PRODUCTION, ISOLATION AND RADIOLABELING METHODS FOR ^{211}AT-LABELING OF BIOMOLECULES.

D. S. Wilbur, D. K. Hamlin, M. Chyan

Department of Radiation Oncology, University of Washington, Seattle, WA 98195
Email: dswilbur@uw.edu

Targeted alpha therapy with ^{211}At-labeled compounds holds great promise for treatment of cancer, particularly compartmentalized cancer (e.g. ovarian cancer), minimal residual cancer after surgery and metastatic disease. Unfortunately, ^{211}At has limited availability and, due to its unique nature, has the potential to be readily dissociated from the cancer-targeting agents used in vivo. Finding methods to circumvent these two problems has occupied a large amount of our efforts over the past few years. ^{211}At is produced at the University of Washington on a Scanditronix MC-50 using a 28 MeV alpha beam. Our initial preclinical studies were conducted using a small target assembly with irradiations of a 10 A alpha beam, but our desire to ultimately conduct clinical studies led to the design and installation of a new target assembly that had much larger irradiation surface and would withstand beam energies of 50 A or more. Prior to this upgrade, ^{211}At was efficiently isolated (60-80%) from the irradiated aluminum-backed bismuth targets by dry distillation at 650°C. However, the dry distillation method gave low recovery yields (e.g. 10-40%) when the much larger new targets were used. After some attempts to improve the distillation yields, we have more recently conducted a wet chemistry approach to the ^{211}At isolation. While this method still needs to be optimized, it has provided good recovery (60-90%) of the ^{211}At. Our radiolabeling methods have undergone a similar transition in the past few years. Until recently our ^{211}At studies were limited to the use of intact monoclonal antibodies (MAb) labeled using conjugates containing aryl-astatine derivatives due to the deastatination of more rapidly metabolized targeting biomolecules. This limitation made it all but impossible to label important biomolecules such as MAb fragments, engineered proteins, peptides and small molecules. This critical shortcoming of labeling methods for ^{211}At led to our investigating boron cage molecules as pendant groups for labeling more rapidly metabolized biomolecules. After extensive study, we found that closo-decaborate(2-) moieties were uniquely suited for ^{211}At labeling, as they underwent very fast (within 30 seconds) and efficient (>70% yields) labeling, were non-toxic, and had minimal affect on the in vivo behavior of the biomolecule that it was attached to. Studies of closo-decaborate(2-) containing different functional groups have been conducted to determine the best candidates for pendant groups to be conjugated with biomolecules. A number of conjugation molecules containing the closo-decaborate(2-) moiety have been prepared and tested. In those studies, intact MAb, Fab´ fragments, a peptide (bombesin), biotin derivatives and a small molecule (PSMA) inhibitor have been labeled with both ^{125}I and ^{211}At to determine in vivo stability and equivalence of biodistributions. In every example, the ^{211}At label was found to be very stable to in vivo deastatination. Likewise, the ^{211}At and ^{125}I distributions were essentially equivalent in all but a couple of examples. Although the ^{125}I and ^{211}At had equivalent distributions, it was found that the closo-decaborate(2-) moiety appears to be retained in some tissues (e.g. kidney and liver) longer than MAb that did not contain that moiety. To circumvent this problem, closo-decaborate(2-) derivatives were prepared that contained a hydrazone functionality between the decaborate(2-) and the biomolecule. In subsequent biodistribution studies, it was shown

that the hydrazone linkage was efficiently cleaved in vivo, releasing the radioactivity from kidney. In the examples where the biodistributions of ^{125}I and ^{211}At were not equivalent, a hydrazone and phenacyl-closo-decaborate(2-) derivative were involved. Studies are planned to determine why the biodistribution in these examples was not equivalent. In the presentation, examples showing equivalency in biodistributions will be presented, as will some promising results from an ^{211}At-labeled intact MAb for therapy of a mouse tibia metastatic prostate cancer model. In the latter studies, dramatic differences in serum PSA were observed between mice treated with ^{211}At-labeled MAb and those that were not.