Research Article

In Vivo 3T Magnetic Resonance Imaging Using a Biologically Specific Contrast Agent for Prostate Cancer: A Nude Mouse Model

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We characterized in vivo a functional superparamagnetic iron-oxide magnetic resonance contrast agent that shortens the T_2 relaxation time in magnetic resonance imaging (MRI) of prostate cancer xenografts. The agent was developed by conjugating Molday ION™ carboxyl-6 (MIC6), with a deimmunized mouse monoclonal antibody (muJ591) targeting prostate-specific membrane antigen (PSMA). This functional contrast agent could be used as a noninvasive method to detect prostate cancer cells that are PSMA positive and more readily differentiate them from surrounding tissues for treatment. The functional contrast agent was injected intravenously into mice and its effect was compared to both MIC6 (without conjugated antibody) and phosphate-buffered saline (PBS) injection controls. MR imaging was performed on a clinical 3T MRI scanner using a multiecho spin echo (MESE) sequence to obtain T_2 relaxation time values. Inductively coupled plasma atomic emission spectroscopy was used to confirm an increase in elemental iron in injected mice tumours relative to controls. Histological examination of H&E stained tissues showed normal morphology of the tissues collected.

1. Introduction

Prostate cancer often remains asymptomatic during its early stages and diagnostic accuracy relies heavily on the detection of elevated serum levels of prostate-specific antigen (PSA). The low specificity of PSA can result in over- or undertreatment of prostate cancer patients [1]. Overtreatment of less aggressive cancers can result in decreased quality of life [2]. Conversely, undertreatment of patients with aggressive cancers can lead to unnecessary fatalities [1].

Recent studies have shown that the use of prostate-specific membrane antigen (PSMA) can help in the treatment of localized and aggressive cases of prostate cancer [3–5]. PSMA is a well characterized prostate cancer biomarker localized in the prostate cancer cell membrane which, when

overexpressed, makes it a strong, robust, and highly specific candidate for strategies that target prostate cancer [6–8]. PSMA receptors are localized to the apical plasma membrane of prostate cancer epithelium and are believed to promote antiapoptotic signaling resulting in cell resilience and cell proliferation [9, 10]. The J591 antibody has proven to be an effective detection and therapeutic tool for targeting PSMA-positive prostate cancer cells [11]. An anti-PSMA antibody has been deimmunized by replacing murine immunoglobulin sequences with human immunoglobulin sequences, resulting in a nonimmunogenic, humanized antibody, huJ591, for which phase I clinical trials have proven to be effective with no adverse host immune response [3, 12]. PSMA has also been used as a marker to detect the occurrence and reoccurrence of prostate cancer using positron emission

tomography (PET), X-ray computed tomography (CT), magnetic resonance imaging (MRI), and optical imaging [13–16].

MRI offers some advantages relative to other techniques used for prostate cancer imaging. No radioactive agents are needed to create images. MRI is frequently used to guide treatments such as high intensity focussed ultrasound (HIFU), which offers a noninvasive approach to treat prostate cancer [17]. Improving the visibility of tumours for diagnosis and treatment would therefore be advantageous. The current gold standard used clinically to localize prostate tumours by MRI uses a combination of dynamic contrast-enhanced (DCE) and diffusion weighted imaging (DWI) to improve diagnosis and targeting [17, 18]. Another approach to improve visibility and diagnosis in MRI would be to use biologically specific contrast agents.

Contrast agents in MRI change the signal intensity by altering the T_1 and T_2 relaxation times of tissues in the affected area [19, 20]. By injecting contrast agents, the ability to differentiate tumours from surrounding tissue is increased, improving contrast and guidance of treatments. Superparamagnetic iron-oxide (SPIO) nanoparticles such as Molday ION carboxyl-6 (MIC6) can reduce the T_2 relaxation time of tissues and affect contrast. Untargeted, contrast agents have limited specificity; however, noninvasive specific detection of cells that express PSMA can be done by linking an anti-PSMA antibody, such as J591 [11], to SPIO nanoparticles such as MIC6.

In a previous study, we developed a functional antibody-labeled MRI contrast agent complex that bound to live cells and was detected in a clinical 3T MRI [21]. Abdolahi et al. [22] proposed and tested a similar J591:SPIO conjugate that targeted PSMA-positive prostate cancer cells in vitro. A comparable conjugate was tested by Tse et al. [13] who showed that use of the conjugate led to a reduction in MRI signal intensity in an in vivo animal model using a 16.4T scanner. Taylor and Sillerud [23] reported the use of paclitaxel-loaded PSMA-targeted iron platinum micelles as an MRI contrast agent, showing that this agent caused tumour volume to significantly decrease compared to paclitaxel alone. In our previous work we showed that a muJ591:SPIO conjugate worked as an MRI contrast agent while reducing cell proliferation and causing apoptosis [21].

This study expands on our previous work [21] by treating prostate-tumour-induced mice with an antibody-labeled contrast agent. In this previous work, Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) spectroscopy were used to characterize the muJ591:MIC6 conjugate and to confirm the presence of organic matter (antibody) and iron nanoparticles. The specificity and uptake of muJ591:MIC6 compared with the nonconjugated muJ591 and a nonspecific muIgG:MIC6 conjugate were also confirmed on live prostate cancer cell lines using flow cytometry [21]. In addition, we confirmed on a 3T MRI that only PSMA-positive cells treated with muJ591:MIC6 presented more rapid T_2 relaxation times relative to PSMA-negative cells and cells treated with a nonspecific muIgG:MIC6 conjugate, with the T_2 relaxation time decreasing as a function of iron concentration, for a relaxivity r_2 of 171 Hz/mM in water [21].

This paper demonstrates the potential of a functional antibody-labeled MRI contrast agent to detect PSMA-positive prostate cancer by monitoring T_2 relaxation time and signal intensity reduction in spin echo images. We have tested muJ591:MIC6 in a clinical 3T MRI scanner to demonstrate feasibility in clinically relevant conditions.

2. Materials and Methods

2.1. Conjugate Preparation. The conjugation method was based on our previous work [21]. Briefly, murine J591 monoclonal antibody (muJ591), at a concentration of 5 mg/mL (Laboratory of Urological Oncology, Weill Cornell Medical College, New York, NY, USA), was conjugated to MIC6 (Cat #: CL-30Q02-7, BioPAL, Inc., Worcester, MA, USA) to prepare the antibody-labeled MRI contrast agent, muJ591:MIC6. MIC6 nanoparticles have a particle size distribution of 35 ± 15 nm as reported by the manufacturer. All the reagents required for the conjugation reactions and analysis, including N-(3-dimethylaminopropyl)-N9-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 2-(4-Morpholino) ethanesulfonic acid hydrate (MES), sodium bicarbonate, HPLC-grade water (ChromaSolv Water), chloroform, acetonitrile, and deuterium oxide, were purchased from Sigma Aldrich, Oakville, ON, Canada, unless otherwise stated. The total protein concentration of the conjugates was determined by a Bradford protein assay using a gradient concentration of BSA and human c-globulin as standards for the determination of antibody content. Flow cytometry confirmed binding of the conjugated muJ591:MIC6 complex to PSMA-positive (LNCaP) cells. The iron concentration of the final product was obtained by using ICP-AES and following the methodology described in Bates et al. [21].

2.2. Conjugate Characterization. Dynamic light scattering was used to determine the particle size and zeta potential of the muJ591:MIC6 conjugate. The muJ591:MIC6 conjugate was dispersed in sodium bicarbonate or saline (Hospira, CAT#0037796, 0.9% sodium chloride) at a protein concentration of 0.01% w/v. Measurements were performed using a Zetasizer Nano ZS instrument (Malvern Instruments, Worcestershire, UK). The particle size results were reported as the harmonic intensity averaged particle diameter (or Z-average) expressed in nanometers and zeta potential reported in mV. Four replicates of each sample were recorded and used to calculate mean and standard deviation.

2.3. Tumour Induction. Prostate tumours were induced in 22 animals by injection of 5×10^6 LNCaP cells (CRL-1740, American Type Cell Culture, Manassas, VA, USA) suspended in a 100 μ L mix of 50 : 50 PBS and Matrigel (Corning, Corning, NY, USA). Cells were subcutaneously injected into the flank of 20 male athymic nude mice (Charles River, Wilmington, MA, USA) and MRI was performed when tumours reached a diameter of 5 mm according to our approved protocol (Lakehead University Animal Care Committee).

2.4. In Vivo MRI. For imaging, animals were anesthetized with 2% isoflurane (Baxter International Inc., Deerfield, USA) and placed in an 8-channel wrist RF coil with a water

TABLE 1: Pulse sequences.

Sequence	Description	Echo time (TE)	Relaxation time (TR)	Field of view (FOV)	Slice thickness	Acquisition matrix	Averages	Turbo spin factor (TSF)	Number of echoes
A	Spin echo (SE)	15 ms	318 ms	70 × 70 mm	3 mm	480 × 480	2	—	—
B	Turbo spin echo (TSE)	70 ms	1100 ms	70 × 70 mm	3 mm	448 × 448	4	15	—
C	Multiecho spin echo (MESE)	Δ8 ms	1396 ms	70 × 70 mm	3 mm	156 × 156	4	—	30
D	Gradient echo (GE)	2.5 ms	5.27 ms	120 × 120 mm	2 mm	240 × 240	2	186	—

heating pad (Multi-T Pad and T/Pump, Gaymar Industries Inc., New York, USA) maintained at 36.5°C that surrounded the animal to maintain body temperature. MR imaging was performed using a 3T MRI scanner (Achieva, Philips, The Netherlands) using the sequences described in Table 1. Spin echo T_1 -weighted images (Seq. A), followed by T_2 -weighted turbo spin echo images (Seq. B), were used to localize tumours. The T_2 relaxation time was obtained using MESE sequences (Seq. C), plotting the echo time (TE) as a function of signal intensity and fitting the signal to

$$M_{xy}(\text{TE}) = A + Be^{-\text{TE}/T_2}, \quad (1)$$

where M_{xy} is the signal intensity at the echo time TE and A, B, and T_2 are the fitting parameters [24]. The images were transferred to an external workstation and processed with custom MATLAB code (MATLAB R2014b, The MathWorks, Inc., Natick, Massachusetts, USA). A region of interest (ROI) was selected by the user for each tumour for T_2 relaxation time calculation. Signal intensity and T_2 relaxation time were obtained within the defined ROI. T_2 relaxation time maps were created by fitting (1) for each pixel within the ROI.

Eleven mice were injected intravenously with muJ591:MIC6, and eight with MIC6 alone. The dose administered to the mice was 0.6 mg of iron per kg and was calculated using ICP data as described in Bates et al. [21]. Three mice were injected with phosphate-buffered saline (PBS) as a control. The iron concentration was chosen to match the recommended clinical dose of 0.56 mg of iron per kg used clinically for the SPIO contrast agent Feridex® (Feridex IV, Berlex Laboratories). T_2 relaxation time, signal intensity, and tumour volume were obtained before injection, immediately after, and 10 min, 20 min, 30 min, 40 min, 1 day, 2 days, and 3 days after injection. Mice were then euthanized and tissues were harvested.

2.5. Histology. The tumours, spleens, kidneys, livers, and brains were harvested immediately after euthanasia, which occurred three days after injection, immediately following MR imaging. After harvesting, the tissues were fixed in 10% formalin and then paraffin-embedded (Thunder Bay Regional Health Sciences Central Laboratory) for hematoxylin and eosin stain (H&E) using 4 μm sections after deparaffinization by three 10-minute xylene rinses, followed

by three 10-minute rinses with 100% ethanol, and then rehydration with 80/70% alcohol and finally with distilled water. Slides were then incubated with Mayer's Hematoxylin solution from Sigma Aldrich for 10 minutes and then rinsed with tap water for 1 minute, followed by two 5-minute washes with distilled water as well as a differentiator (0.05% acid alcohol, 2 mL hydrochloric acid concentrate diluted in 2800 mL of 95% ethanol and 1200 mL of distilled water, and TRIS buffer as a bluing agent). Slides were counterstained with Mayer's Eosin solution for 30 seconds. Samples were then dehydrated with one 3-minute 95% ethanol rinse, followed by three 3-minute 100% ethanol rinses and three 3-minute xylene rinses. The slides were imaged using a bright field microscope (Axioskop, Zeiss, Oberkochen, Germany) and then captured using an RGB camera (Retiga 1300, Qimaging, Surrey, BC, Canada).

2.6. Elemental Iron Content Analysis. Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to determine the elemental iron content of tissue samples. Tumour, liver, kidney, spleen, and brain tissue samples were stored in cryo-vials in a liquid nitrogen tank and were digested with nitric acid overnight in a 70°C water bath. Sample weight was determined by weighing the empty vials and subtracting the value from the total weight of the vial containing the sample. Once digested, samples were transferred using graduated pipettes into falcon tubes. Final volumes were recorded to calculate the concentrations. Chromatography-grade water was added to the tubes to obtain a final volume of 11 mL, and 1 mL of this sample was added to a second falcon tube containing 9 mL of chromatography-grade water to make up a diluted sample. Diluted and undiluted samples were all run on a Varian Vista Pro Radial ICP-AES instrument (Varian Vista Pro CCD Simultaneous ICP-OES, Varian Inc., Palo Alto, CA, USA) using TMDA-64.3 (National Water Research Institute, Environment Canada, Canada) as a quality control standard for iron.

2.7. Statistical Analysis. SPSS software (2013 release, IBM SPSS Statistics for Windows, Version 22.0, Armonk, NY, IBM Corp.) was used to perform student's *t*-tests and analysis of variance (ANOVA) on select data sets. A *p* value of 0.05 was used when considering statistical significance.

FIGURE 1: In vitro characterization. Size (nm), zeta potential (mV), and pH for the muJ591:MIC6 conjugate suspended in saline or sodium bicarbonate ($n = 4$).

3. Results

3.1. In Vitro Characterization. Zeta potential was used to address potential stability issues while formulating the conjugates in different buffers. Figure 1 shows the zeta potential, the pH, and the size of the muJ591:MIC6 antibody-labeled complexes in the different solutions tested. The size of the conjugate closely matched the size of the MIC6 nanoparticles when suspended in either sodium bicarbonate or saline (32.38 ± 5.06 nm and 39.14 ± 6.03 nm, resp.), as previously reported in Bates et al. (34.75 ± 14 nm and 36.64 ± 10.83 nm, resp.) [21]. When using sodium bicarbonate as a solution for the conjugate, the zeta potential was significantly higher, 11.00 ± 2.56 mV compared to 1.44 ± 2.88 mV for saline. The pH of the muJ591:MIC6 conjugate solution was $5.06 \pm .01$ and $6.03 \pm .01$ for saline and sodium bicarbonate, respectively. Complexes suspended in saline were likely unstable and when injected into animals caused mortality. This resulted in the decision to use sodium bicarbonate as a buffer for subsequent animal injections of muJ591:MIC6 conjugates and the use of PBS for control injections.

3.2. Tumour Localization. Imaging in the clinical MRI required the development of a method to locate tumours systematically. To the best of our knowledge, there is no standard protocol to localize small tumours in animals in a clinical 3T MR scanner. Using typical localizer gradient echo images (Seq. D), it was not possible to pinpoint tumours in the images due to the small size of the tumours. Other groups have reported successful use of low resolution gradient echo images to localize tumours in high field 16.4T animal scanners [13]. We first used turbo spin echo images at higher resolution in our 3T scanner (Seq. B). In these T_2 -weighted images it was possible to locate subcutaneous hyperintense (bright) regions that were presumed tumours. However, it was common to find more than one subcutaneous hyperintense region, which could correspond to either a tumour or lymph nodes. High resolution T_1 -weighted spin echo images were acquired (Seq. A). Tumours could easily be distinguished by their hypointense (dark) appearance;

lymph nodes appeared hyperintense (bright). This systematic approach provided a way to localize tumours. Unidentified masses were first located as hyperintense regions using T_2 -weighted images and tumours were subsequently confirmed by their hypointense (dark grey) appearance in T_1 -weighted images.

A trend in reduction of tumour volume was observed for the animals injected with muJ591:MIC6 suggesting an effect on tumour growth rate as reported by other groups [4]. However, the use of a single injection and short time follow-up (maximum 72 h) is not sufficient to draw a conclusion. Additional treatments may be able to show a noticeable effect on growth as reported in Milowsky et al. [4].

3.3. Signal Intensity. Figure 2 shows a T_2 -weighted spin echo image at 3 different time points: before and 40 minutes and 1 day after injection of muJ591:MIC6 compared to animals injected with PBS. A signal reduction of 17% within the tumour was calculated from the animal injected with muJ591:MIC6 40 minutes after injection while no signal change was measured in the animal injected with PBS. One day after injection, no reduction in signal intensity was observed in any animal. Figure 3 shows the decrease in signal intensity in tumours over time for all the animals injected with muJ591:MIC6 compared to MIC6 and PBS. T_2 -weighted images of tumours after injection revealed an average signal intensity reduction of $14 \pm 3\%$ for animals injected with muJ591:MIC6 compared to an average signal intensity increase of $2 \pm 11\%$ for animals injected with PBS at 40 min after injection ($p = 0.048$). A similar trend was also observed for the group of mice injected with MIC6 but was not statistically significant at any time point. A different representation of changes in signal intensity as fractional signal loss can be observed in the supplementary data (Figure S1 in Supplementary Material available online at <https://doi.org/10.1155/2017/8424686>).

3.4. Tumour T_2 Relaxation Time. Parametric imaging was performed as spatial maps of T_2 relaxation time of the tumour at different time points before and after injection of muJ591:MIC6. Such maps can potentially help differentiate healthy prostate tissue from PSMA-positive prostate cancer tissue following injection of the contrast agent. The T_2 relaxation time values are typically similar for these tissues [25] but PSMA-positive cells will be modified due to the contrast agent. Figure 4 shows the T_2 relaxation time maps for animals injected with muJ591:MIC6 and PBS. T_2 relaxation time maps are overlaid only over the tumour area. A 26% decrease in T_2 relaxation time 40 min after injection can be observed for the animal injected with muJ591:MIC6 where none is observed after injection of PBS.

The average T_2 relaxation time changes over time for the animals is shown in Figure 5. At 20 minutes after injection a statistically significant reduction in T_2 relaxation time can be observed for animals injected with muJ591:MIC6 compared to PBS ($p = 0.05$) but this reduction was no longer observed at other time points. A similar trend was also observed for animals injected with MIC6 but was never significant at any time point. T_2 relaxation times for all tumours at each of the

FIGURE 2: In vivo signal intensity image. T_2 -weighted spin echo image showing tumours (circle) before and 40 min and 1 day after injection for a mouse injected with muJ591:MIC6 and PBS (Seq. B).

FIGURE 3: Normalized mean signal intensity ratio of muJ591:MIC6, MIC6, and PBS groups at different time points after injection. The normalized mean signal intensity for each animal was obtained by averaging the signal intensity over an ROI and then dividing by the mean signal intensity before injection over the same ROI (* indicates a significant difference in the tumour mean signal intensity ratio between muJ591:MIC6 and PBS at 40 min after injection, $p = 0.048$) (Seq. C, TE = 8 ms).

FIGURE 4: T_2 relaxation time map. T_2 relaxation time maps of tumours before and 40 min and 1 day after muJ591:MIC6 (top) or PBS injection (bottom) (Seq. C).

time points and treatments are shown as supplementary data (Table S1).

T_2^* relaxation time is more sensitive to susceptibility effects caused by MIC6 and similar SPIOs, when compared to T_2 relaxation time. T_2^* relaxation time was calculated and analyzed between the different groups; however, because the tumours were close to the skin, strong susceptibility gradients affected the images and no signal was visible on the tumour after the first echo, making the calculation of T_2^* unreliable.

3.5. Elemental Iron Content Analysis. The contrast agent used, MIC6, is a SPIO nanoparticle consisting of an iron based core. ICP-AES was used to detect elemental iron at concentrations lower than could be observed by MRI. Figure 6 shows the iron levels of the tissue samples as obtained by ICP-AES. There was a significant increase in iron in the animals injected with muJ591:MIC6 relative to those injected with PBS as assessed

by one-way ANOVA ($p = 0.04$). No significant increase of iron mass percentage was observed for tumours from MIC6 injected animals ($p = 0.28$). Spleen tissues were also analyzed using ICP-AES and no significant increase in iron levels was found for any group.

3.6. Histology. Normal morphology was observed in all the organs examined with H&E staining which indicates that the contrast agents did not grossly alter tissue structure (author PJ). All nontumour tissue demonstrated normal cellularity. Figure 7 shows an H&E stained section of spleen from an injected mouse. Normal structure of the spleen is observed with the white and red pulp clearly visible, as well as the capsule. In Figure 7(b) the white and red pulps can be seen in closer detail, as well as the central artery. Normal structure of the liver is observed in Figure 8 from a treated mouse. The hepatocytes are arranged in thick plates, coming from

FIGURE 5: Mean ratio on T_2 relaxation time of muJ591:MIC6, MIC6, and PBS groups at different time points after injection. The mean change on T_2 relaxation values for all animals was averaged at each imaging time point (* indicates a significant difference in the tumour mean T_2 ratio between muJ591:MIC6 and PBS at 20 min after injection, $p = 0.05$) (Seq. C).

FIGURE 6: ICP iron content measurements. Tumour tissue iron content 72 hours after injection from animals injected with muJ591:MIC6, MIC6, and PBS (* indicates a significant difference in tumour iron mass content between muJ591:MIC6 and PBS: $p = 0.04$) Ω .

the central vein towards the periphery of the lobule, as well as the sinusoids that occupy the spaces between the hepatic plates. Normal structure of the kidney from a muJ591:MIC6 injected mouse can be observed in Figure 9. The H&E section from the kidney shows normal proximal and distal collecting ducts/tubules. They are lined by cuboidal epithelium. There is no evidence of any nuclear or cytoplasmic atypia. The interstitial connective tissue is unremarkable. There is no evidence of any inflammation in the examined sections. The section in Figure 9 also shows part of the normal renal pelvicalyceal system. PBS-injected control mice also showed normal morphology in all organs harvested and showed no visible differences compared to the muJ591:MIC6 group.

All tumour samples observed had well to moderately differentiated cell morphology. The tumour cells showed enlarged hyperchromatic nuclei, high nucleus to cytoplasmic

ratio, prominent nucleoli, and irregular nuclear outlines. Necrosis was observed from mild to moderate degree for all tumours.

4. Discussion

A conjugated superparamagnetic iron-oxide nanoparticle, muJ591:MIC6, was used as a functional contrast agent targeting PSMA-positive prostate cancer cells. The contrast agent was tested in vivo and magnetic resonance images detected the effect of the contrast agent after injection.

The low zeta potential that was measured for muJ591:MIC6 suspended in saline suggests that particles may have agglomerated once injected into the bloodstream of the mice, resulting in mortality. Complexes suspended in 0.01 M sodium bicarbonate buffer were better tolerated by the mice following injection. The use of saline (unbuffered) likely caused the pH to change after the addition of our conjugate. This also caused the zeta potential to be lower compared to the one obtained using sodium bicarbonate.

Our MESE images have a T_1 -weighting caused by a TR of 1396 ms which is shorter than 4 times the expected T_1 relaxation time. In order to approach a steady state with no T_1 effects, a pulse sequence requires a TR 4 to 5 times the expected T_1 [24]. However, when calculating the value of T_2 using multiple echoes, the T_1 weighting equally affects each echo. Each echo has T_1 weighting, but the exponential decay on intensity between echoes is primarily caused by T_2 decay. Previous in vitro experiments using various concentrations of MIC6 (data not shown) confirmed that calculated T_2 relaxation times remained the same in samples with T_1 relaxation times, as long as 1500 ms. Considering the T_1 relaxation time of the tissues of interest is within this range [26], we chose a TR value of 1396 ms which resulted in T_2 relaxation time measurements consistent with literature (88.7 [71.3, 136.7] ms) [25].

The reduction in signal intensity observed in tumour images shows no statistically significant difference between animals injected with muJ591:MIC6 and the controls after 24 h. Seventy-two hours after injection, the average signal intensity ratio of animals injected with muJ591:MIC6 has returned to the values before injection. Sillerud [27] reported that MRI has a low iron concentration detection limit of $2 \mu\text{M}$ for iron-oxide nanoparticles. Based on the ICP-AES results in this paper, the iron concentration present in the tumour 72 h after injection was below this detection limit. This explains that a signal reduction could not be observed at this time point.

Nontargeted SPIO MR contrast agents have been reported to wash out from tissues a few hours following injection [28], which is also observed for the MIC6-injected animals. We expect our muJ591:MIC6 conjugate to have a prolonged persistence in PSMA-positive tumours compared to nontargeted MIC6 particles as suggested from our ICP-AES results, although not statistically significant. Note that future studies involving ICP-AES at various time points would confirm this observation by determining if the muJ591:MIC6 injected mice had an increased iron content at earlier time points compared to nonconjugated MIC6.

FIGURE 7: H&E stained spleen. Mouse previously injected with muJ591:MIC6 during in vivo experiments at 50x and 200x magnifications, respectively.

FIGURE 8: H&E stained liver. Mouse previously injected with muJ591:MIC6 during in vivo experiments at 50x and 200x magnification, respectively.

FIGURE 9: H&E stained kidney. Mouse previously injected with muJ591:MIC6 during in vivo experiments at 50x and 200x magnification, respectively.

The significantly higher iron content measured by ICP-AES in muJ591:MIC6-injected animals suggests that the iron-containing MIC6 nanoparticle was present in the tumours 3 days after injection but that the levels were below the MRI detectable threshold. Although animals injected with MIC6

had an iron content that was not significantly different from the PBS control, the iron content was half of the measured value for the muJ591:MIC6, showing there is a possible nonspecific retention of the nanoparticles in the tumours. The target iron dosage for our experiment was based on the

clinical dose of Feridex. Although Feridex is a SPIO, Feridex is reported to be 120–180 nm in size [29], which is larger than the 35 ± 15 nm for MIC6. The discrepancy in size suggests that it may be necessary to increase the concentration of MIC6 to increase our contrast. Increasing the concentration of the contrast agent would increase its effectiveness and potentially the length of time it remains effective in vivo. Further studies would help elucidate the ideal concentration that would also yield safe toxicology results. Alternatively, multiple injections could be administered over time.

H&E staining confirmed healthy morphology of tissues 3 days after injection of the contrast agent. Further testing would be needed to determine if there are long-term effects. All pathology for control and treated animals was normal. Only moderate to low necrosis was observed in the presented cases since any animal with a high necrotic volume was not included in the study to ensure uniformity among tumours. Immunohistochemistry (IHC) staining did not conclusively determine that the antibody was present 3 days after injection. Based on our MRI results, we hypothesize that very little contrast agent was present in the tumours 3 days after injection, making IHC staining of muJ591 difficult. Bander et al. [3] suggest that J591 may be present in liver and spleen tissue; however we did not observe any IHC staining in these organs. Morris et al. [30] reported a half-life of low dose (5 mg) J591 in humans of 0.71 days. Doses of 10, 20, 40, 60, and 100 mg yielded half-lives of 0.84, 1.86, 1.83, 3.32, and 3.56 days, respectively. Based on these data, the muJ591:MIC6 contrast agent would mostly be cleared 3 days after injection. In future studies, a time course will be performed; organs will be harvested at various time points to determine the presence of the muJ591 antibody histologically. Treatments at different doses are planned to evaluate if changes in MRI and histology can be detected 3 days after injection.

5. Conclusion

A muJ591:MIC6 conjugate was tested as a contrast agent in vivo to target and detect PSMA-positive prostate cancer cells specifically using a 3T clinical MRI. The contrast agent combined an antibody against PSMA with a superparamagnetic iron-oxide (SPIO) nanoparticle that affects T_2 relaxation times. T_2 relaxation times, signal intensities, and the tumour growth rates were reduced after injection of the contrast agent. This reduction was not observed 3 days after injection, suggesting that the level of the effect was below the detection threshold of MRI at this time point. The high sensitivity of ICP-AES confirmed an increase in iron levels in animals injected with muJ591:MIC6 relative to PBS and nonconjugated MIC6. Histology on the isolated organs did not show any morphological changes related to injection of the contrast agent. These data suggest that muJ591:MIC6 may be a promising contrast agent for PSMA-positive prostate cancer tumours.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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