Site-specific conjugation and labelling of prostate antibody 7E11C5.3 (CYT-351) with technetium-99m

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Abstract. Attachment of chelating agents to the sugar residues of antibodies for subsequent radiolabelling is an attractive approach since it may have less effect on the immunoreactivity than attachment through lysine residues, which are distributed throughout the antibody and may be present near the antigen binding site. We have attached a new hydrazide-linked chelator CYT-395 (Cytogen Corp., Princeton, N.J.) to the sugar residues of the anti-prostate monoclonal antibody 7E11C5.3 and optimised the conditions for labelling the conjugate with technetium-99m in order to compare the conjugate to 7E11C5.3 antibody labelled directly with technetium using a mercaptoethanol reduction technique. Labelling yields of 70%-90% were obtained at specific activities up to 2000 MBq/mg antibody. The stability of the technetium-labelled conjugate in plasma or to a challenge with 0.1 or 1.0 mM cysteine was similar to that of direct-labelled antibody. In nine patients with prostate cancer, the plasma clearance of the labelled conjugate followed a two-compartment model, with an average β-phase half-life of 31.4±3.9 h. The average urinary clearance at 24 h was 15.3±5.0% of the injected dose. In this group of patients there was no significant difference between the blood and urine clearance of the labelled conjugate, and the clearances of the direct-labelled antibody.

Key words: Monoclonal antibody – Prostate cancer – Radioimmunoscintigraphy – Radiolabelling – Technetium-99m


Introduction

Most methods used for attaching chelating agents to antibodies make use of the side chains on amino acid residues such as lysine, which are distributed throughout the antibody and may be present near the antigen-binding sites, so that conjugation may have adverse effects on the antibody’s antigen-binding ability. In contrast, the sugar residues on IgG antibodies are attached to the CH2 domain of the heavy chains, away from the antigen-binding sites, so that conjugation through the sugar residues should have little effect on antigen binding. Cytogen has developed a glycyl-tyrosyl-lysyl-diethylene triamine penta-acetic acid (GYK-DTPA) linker, which is attached to the antibody sugars through the glycine amino group, for labelling antibodies with indium-111 [1], and clinical studies of antibodies labelled with this chelator have been performed in colorectal, breast, ovarian and prostate cancer.

Since technetium-99m is often the preferred isotope for nuclear medicine imaging, Coughlin and Belinka [2] synthesised a new bis(thiosemicarbazone) technetium chelator CYT-395 (Fig. 1), which has a hydrazide side chain for conjugation to antibody sugar residues. We have conjugated the CYT-395 chelator to the prostate antibody 7E11C5.3, and optimised the conditions for labelling the conjugate with 99mTc. We have used the 99mTc-labelled conjugate for radioimmunoscintigraphy in prostate cancer patients, and compared the pharmacokinetics with those obtained in patients imaged with 99mTc-labelled mercaptoethanol-reduced 7E11C5.3 [3].

Materials and methods

Monoclonal antibody. 7E11C5.3 (CYT-351), a mouse IgG1 monoclonal antibody, was obtained from Cytogen Corporation, Princeton, N.J. The antibody has been shown to react with epithelial cells from prostatic carcinoma, benign prostatic hypertrophy and, to a lesser extent, normal prostate glands [4].

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Fig. 1. Structure of the CYT-395 technetium chelator
Oxidation and conjugation of antibody. Ten mg of 7E11C5.3 antibody, concentrated to 5 mg/ml in phosphate-buffered saline (PBS) pH 6, was placed in a foil-wrapped vial, and 133 μl of an 80 mg/ml solution of sodium periodate in PBS pH 6 was added with stirring. The reaction mixture was incubated for 1 h in the dark at room temperature. The excess periodate was removed by chromatography on a 20-ml column of Sephadex G-50 eluted with 0.1 M sodium acetate pH 5; the antibody-containing fractions were collected and concentrated to 5 mg/ml. Seven mg of the CYT-395 chelator was dissolved in 262 μl water, and 106 μl of the solution was added, with stirring, to 8.7 mg of oxidised 7E11C5.3 antibody at a concentration of 5 mg/ml in 0.1 M sodium acetate buffer pH 5.0 (chelator/antibody molar ratio 70/1). The reaction mixture was incubated with continuous rotation for 4 h at room temperature, stored at 4°C overnight and then analysed by size-exclusion high-performance liquid chromatography (HPLC). Fast protein liquid chromatography (FPLC) was used to purify the antibody conjugate from aggregated antibody and excess chelator. The purified antibody was concentrated to 5 mg/ml, filtered through a sterile 0.2-μm syringe filter and divided into 0.5-mg aliquots which were stored at -70°C.

Optimisation of labelling efficiency and characterisation of antibody conjugate. The following parameters were varied in order to determine their effect upon the labelling efficiency of the antibody conjugate: conjugate concentration, amount and concentration of stannous ion, amount and concentration of gluconate, incubation time and incubation temperature. This exercise resulted in the development of the following optimised labelling method which was employed for further studies with the 7E11C5.3-CYT-395 conjugate.

One hundred μl of a 300 mg/ml solution of sodium gluconate in citrate-buffered saline (CBS) pH 7 was placed in a 1.8-ml sterile CryoTube. Two μl of a freshly prepared 113 mg/ml solution of stannous chloride dihydrate in 1 M HCl was then added, followed by 300 μl of sodium [99mTc]pertechnetate in saline, containing up to 2500 MBq 99mTc. The mixture was vortexed, and incubated at room temperature for 2 min. A 100-μl (5 mg/ml, 0.5 mg) aliquot of the 7E11C5.3 conjugate in CBS pH 7 was thawed and added to the vial containing the technetium gluconate. The mixture was incubated for 1 h with continuous rotation at 37°C. A 25-μl aliquot of the reaction mixture was removed and analysed by size-exclusion HPLC. The labelled antibody conjugate was purified by chromatography on a 10-ml column of sterile Sephadex G-50, eluting with CBS pH 7. The antibody-containing fractions were combined and filtered through a sterile 0.2-μm syringe filter into a sterile 10-ml glass vial. A 50-μl aliquot was withdrawn from the vial and analysed by size-exclusion HPLC.

The characteristics of the labelled antibody were compared with those of the native 7E11C5.3 and antibody labelled using the mercaptoethanol reduction technique [5]. To ensure that labelling was occurring through the chelator and not through adventitious binding to the amino acid side-chain of the antibody, the labelling efficiency obtained for the 7E11C5.3-CYT-395 conjugate was compared to that of native 7E11C5.3 and periodate oxidised 7E11C5.3. The stability of the labelled antibodies was compared in plasma and following challenge with cysteine [6], and the effect of conjugation and labelling on the immunoreactivity of the antibody was determined by an ELISA assay on LNCaP lysate [4].

Clearance of 99mTc-labelled 7E11C5.3-CYT-395 conjugate. Patients were injected intravenously with 0.5 mg of 7E11C5.3-CYT-395 conjugate labelled with 600 MBq of 99mTc. Blood samples were obtained at 15 min, 4 h and 24 h post-infusion and complete urine collections were obtained for 24 hr post-infusion. Samples were counted in an LKB gamma counter and compared with the counts of a known dilution of the injected material.

Results

Antibody conjugation and radiolabelling optimisation

The antibody oxidation and conjugation steps resulted in 10%–20% aggregation and FPLC purification was used to separate the antibody conjugate from aggregated antibody and excess chelator. Analysis of the purified conjugate by size-exclusion HPLC showed that most of the aggregated antibody had been removed, and only 1% aggregated antibody remained.

To optimise the labelling efficiency we examined the effect of several parameters including antibody concentration, temperature and tin and gluconate concentrations. Higher labelling yields were obtained at 37°C than at room temperature, and using a 5 mg/ml concentration of the antibody conjugate rather than 1 mg/ml. The optimum amount of gluconate was 30 mg. The results showed that labelling efficiencies were higher when smaller amounts of tin were used, but the amount of tin used had to be high enough to give reproducible labelling efficiencies time after time.

Fig. 2. a Size-exclusion HPLC radiochromatogram of 99mTc-labelled 7E11C5.3-CYT-395 conjugate. b Size-exclusion HPLC radiochromatogram of 99mTc-labelled 7E11C5.3 conjugate after Sephadex purification. Beckman SEC 3000 column; mobile phase 0.2 M sodium phosphate pH 7.0, 2 mM EDTA; flow rate 1.0 ml/min; retention times, 99mTc-labelled aggregated antibody 400 s, 99mTc-labelled antibody 450 s, [99mTc]pertechnetate 624 s, [99mTc]pertechnetate 860 s.