Targeting properties of an anti-CD16/anti-CD30 bispecific antibody in an in vivo system

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Abstract Bispecific antibodies are currently being used in clinical trials in increasing numbers in the areas of breast cancer, prostate cancer, non-Hodgkin’s lymphoma and Hodgkin’s lymphoma. We have previously performed two clinical trials in patients with Hodgkin’s disease with an anti-CD30/anti-CD16 bispecific antibody and demonstrated a 30% response rate in a cohort of patients otherwise resistant to standard therapeutic modalities. However, no surrogate marker could be defined in these trials indicative of optimal antibody dosing/scheduling or predictive for favorable response. In order to evaluate accurately the potential biodistribution properties of bispecific antibody in patients, we have performed a detailed analysis of the binding properties and animal model in vivo characteristics of these constructs. For this purpose, the parental antibodies (anti-CD30 and anti-CD16) and the bispecific antibody (anti-CD30/anti-CD16) were radiolabeled with either $^{125}$I or $^{111}$In. Antibody integrity and binding properties after labeling were confirmed by Scatchard plot and Lindmo analysis. $^{111}$In-labeled antibodies revealed superior targeting properties in a standard SCID mouse tumor model. Both the bivalent parental anti-CD30 monoclonal antibody and the monovalent anti-CD30/anti-CD16 bispecific antibody showed excellent uptake in CD30$^+$ tumors which did not differ significantly between the two (maximum uptake 16.5% ± 4.2% vs. 18.4% ± 3.8% injected dose/gram tissue). The equivalent targeting properties of the bispecific antibody compared with the parental anti-CD30 antibody encourages the further clinical development of this bispecific antibody, and might help to explain the clinical responses seen with this antibody so far in patients suffering from Hodgkin’s disease.

Key words Bispecific antibodies · Hodgkin’s lymphoma · Tumor targeting

Introduction

With the re-emerging clinical interest for antibody-based approaches in cancer therapy [18, 27], attention is now focusing on strategies to improve the genuine cytotoxic activity of monoclonal antibodies, in order to achieve higher and longer-lasting remission rates. The major handicap of unconjugated monoclonal antibodies (Mabs) is their variable and usually low cytotoxic potential. Therefore, concepts have been developed that take advantage of the high tumor-specificity of Mabs for the specific delivery of cytotoxic drugs, enzymes or radioisotopes [6, 28]. In addition, bispecific antibodies (Bi-Mabs) were generated which are able to redirect potential cytotoxic effector cells to the tumor site [7, 22, 35]. Immunological effector cells that can be activated by such Bi-Mabs include granulocytes, macrophages, natural killer (NK), and T-cells [8, 10, 30]. The advantage of Bi-Mabs is their ability to bridge tumor and effector cells together and to induce local cell destruction subsequently. This concept of Bi-Mabs has proven its efficacy in multiple tumor systems in vitro, and in pre-clinical studies with animal models in vivo [13, 23, 25].

We have performed two clinical trials with an anti-CD30/anti-CD16 Bi-Mab in a total of 31 patients with Hodgkin’s disease so far [12, 26]. In both studies, an objective response of 30% following antibody treatment...
had been demonstrated. However, neither the first trial which was performed as a dose escalation trial nor the second one where two different infusion regimens were compared, allowed us to design larger phase II/III clinical trials. No surrogate marker emerged in these trials that could be used to optimize antibody dosing and scheduling in order to improve efficacy. This dilemma is not unique to our Hodgkin studies but is typical for almost all antibody-based trials [2, 36, 37].

As a consequence, clinical trials with Bi-Mabs and other biological response modifiers have to be re-designed. Most importantly, a clear distinction between chemotherapy and antibody trials has to be made, as maximum tolerated dose is not an appropriate goal in the field of immunotherapy. The basic fundamental of antibody trials is the fact that the antibody to be tested has to target the tumor specifically with an optimal tumor to normal tissue ratio [29]. Therefore, we initiated the present study to define the binding properties, and conditions needed for radiolabeling of our anti-CD30/anti-CD16 Bi-Mab. We also sought to examine in a SCID mouse model the biodistribution properties of the Bi-Mab in comparison with the parental antibodies.

Material and methods

Antibodies and cell lines

The generation, purification and characterization of the parental anti-CD30 (HRS-3) and anti-CD16 (A9) antibodies and of the bispecific anti-CD16/CD30 antibody have been described previously [12, 13]. A33, a murine antibody directed against a novel antigen found in over 95% of colorectal cancers [16], was used as a sub-class specific control. The recombinant CD30 antigen was generated in our laboratory and has been described previously [24]. Recombinant CD16 antigen was kindly supplied by Dr. C. Sautès, Institut Curie, Paris, France.

The established CD30+ human tumor cell line L540CY has been described elsewhere [4]. SW1222, a CD30-antigen-negative human colonic cancer cell line, was used as a control cell line. All cell lines were cultured under standard conditions in 5% CO2 atmosphere at 37°C. RPMI 1640 supplemented with standard antibiotics and 10% FCS (all Gibco, Karlsruhe, Germany) was used, and is referred as complete medium throughout the text. Cell viability in all experiments, as determined by trypan blue exclusion, exceeded 90%.

Plasmon resonance analysis (BIAcore)

Analyses were performed with a BIAcore 2000 biosensor (Pharmacia Biosensor, Uppsala, Sweden) [19]. Carboxymethylxtraneous coated sensor chip, CM5 (Research grade) and the amine coupling reagents (N-ethyl-N'-dimethylaminopropyl-carbodiimide (EDC), hydroxysuccinimide (NHS) and ethanolamine) were obtained from Pharmacia Biosensor. CD30 and CD16 antigens and the related Mabs were re-purified, immediately prior to use in immobilization and kinetic studies, by micropreparative HPLC using a Supersose 12 HR 3.2/30 size-exclusion column equilibrated in HBS (10 mM HEPES (pH 7.4), 3.4 mM EDTA, 150 mM NaCl) to ensure homogeneity. The column was connected to a SMART μHPLC system (Pharmacia Biotech, Uppsala, Sweden) and eluted at a flow rate of 100 μl/min. Monoclonal antibody concentrations were determined by ultra-violet absorption at 280 nm using A280 (1 mg/ml of 1.46. CD16 and CD30 antigen were immobilized onto the biosensor surface using the conventional NHS/EDC chemistry. Purified antibodies were diluted in HBs buffer prior to analysis. Samples (30 μl) were injected over the sensor surface at a flow rate of 10 μl/min. Following completion of the injection phase, dissociation was monitored in HBS buffer for 360 s at the same flow rate. Residual bound antibody was desorbed, and the surface re-generated between injections, using 30 μl of 10 mM NaOH. This treatment did not denature the surface, as shown by equivalent signals on re-injection of an antibody-containing sample.

Kinetic analysis of the biosensor data

Binding data were generated by passing varying concentrations of the antibodies (20, 40, 60, 80 and 100 nM) over the immobilized CD16 and CD30 antigens. For the Bi-Mab, which should exhibit monovalent binding, the apparent association and dissociation rate constants were calculated from regions of the sensograms where 1:1 Langmuirian interactions appeared to be operative, with BIAevaluation version 3.0 software that was supplied by the manufacturer.

The goodness of fit between experimental data and fitted curves was estimated by chi-squared analysis using the equation:

\[ \chi^2 = \sum (r_i - r_j)^2 / n - p \]

Radiolabeling and quality assurance

The parental anti-CD30 antibody HRS-3 and the anti-CD16/anti-CD30 bispecific A9/HRS-3 antibody were labeled with 125I or 111In isotopes, respectively. Isotopes were obtained from Dupont, Life Science Products, Boston, Mass. Radiiodination was performed using a modification of a previously published chloramine-T reaction [14] using a 2.5-fold molar excess of chloramine-T (Merck, Darmstadt, Germany) over antibody, dissolved in 0.5 M potassium phosphate buffer (pH 7.2). After a 2-min incubation period the reaction was stopped by the addition of a 10-fold excess of sodium metabisulfite, again dissolved in a 0.5 M phosphate buffer, and then purified through a desalting column (P6DG BioRad, Australia) equilibrated with phosphate-buffered saline (PBS).

Antibody labeling with 111In was achieved via a bifunctional metal-ion conjugate, CHX-A'+DTPA [38] using a modification of a previously published method [20]. In brief, a dialysis bag with an MW cutoff of 10 000 kD was immersed, Sydney, Australia was treated with 10 mM sodium bicarbonate, soaked in deionized water and 1 mM EDTA (pH 7.0) to remove heavy metals. Antibodies were dialyzed against a 50 mM sodium bicarbonate buffer (pH 8.6) containing 0.15 M NaCl for 6 h. CHX-A'+DTPA was added in a molar excess of 5:1 and incubated at room temperature (RT) overnight in the dark. Excess unbound antibody was removed by 8-h dialysis with 20 mM sodium acetate buffer containing 0.15 M NaCl (pH 6.3). 111In was bound to CHX-A'+DTPA antibody conjugate under acidic conditions (pH 5.5, 20 min), then the pH was raised to 7.0 by the addition of 2.0 M sodium acetate followed by 10 mM EDTA. The radiolabeled mixture was purified by centrifugal desalting on a Sephadex G50 column equilibrated with PBS.

Radiolabeling was performed on the day of the experiment or of injection into mice, respectively. Prior to use, percentage of unbound radionuclide content was determined by instant thin-layer chromatography (ITLC) [20, 34], and binding ability of the final radiolabeled product was tested by a cell-binding assay. Scatchard analysis was used to determine the binding constant (Kd) and number of antibody molecules bound per cell for 125I- and 111In-labeled antibody. The percentage of antibody-bound isotope was >95% in all experiments detailed.

Immunoreactivity assay

Immunoreactivity assay (IR) of radiolabeled antibodies to L540CY target cells was determined by linear extrapolation to binding at infinite antigen excess using a ‘Lindmo’ assay [17]. Twenty nano-