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LABELING OF BIOTIN WITH ^{166}Dy/^{166}Ho AS A STABLE IN VIVO GENERATOR SYSTEM

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Biotin (*cis*-tetrahydro-2-oxothieno[3,4-d]imidazoline-4-valeric acid) is a 244 Da vitamin found in low concentration in blood and tissue (vitamin H). In radioimmunodiagnosis and radioimmunotherapy practice, the pretargeting avidin-biotin strategy has shown that target-to-nontarget radioactivity ratios can be significantly improved. In addition, the biotin content of cancerous tumours is higher than that of normal tissue and it has been found in the cellular nucleus due to a specific transfer of biotin to histones by human serum biotinidase. Because of its nuclear properties, the ^{166}Dy/^{166}Ho radionuclide pair is considered an *in vivo* generator system. The aim of this work was to synthesize ^{166}Dy/^{166}Ho-DTPA-*bis*Biotin to evaluate its potential as a new radiopharmaceutical for targeted radiotherapy. Dysprosium-166/ holmium-166 chloride was obtained by neutron irradiation of 20 mg of enriched Dy_2O_3 (^{164}Dy, 99 %, from Oak Ridge NL.) in a TRIGA Mark III reactor at a flux in the central thimble of $3 \cdot 10^{13}$ n· cm^{-2}· s^{-1} for 20 h. Following irradiation, the target was allowed to decay for 2 days, then 100 µL of 12 N chloride acid were added and stirred for 1 min. To this solution 500 µL of injectable water were added and also stirred for 2 min. The average radioactive concentration was 332 MBq/mL. The biotin used in this investigation was covalently conjugated to diethylenetriamine pentaacetic acid (DTPA) through the use of the cyclic anhydride and lysine conjugate to biotin (biocytin) to produce DTPA-α,ω-bis(biocytinamide)(DTPA-*bis*Biotin). Sterile and apyrogenic V-vial was prepared to contain 2.0 mg (1.9 X 10^{-3} mmol) of the DTPA-*bis*Biotin compound in 1.0 mL of 0.05 M bicarbonate buffer (pH 8.0) and then 20 µL of $^{166}Dy_2Cl_3$ solution were added to the preparation. Thin Layer Chromatography aluminum cellulose sheets were utilised as the stationary phase and a ternary mixture of methanol:water:ammonium hydroxide (20 :40 :2) as the mobile phase. ^{166}Dy/^{166}Ho-DTPA-*bis*Biotin travelled with the solvent front R_f 0.9-1.0 and the Dy^{+3} / Ho^{+3} species remained at the origin (R_f = 0). The biological integrity of labelled biotin was achieved evaluating its avidity for avidin in an agarose column. Stability studies against dilution were carried out by diluting the radiocomplex solution with saline and with human serum at 310 K. After 10 min and 24 h the radiochemical purity of each ^{166}Dy/^{166}Ho complex solution was determined by TLC. The complex ^{166}Dy/^{166}Ho-DTPA-*bis*Biotin was obtained with 99% radiochemical purity. *In vitro* studies demonstrated that the complex is stable after dilution in saline and in human serum. Avidity of labelled biotin for avidin was not affected by the labelling procedure. This radiocomplex could work as a stable *in vivo* generator system for targeted radiotherapy.