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Imaging, biodistribution, and toxicology evaluation of ^{212}Pb-TCMC-trastuzumab in nonhuman primates

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ABSTRACT

Introduction: The biodistribution and toxicology of a radiotherapeutic ^{212}Pb-trastuzumab conjugate were evaluated in nonhuman primates to meet the investigational new drug requirements prior to a phase I clinical trial in human subjects.

Methods: Male cynomolgus monkeys (n = 3/group) were injected intraperitoneally with the ^{212}Pb-trastuzumab conjugate and terminated at 8 h, 10 d, and 90 d post-injection. Quantitative imaging studies in phantoms and monkeys were conducted using a planar gamma camera and a high purity germanium (HPGe) detector out to 48 h following injection. Biodistribution analyses were conducted at 8 h; all tissues and time points were evaluated for macroscopic and microscopic pathology. Blood samples were taken throughout the 90 d study period for assessment of hematology parameters and serum chemistry parameters.

Results: Quantitative gamma camera imaging and region-of-interest analyses of phantoms and monkeys indicated that 95.5 ± 5.0% of the decay-corrected ^{212}Pb activity was retained in the peritoneal region up to 48 h following administration of the ^{212}Pb-trastuzumab. Gamma-ray spectroscopy analyses confirmed that 87.6 ± 4.5% of the decay-corrected ^{212}Bi activity was also retained in the peritoneal cavity during this time. Serum chemistry parameters for all groups always fell within normal ranges. Gross and histopathology evaluations showed no radiation-related toxicity in any tissue at any time.

Conclusion: In vivo imaging and biodistribution analyses showed that about 90% of both ^{212}Pb and decay product ^{212}Bi remained in the monkey peritoneal cavity. The imaging methods could also be applied to human subjects. The lack of toxicity observed in monkeys following intraperitoneal injection of the ^{212}Pb-trastuzumab conjugate supports its clinical assessment in humans.

1. Introduction

A growing number of studies have employed the beta-emitter ^{212}Pb ($t_{1/2}$ = 10.64 h), which decays to the alpha-emitter ^{212}Bi ($t_{1/2}$ = 60.5 min), complexed with antibodies in targeted radiotherapeutic approaches for cancer applications [1–8]. Most preclinical studies have employed trastuzumab as the immunotargeting molecule for treating models of disease that express the human epidermal growth factor type 2 (HER2). As HER2$^+$ malignancies are associated with poor patient prognosis, additional research is needed to develop therapeutic regimens for managing these diseases. ^{212}Pb-TCMC-trastuzumab (TCMC, *S*-2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraaza-1,4,7,10-tetra(2-carbamoyl-methyl)cyclododecane) [9] radioimmunoconjugates have shown efficacy in establishing therapeutic responses in HER2$^+$ tumor xenograft models in rodents [2,4,10–12]. Trastuzumab-mediated binding to HER2$^+$ cells subjects these cells to high dose rates of the short-range (<100 μm), high linear energy transfer alpha-particles (>6 MeV) emitted by the short-lived daughter radionuclides (^{212}Bi, ^{212}Po) from decay of ^{212}Pb. Non-specific toxicity to HER2$^-$ tissues is minimized since the alpha-particles emitted during decay do not penetrate more than about 60 μm. Subsequent to the successful preclinical data, ^{212}Pb-TCMC-trastuzumab has been evaluated in a phase I clinical trial in patients presenting with HER2$^+$ malignancies (ovarian and colon cancers) in the peritoneal cavity [13,14].

This report presents the imaging, biodistribution and toxicology analyses of ^{212}Pb-TCMC-trastuzumab following intraperitoneal (i.p.) administration in normal nonhuman primates (cynomolgus monkeys). The experiments reported here, which were performed prior to commencement of the first phase I human trial with ^{212}Pb-TCMC-trastuzumab mentioned above, were required to fulfill the investigational new drug requirements for the subsequent studies in human patients. In addition to standard analyses of toxicology and biodistribution of radioactivity in excised tissues, *in vivo* localization

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and retention of ^{212}Pb and ^{212}Bi were examined using planar gamma camera imaging and regional imaging of selected organs using a high purity germanium (HPGe) detector.

2. Materials and methods

2.1. Test article ^{212}Pb-TCMC-trastuzumab

The source of ^{212}Pb, TCMC-trastuzumab (14 chelates/antibody), radiolabeling procedures, and product validations [instant thin layer chromatography (ITLC), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE), endotoxin testing, sterility and Scatchard analyses] have been published elsewhere [13–16]. The specific activity of ^{212}Pb-TCMC-trastuzumab used in these studies was 5760 MBq/μmol (153 mCi/μmol) immunoconjugate. Each monkey was administered 3.7 MBq/kg (0.1 mCi/kg) [approximately 44 MBq/m^2 (1.2 mCi/m^2)] ^{212}Pb-TCMC-trastuzumab *via* i.p. injection to be consistent with the route of administration planned for the human studies (patients with peritoneal disease). This dose was 60% greater than the maximum activity planned for the subsequent dose-escalation clinical trial in humans [13] and was utilized for safety considerations prior to performing the human studies. All radioactive measurements and analyses were performed with instruments cross-calibrated to a National Institute of Standards and Technology certified ^{228}Th standard (Eckert and Ziegler, source #1388-64), which included all of the radionuclides of interest in its decay chain. Unless stated otherwise, radioactivity analyses were decay-corrected to the time of administration into the animals (initial time point).

2.2. Animal subjects and husbandry

All animal experiments were reviewed and approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee before they were started and were performed in compliance with national regulatory agency guidelines. Male cynomolgus monkeys (*Macaca fascicularis*) were obtained from Harlan Laboratories (Blue Mounds, WI) and were quarantined and acclimated before initiation of the study. The monkeys were approximately 3.5 y of age and weighed 2.6 to 3.2 kg at initiation of the studies. Animals were cared for and evaluated throughout the study period by trained Animal Resource Program (ARP) personnel and the attending Laboratory Animal Veterinarian. Additional details for animal husbandry are given in the supplementary information. Animals were sedated with 0.1 mL/kg ketamine/acepromazine cocktail (10:1) for all sedation and anesthesia procedures. As was scheduled for the phase I human clinical protocol, an intravenous pre-dose injection of 4 mg/kg of unlabeled trastuzumab was performed on the date of the start of the study (up to 24 h prior to administration of ^{212}Pb-TCMC-trastuzumab). An i.p. catheter was placed on the left side of the mid lower abdomen and flushed with 10 mL of saline to ensure proper placement. The injection site was marked for collection at termination.

The monkeys were divided into three groups (n = 3 per group) and were euthanized at 8 h, 10 d, and 90 d post-injection of ^{212}Pb-TCMC-trastuzumab. The 8 h group was used for biodistribution analysis, and all groups were used to determine the acute (8 h and 10 d) and chronic (90 d) toxicology of the test article. At the designated time points, animals were euthanized with a 1–1.5 mL/kg ketamine/acepromazine cocktail and exsanguinated by severing the posterior humeral circumflex artery under each armpit. After cessation of bleeding, selected tissues were dissected, weighed, sectioned, and processed for biodistribution analysis or histopathology evaluation as described below.

2.3. Blood sample and tissue radioactivity analyses

Blood samples for radioactivity measurements were obtained from monkeys in all groups prior to injection and at 0, 4, and 8 h after administering ^{212}Pb-TCMC-trastuzumab; samples from the 10 d and 90 d groups were also taken 24 and 48 h after administering ^{212}Pb-TCMC-trastuzumab. Up to 3 mL of blood from each animal was collected in Vacutainer tubes with EDTA as an anticoagulant prior to counting on an auto-gamma counter (Hewlett Packard Minaxi 5000).

Organ and tissue biodistribution analyses were performed using tissues from the 8 h group. Following euthanization, sections of the excised tissues were placed in pre-weighed 30 mL plastic liquid scintillation vials and assayed for ^{212}Pb and ^{212}Bi using an HPGe detector (ISOCS model, Canberra) and the auto-gamma counter. The remaining tissues from this group were processed for histopathology evaluation as described below. The radiation absorbed doses to specified organs and tissues were determined from analysis of the administered activity, measured activities in tissues from the 8 h biodistribution data, and organ weights, using standard methods recommended by the Medical Internal Radiation Dose (MIRD) Committee of the Society of Nuclear Medicine and Medical Imaging. Further details of the dosimetry approach are given in the supplementary information.

2.4. Imaging analyses

Planar imaging studies were conducted with a gamma camera equipped with a parallel-hole, high-energy collimator (Picker Axis single photon emission computed tomography [SPECT] camera, Philips). Gamma camera settings employed a ±20% window centered at 238.6 keV to detect the dominant photon emitted by ^{212}Pb (43.6%). 500,000 net counts were collected for each imaging session (two to thirty min depending on the time point), and data were analyzed using ImageJ (version 1.43). A phantom imaging study was conducted using a plastic intravenous saline bag, which corresponded to the average dimensions of the peritoneal cavity (220 × 100 mm) in monkeys. The phantom was filled with 400 mL 0.9% saline to correlate to the summed mass of the peritoneal cavity organs (385 g).

At designated time points, the anesthetized animals were placed in a plastic 56.5 × 31.5-cm basin that was 2.3 mm thick. The basin enabled the subject to be positioned so that its entire body fit within the field of view (FOV) of the SPECT camera head (Fig. 1A). The subject geometry enabled reproducible positioning of the subject for imaging close to the camera head for optimal efficiency of photon capture and image quality. The monkeys in all groups were imaged at 0, 4, and 8 h after administering ^{212}Pb-TCMC-trastuzumab; animals in the 10 d and 90 d groups were also imaged 24 and 48 h after administering ^{212}Pb-TCMC-trastuzumab.

Regional anatomical imaging studies were conducted with the HPGe detector equipped with a lead cone collimator (conical aperture dimensions: 37.0 mm to 13.7 mm over a vertical height of 54.5 mm); the shielded HPGe detector is shown in Fig. 1B. The monkeys were kept in the same positional geometry in the basin employed for the gamma camera imaging. The basin was positioned above the orifice of the detector shielding so that a representative section of the monkey's anatomy could be evaluated for ^{212}Pb and ^{212}Bi content. The following five anatomical regions were selected: injection site, heart, liver, kidney and femur. The kidneys and femurs were stacked in the FOV of the HPGe detector, as represented in Fig. 1C for the regional imaging of the femurs.

2.5. Hematology and clinical chemistry sample preparation

Blood samples for hematology were collected in Vacutainer tubes with EDTA as an anticoagulant. Samples for blood chemistry were collected in Vacutainer tubes without anticoagulant and allowed to clot at room temperature for at least 45 min. The resultant serum was separated, transferred to clear polypropylene tubes, frozen on dry ice, and stored at −80 °C until analyzed. Samples were taken prior to initiation of study (for all three groups), 4 to 6 d after injection (for the 10 d and 90 d groups only), and at time of sacrifice (for the 10 d and 90 d groups only).