An Effective Chelating Agent for Labelling of Monoclonal Antibody with ²¹²Bi for α -Particle Mediated Radioimmunotherapy

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The ligand *N*-[2-amino-3-(*p*-isothiocyanatophenyl)propyl]- (\pm) -*trans*-1,2-diaminocyclohexane-*N*,*N'*,*N''*pentaacetic acid has been synthesized and linked to IgG and to monoclonal antibody B72.3, and labelled with ²⁰⁶Bi and ²¹²Bi to demonstrate the *in vivo* stability of the label and its utility for ²¹²Bi-radioimmunotherapy.

When tumour-localizing monoclonal antibodies (mAb) are radiolabelled for use in therapy, it is essential that the radionuclide remain linked to the protein for at least five half-lives. Despite observations *in vitro* of remarkable tumour cell specific cytotoxicity of targeted ²¹²Bi-radioimmunoconjugates,¹ the almost instantaneous loss in circulation *in vivo* of ²¹²Bi (α 6.05 MeV; $t_{1/2}$ 60.6 min) from conventional diethylenetriaminepentaacetic acid (DTPA) anhydride chelators² linked to mAb has precluded their clinical use. Even ²¹²Bi-mAb studies in animal tumour model systems have been limited to investigations designed to avoid the circulatory system, one by using subcutaneous injection,³ another by employing an intracavitary (i.p.) infusion.⁴

A less well appreciated chemical constraint on the chelator to be used is that formation of the labelled immunoconjugate should be rapid and efficient in order to effectively utilize carrier-free metal solutions and to minimize radiation damage.[†] Thus, we previously reported that a macrocyclic tetraazacyclododecane tetraacid (DOTA, **1a**) formed a kinetically inert Bi^{III} complex⁵ and we showed that a C-functionalized DOTA derivative **1b**, when linked to mAb and labelled with ²⁰⁶Bi, prevented the release *in vivo* of bismuth from the radioimmunoconjugate.² However, when we attempted to label mAb-linked **1b** with carrier-free ²¹²BiI₄⁻ according to established procedures,¹ radiochemical yields of less than a few percent were found. Inefficient labellings of DOTA derivatives with ⁹⁰Y have been investigated by Broan *et al.* who observed that divalent cations like Ca^{II} or Zn^{II} compete effectively for the ligand.⁶

In order to test the incorporation of Bi^{III} into the DOTA ligand in the absence of divalent metals, we measured the rate of reaction of Bi₄⁻ with DTPA **2a** and compared this rate with that for DOTA **1a**. Under similar reaction conditions, $[Bi]_T = 5.0 \times 10^{-6} \text{ mol dm}^{-3}, [L] = 5.0 \times 10^{-5} \text{ mol dm}^{-3}, 0.1 \text{ mol dm}^{-3} \text{ NaI}, \text{ pH } 4.0, T 25 °C, the half-life for formation of the Bi(DTPA)²⁻ was 0.06 s whereas Bi(DOTA)⁻ formed more than 10⁴ times more slowly. These measurements, together with the suggestion that the DOTA ligand may be immunogenic,⁷ required us to design an improved ligand for ²¹²Bi-immunotherapy.$

In considering new chelators, we recalled that the substituted DTPA's **2b,c** we studied earlier, while not useful for Bi^{III} *in vivo*, increased in stability in circulation with the addition of methyl groups on the carbon backbone.² We also noted that alkaline earth cation DTPA complexes are markedly less stable than those of DOTA and so could compete less well with trivalent metals in labelling reactions. We therefore synthesized the ligand cyclohexyl DTPA‡ (CyDTPA), **3** to

[†] We and others have reported that exposure of mAb to 212 Bi for 20–30 min labelling reaction time does not in itself compromise the immunoprotein.^{1,3,4}

[‡] The CyDTPA ligand was prepared by using CBZ-glycine in place of the protected *p*-nitrophenylalanine in the synthesis described for **6**.



increase backbone substitution and complex rigidity and found the half-life for reaction of BiI_4^- to $[Bi(CyDTPA)^{2-}]$ to be 0.27 s under the reaction conditions described above.

For linkage to mAb, we prepared N-[2-amino-3-(p-isothiocyanatophenyl)propyl]- (\pm) -trans-1,2-diaminocyclohexane-N,N',N"-pentaacetic acid **6c**,c'. Reaction of trans-1,2-diaminocyclohexane with the N-hydroxysuccinimidyl ester of N-t-Boc-p-nitrophenylalanine⁸ (t-Boc = tert-N-butoxycarbonyl) generated amide **4**. The carbamate was removed with dioxane saturated with HCl(g) and the amide reduced with diborane-tetrahydrofuran (THF) to produce triamine **5**. Alkylation of **5** with tert-butyl bromoacetate⁹ followed by treatment with anhydrous trifluoroacetic acid provided two pairs of enantiomers **6a**,**a**'.

Preparative HPLC separation of the diastereoisomers was effected by employing a linear gradient of 0.5 mol dm⁻³ aqueous triethylammonium acetate to methanol and a C₁₈ reverse phase column. The separated ligands were further purified by anion-exchange chromatography (AG1X8, 200–400 mesh, chloroacetate form).§ Hydrogenation of the nitro group (10% Pd/C) and reaction of the anilines **6b,b**' with thiophosgene was used to prepare the isothiocyanate derivatives **6c,c'** which were linked to mAb–B72.3¹⁰ by well-defined methods.¹¹

After labelling with ²⁰⁶Bi ($t_{1/2} = 6.24$ days), a γ -ray tracer for the short-lived ²¹²Bi, the **6c,c**'-B72.3 radioimmunoconjugates were separately injected intraperitoneal (i.p.) into athymic mice. The accretion of the radionuclide in the kidney was compared in tissue distribution studies with that exhibited by similarly labelled B72.3 radioimmunoconjugates formed with ligands **2b,c** and with ¹²⁵I-B72.3 and to results obtained by intravenous (i.v.) injection in the earlier study.²¶ When ligand **2b** was used, within one hour after injection, kidney levels rose to 25.7% of the injected dose per gram of tissue (% ID g⁻¹) and remained above this level for 6 h. Similarly, if **2c** was employed, kidney levels rose to 18.6% ID g⁻¹ at 1 h and

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stayed high over 6 h. The labelled macrocycle immunoconjugate ²⁰⁶Bi–**1b**–B72.3 showed kidney levels of 10.6% ID g⁻¹ and 11.3% ID g⁻¹ at 1 h and 4 h after injection. Levels of ¹²⁵I and those for ²⁰⁶Bi complexed by ligands **6c**,**c**' were statistically identical over 6 h, did not rise above 6.88% ID g⁻¹ in the kidney at 1 h and the ²⁰⁶Bi level remained below 9.25% ID g⁻¹ at 6 h. The close parallel between ¹²⁵I levels and those for the ²⁰⁶Bi levels seen for immunoconjugates formed with ligands **6c**,**c**' indicates that the bismuth label remains with the antibody for at least six hours, *i.e.* six half-lives of ²¹²Bi. Liver levels for ²⁰⁶Bi immunoconjugates with **6c**,**c**' were normal and paralleled those for ¹²⁵I in rising from 2–7% ID g⁻¹ from 0.5 to 3 h post injection and dropping slightly thereafter as the antibody label cleared the blood.

Immunoconjugates of bovine IgG with **6c** were then tested for effectiveness in labelling immunoproteins with ~ 3 mCi of ²²⁴Ra generator^{12,13} produced ²¹²BiI₄⁻ in 0.1 mol dm⁻³ HI. The solution was neutralized to pH 4 with 2 mol dm⁻³ acetic acid and reacted with 450 µg (45 µl) of IgG-**6c** conjugate in 0.02 mol dm⁻³ 2-morpholinoethanesulphonic acid (MES)-0.15 mol dm⁻³ NaCl solution for 20 min. After purification, 1 mCi (400 µg) of labelled protein was recovered. Decay corrected, the radiochemical yield was *ca*. 60%. These amounts of ²¹²Bi when used to label mAb-103a-**6c** immunoconjugate were sufficient to perform a study of ²¹²Bi targeting and radioimmunotherapy by i.v. injection in the Rauscher leukaemia model system.¹⁴⁻¹⁶

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[§] All compounds gave satisfactory NMR (¹³C and ¹H) and mass spectral data. HPLC, $t_{\rm R} = 11.59$, 12.48 min for **6a,a'**, respectively. Satisfactory chemical analyses were found for **6a,a'**.

[¶] Since any free bismuth in circulation is rapidly cleared to the kidney, the levels of ²⁰⁶Bi radiolabel found there in excess of those of ¹²⁵I-mAb in blood may be used to quantitate the amount of ²⁰⁶Bi released from the chelate immunoconjugate. See refs. 2, 4.