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Preparation of Single Patient Dose of Lu-177-DOTA-Rituximab –Using Low Specific Activity Lu-177-Chloride

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Background:

Limited access to high specific activity Lu-177 in India and its prohibited cost provide the necessary impetus for the present work on development of Lu-177-DOTA-Rituximab using low specific activity Lu-177 Based on the advantageous/favorable nuclear properties of Lu-177 over the I-131, the development of Lu-177-DOTA-Rituximab was obviously pertinent. Towards this we attempted to optimize the radiolabeling of Lu-177-DOTA-Rituximab using low specific activity(<15 mCi/μg) and carrier added Lu-177-Chloride. The physicochemical, biological quality control parameters, *in-vitro* stability, immunoreactivity and cell binding studies carried out in Daudi cell-lines. *In-vivo* biodistribution studies were carried out in suitable animal model.

Methodology:

Lu-177-Chloride produced at our research reactor. Rituximab(10mg/mL) pre-concentrated from 500μL to 100μL using 30kDa MW cut-off filtration device at 5000rpm for 22minutes. Coupling of rituximab(5mg/100μL, 34.75nM) with p-NCS-benzyl-DOTA(240μg/24μL, 347.56nM) carried out at 1:10 molar ratio incubating at 37degC for 22hr. The conjugated reaction mixture purified using pre-conditioned PD-10. The DOTA-benzyl-Rituximab eluted from PD-10 using 0.2M sodium-acetate buffer(pH˜5.5) and its concentration was estimated by Bradford's assay at 570nm. Prior to radiolabeling, pH of Lu-177-Chloride(285-300mCi in 250-275μL) adjusted to 6.5-7.0 using 0.2M sodium-acetate solution. Lu-177-Acetate incubated with 124μL of DOTA-benzyl-Rituximab at 37degC for 80minutes. After incubation the radiolabeled reaction mixture purified using PD10(pre-conditioned with 0.2M sodium-acetate solution). In-vitro stability of the Lu-177-DOTA-Rituximab was ascertained by adding ascorbic acid(40mg/0.5mL of 0.2M sodium-acetate solution). The RCP was evaluated using TLC-SG{(0.1M sodium-citrate buffer(pH-5.0)} and HPLC(size-exclusion column, 0.05M phosphate-buffer,pH˜6.8). Gel-clot BET-assay and sterility test were performed.
Human Leukemia cell-line Daudi expressing CD20, used for *in-vitro* evaluation, grown in IMDM with 10%FBS at 37degC. *In-vitro* cell-binding studies performed by incubating Daudi cells in 1mL of internalization buffer(IMDM, 0.2%BSA) containing radioligand(˜5pmol peptide) for 15, 30, 60 & 120minutes and washed with PBS. Non-specific internalization assessed by addition of cold rituximab(5nmol). For membrane receptor binding assay, cells homogenates incubated at above time points. Biodistribution studies carried out in Daudi cell-lines xenograft tumor bearing nude mice at 6h, 24h, 48h & 72h intervals and quantified by γ-spectrometer.

Results and Discussions:

Using Lu-177 of low specific activity (< 15 mCi/μg), 60-65mCi of Lu-177-DOTA-Rituximab(single patient-dose) was prepared using ˜300mCi of Lu-177-Chloride. Lu-177-DOTA-Rituximab was found to be clear, colorless, pH between 5.5-6.0 and RAC between 8-10 mCi/mL. The RCP of Lu-177-DOTA-Rituximab estimated by TLC was >98% with retention-factor 0.00-0.10. RCP derived by HPLC was >95% with retention-time of labeled-product between 14.5–15.5minutes. EL <6EU/mL, radiopharmaceutical was sterile. *In-vitro* and serum stability of the product indicated stability upto 96hr upon storage at -20degC with stabilizer.
Lu-177-DOTA-Rituximab showed rapid binding in Daudi cells(25%), reaching a plateau after 30-60minutes. In biodistribution study, radioactivity decreased from most organs after 24h post-injection. High uptake and long-term retention of radioactivity found in tumor model which corroborates with scintigraphy studies.

Conclusion:

Single patient dose of Lu-177-DOTA-Rituximab could be produced in optimum yield using 12-15 mCi/μg Lu-177. The product compares well with the preparation documented using nca Lu-177. Further studies towards clinical translation of this promising radiopharmaceutical in patient are underway.

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