Rica, Panamá, República Dominicana, etc. Conclusão: Radiopharmacy is a flourishing specialty of increasing complexity that requires solid theoretical knowledge and specialized practical skills. The Radiochemistry Area in the public University of Uruguay is fostering the development and generational replacement in our continent with the objective to improve the quality of the Radiopharmaceuticals received by our population. **Acknowledgments:** Centro Uruguayo de Imagenología molecular, CUDIM and Centro de Medicina Nuclear e Imagenología Molecular del Hospital de Clínicas.

Keywords: Education, Online courses, Posgraduate, Radiopharmacy.

https://doi.org/10.1016/j.htct.2024.04.060

EVALUATION OF POTENTIAL PEPTIDE INHIBITORS THAT INTERACT WITH THE EGF RECEPTOR. RELEVANCE TO GLIOBLASTOMA

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Introduction/Justification: Peptides are implicated to various physiological responses and exhibit considerable potential for disease treatment, encompassing diverse types of tumors. The significant therapeutic promise of peptides is related from their characteristics, including the ability to inhibit angiogenesis, induce tumor apoptosis and block of epidermal growth factor receptor (EGFr) signaling. Their relevance is underscored by overexpression in a range of human cancers, notably glioblastoma, which represents the most prevalent and aggressive form of malignant brain tumors. **Objectives:** This study aims to assess the growth inhibition of rat (C6 cells) and human (U-87 MG cells) glioblastoma tumor cells using peptides that interact with the EGFr. **Materials and Methods:** The anti-EGFr peptides were synthesized through the 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. C6 and U-87 MG cell lines were cultured in supplemented DMEM F-12 medium at 37°C and 5% CO2 until reaching 90% confluence. To assess the effect of peptides on cell proliferation, cells were seeded at a concentration of 5×103 in 6-well plates, with the presence of 80 μM of each proposed peptide. Growth curves were performed in sextuplicate over a 7-day period, with cell counts conducted on days 1, 3, 5, and 7. Cell viability in the presence of peptides was determined using the MTT test. For this analysis, cells were plated at a concentration of 5×103 in 96-well plates, with peptide concentrations of 80, 120, and 160 μM. Spectrophotometric analyses were performed after 24 h and 7 days of incubation at 595 nm. **Results:** Anti-EGFr-LP and anti-EGFr-LG peptides were synthesized efficiently with yields of approximately 45 and 98%, respectively. 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 peptides. Following the determination of growth curve profiles of C6 and U-87 MG cell lines, without the presence of peptides, the interaction of the peptides with both tumor cell types was assessed. The results demonstrated that both anti-EGFr-LP and anti-EGFr-LG peptides significantly interacted with and inhibited the growth of C6 and U-87 MG strains ($p < 0.0001$). Studies conducted with C6 cells showed inhibition percentages of approximately 55.3% and 99.1% for the Anti-EGFr-LP and anti-EGFr-LG peptides, respectively. On the other hand, an inhibition percentage of growth of U-87 MG cells was 44.4% for the Anti-EGFr-LP and 46.4% for the anti-EGFr-LG. Finally, based on the MTT test, the peptides exhibited no toxicity at any of the three concentrations tested. **Conclusion:** The findings indicate that both proposed peptides, at a minimum concentration (80 μM) effectively reduced the proliferation of tumor cells without inducing toxicity. While further experiments are warranted, the peptides have demonstrated the capability to inhibit tumor cell growth associated with glioblastoma, suggesting a potential therapeutic alternative.

Keywords: C6 cells, EGFr-targeting peptides, glioblastoma, U-87 MG cells.

https://doi.org/10.1016/j.htct.2024.04.061

INVESTIGATION OF THE IN VITRO ASSESSMENT OF 99MTC-LABELED LAMININ-111 PEPTIDES AS PROSPECTIVE BIOMARKERS FOR BREAST CANCER

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Introduction/Justification: Breast cancer constitutes a significant public health issue as the second most prevalent type of tumor among women. In the past decade, radiolabeled peptides have been employed in both therapeutic interventions and tumor imaging, representing a substantial promise in the specific targeting of tumorigenic cells. Several studies demonstrate that biologically active peptides derived from laminin-111 regulate gene expression in breast cancer-derived cells, including the YIGSR and IKVAV peptides. **Objectives:** To synthesize the HYIGSR and HIKVAV fragments, derived from laminin-111, standardize and optimize their radiolabeling process with technetium-99m (99mTc), as well as, to assess the in vitro biological characteristics of these radiolabeled peptides as potential biomarkers for breast cancer. **Materials and Methods:** The HYIGSR and HIKVAV peptides were

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.

https://doi.org/10.1016/j.htct.2024.04.062

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