ISTRAŽIVANJE *p-SCN-Bn-DOTA*-TRASTUZUMAB-a OBELEŽENOG RADIOAKTIVNIM I NERADIOAKTIVNIM LUTECIJUMOM I ITRIJUMOM: ZNAČAJAN KORAK ZA BUDUĆE PRIMENE

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Značaj leži u pripremi stabilnog, konjugata trastuzumaba korišćenjem neradioaktivnih $LuCl_3$ i YCl_3, preko *p*-SCN-Bn-DOTA. Ovaj pristup je ključan za određivanje potencijalnih fizičko-hemijskih promena u strukturi imunokonjugata nakon vezivanja metala. Nakon konjugacije, koristeći molarni odnos 1:20, izvedeno je sušenje zamrzavanjem da bi se dobili stabilni imunokonjugati za naknadnu analizu. Nekoliko hemijskih metoda je korišćeno da se karakteriše stabilnost antitela i zadržana imunoreaktivnost unutar formulisanih imunokonjugata. Dokaz integriteta proteina došao je iz SDS-PAGE elektroforeze, sa ujednačenim intenzitetom fragmenata (25 kDa za laki lanac, 50 kDa za teški lanac) što ukazuje na nerazgradnju antitela (1). IR i Ramanova spektroskopija su služile za verifikaciju sekundarnih strukturnih promena, uz prisustvo karakterističnih amidnih traka u oba spektra što ukazuje na zadržavanje prirodne sekundarne strukture (2). Koristeći MALDI-TOF-MS, određeno je 4,9 *p*-SCN-Bn-DOTA molekula po molekulu antitela. Obećavajući rezultati neradioaktivnog obeležavanja pružaju priliku za potencijalno obeležavanje radioaktivnim lutecijumom-177 i itrijumom-90, svaki sa specifičnom aktivnošću od 200 μCi/mL. Radioizotopi su inkubirani sa *p*-SCN-Bn-DOTA-trastuzumabom sat vremena na 40 ºC. Procena radiohemijske čistoće i stabilnosti je sprovedena korišćenjem ITLC-SG sistema. Optimalne mobilne faze, posebno 0,4 M metanol:natrijum acetat (1:1) za itrijum-90 i 0,9% NaCl za lutecijum-177, olakšale su detaljno ispitivanje. Postignuta je izuzetna efikasnost radioaktivnog obeležavanja, >96% za itrijum-90 i <99% za lutecijum-177. Procene stabilnosti posle 72 sata pokazale su veću stabilnost 177Lu-p-SCN-Bn-DOTA-trastuzumaba (<1,5% oslobađanja lutecijuma-177) u poređenju sa 90I obeleženim kolegom (<17% otpuštanje itrijuma-90). Ova studija naglašava uspešan razvoj radioimunokonjugata, pozicionirajući ovaj agens za potencijalnu primenu *in vivo* istraživanja.

Literatura

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INVESTIGATION OF *p*-SCN-Bn-DOTA-TRASTUZUMAB LABELED WITH RADIOACTIVE AND NON-RADIOACTIVE LUTETIUM AND YTTRIUM: A CRUCIAL STEP FOR FUTURE APPLICATIONS

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The significance lies in preparing stable, trastuzumab-immunoconjugate through the utilization of non-radioactive $LuCl_3$ and YCl_3, via *p*-SCN-Bn-DOTA. This approach is crucial to determine potential physicochemical alterations in immunoconjugate structure following metal binding. Post-conjugation, employing a 1:20 molar ratio, freeze-drying was performed to obtain stable immunoconjugates for subsequent analysis. Several chemical methods were employed to characterize antibody stability and retained immunoreactivity within the formulated immunoconjugates. Proof of protein integrity came from SDS-PAGE electrophoresis, with uniform fragment intensities (25 kDa for light chain, 50 kDa for heavy chain) indicating antibody non-degradation (1). IR and Raman spectroscopy verified secondary structural changes, with the presence of characteristic amide bands in both spectra indicating the retention of native secondary structure (2). Employing MALDI-TOF-MS, 4.9 *p*-SCN-Bn-DOTA molecules were determined per antibody molecule. The promising outcomes from non-radioactive labeling provide an opportunity for potential labeling with radioactive lutetium-177 and yttrium-90, each with a specific activity of 200 μCi/mL. Radioisotopes were incubated with *p*-SCN-Bn-DOTA-trastuzumab for an hour at 40 ˚C. Evaluation of radiochemical purity and stability was conducted using the ITLC-SG system. Optimal mobile phases, specifically 0.4 M methanol:sodium acetate (1:1) for yttrium-90 and 0.9% NaCl for lutetium-177, facilitated thorough examination. Remarkable radiolabeling efficiency was achieved, >96% for yttrium-90 and >99% for lutetium-177. Stability assessments after 72 hours demonstrated greater stability in ^{177}Lu-p-SCN-Bn-DOTA-trastuzumab (<1.5% lutetium-177 release) compared to the ^{90}Y-labeled counterpart (<17% yttrium-90 release). This study demonstrates the successful development of radioimmunoconjugates, positioning this agent for potential application *in vivo* investigations.

References

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