1.67 and 54.57% ± 0.90 (n = 5), respectively. On the other hand, the binding percentage of 12.45% ± 0.90 and internalization of 28.41% ± 3.15 (n = 5) were achieved with neoplastic tissue homogenate within a 3-hour period. **Conclusion:** The proposed peptide was efficiently synthesized, and radiolabeling studies with [131I]NaI exhibited a high radiochemical yield. Binding and internalization studies revealed that the [131I]I-DEDEYFELV peptide has a good

affinity for both U-87 MG and neoplastic tissue homogenate, demonstrating higher internalization of [131I]I-DEDEYFELV in human glioblastoma cells. Nevertheless, it is crucial to emphasize that additional in vivo investigations are necessary.

Keywords: EGFr-targeting peptide, Glioblastoma, U-87 MG cells, [131I]I-labeled-peptide.

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SYNTHESIS AND EVALUATION OF BIOACTIVE PEPTIDES FROM LAMININ-111 IN TRIPLE-NEGATIVE BREAST CANCER CELLS

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Introduction/Justification: Breast cancer stands as the neoplasm group with the highest incidence rate among women worldwide. Laminin-111 a constituent of the tissue basement membrane, is implicated in the development of breast tumors. Biologically active peptides of laminin-111 such as YIGSR and IKVAV, play crucial roles in tumor growth, metastasis, protease secretion, and angiogenesis. These peptides exert significant influence on various aspects of cancer progression, emphasizing their potential as key targets for therapeutic intervention. **Objectives:** The aim of the study was to synthesize the bioactive peptides of laminin-111 (YIKVAV and YIGSR) and assess their interactions in triple-negative breast cancer cells. **Materials and Methods:** The YIKVAV and YIGSR peptides were synthesized through solid-phase peptide synthesis using the Fmoc/tBut strategy. Subsequently, the peptides underwent a characterization and purification process employing high performance liquid chromatography (HPLC) and mass spectrometry. The MDA-MB-231 breast tumor cell line was cultured in supplemented RPMI-1640 medium at 37°C and 5% CO2 until reaching 90% confluence. The growth curve of the MDA-MB-231 cell line was conducted in sextuplicate over a 7-day period, with cell counts performed on days 1, 3, 5, and 7. To assess the effect of peptides on cell proliferation, cells were seeded at a concentration of 2×104 in 6-well plates, with the inclusion of both YIKVAV and YIGSR peptides at 50 μM (n = 6). Cell viability in the presence of YIKVAV and YIGSR was determined using the MTT assay. For this analysis, cells were plated at a concentration of 2×104, with peptide concentration of 50 μM. Spectrophotometric analyses were realized after 24 h and 7 days of incubation at 595 nm. **Results:** The YIKVAV and YIGSR peptides were efficiently synthesized, yielding approximately 80% for both. Chromatographic analyzes conducted by HPLC and mass spectrometry confirmed the efficiency of the entire synthesis, cleavage, and characterization process of the peptides by the presence of only a single peak corresponding to the synthesized peptides. The growth curve profile determination of MDA-MB-231 cell line revealed exponential growth between days 5 and 7 of cell culture. The results indicate that the YIGSR peptide significantly inhibited cell growth by approximately 45%, whereas the YIKVAV fragment promoted cell growth by approximately 38% and ($p < 0.0001$) on the seventh day of cell culture. Regarding the MTT analysis after 24 h, no significant differences were observed between the control and treated groups for both fragments, suggesting that the peptides exhibited no toxicity at concentration of 50 μM. Additionally, on the 6th day, a reduction in tumor cell viability was observed for the YIGSR fragment, while an increase in viability was noted for YIKVAV ($p < 0.0001$). **Conclusion:** The YIKVAV and YIGSR peptides were effectively synthesized, characterized, and purified. The YIKVAV peptide, at a concentration of 50μM, promoted cell growth, while the YIGSR peptide significantly hindered the growth of the MDA-MB-231 cell line. This observation is consistent with the findings of cell viability assays conducted using MTT. This study holds significance for enhancing our comprehension of peptide actions in their interaction with triple-negative breast cancer cells, with the ultimate aim of proposing more effective targets for treatment.

Keywords: Breast cancer, Laminin-111-peptides, MDA-MB-231 cells.

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ASSESSMENT OF IN VITRO INTERACTIONS BETWEEN RADIOLABELED EGFR-TARGETING PEPTIDE INHIBITORS AND GLIOBLASTOMA CELLS

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Introduction/Justification: Peptides are implicated in various physiological responses and hold significant potential as targeting molecules, especially in cancer diagnosis or treatment. Radiolabeled peptides have been investigated for their potential as theranostic agents, holding considerable promise for precisely targeting tumorigenic cells. Previous studies indicate that biologically active peptides exhibit a high affinity for the Epidermal Growth Factor receptor (EGFr), which is overexpressed in various tumor cells, including glioblastoma, the most prevalent and aggressive malignant brain tumor. **Objectives:** To evaluate the in vitro interactions involving two radiolabeled peptide inhibitors targeting the EGFr overexpressed in glioblastoma cells. **Materials and Methods:** Two EGFr-targeting peptide inhibitors, anti-EGFr-LP and anti-EGFr-LG, were radiolabeled with [131I]NaI (11.1–14.8 MBq) using