30

Re-188 Labelling of DD-3B6/22 Fab' Monoclonal Antibody Fragment for Radio Immuno Therapy.

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The chemical similarity of technetium and rhenium has created much interest in the nuclear medicine field to make a "matched pair" of radiopharmaceuticals for radioimmuno- diagnosis and therapy. Clinical trials with the ^{99m}Tc-DD-3B6/22 Fab' has shown promise in the diagnosis of ovarian cancer. The design of the analogous therapeutic agent with rhenium-188 (155 keV γ 15 % abundant, β^- E_{max} 2.1 MeV, $T_{1/2}$ 17 h) is under investigation. The present study describes the approach taken for direct radiolabelling of the DD-3B6/22 Fab' with carrier-free ^{188}Re and its biological evaluation in balb/c and nude mice.

It was necessary to optimise complexation conditions to maximise the availability of ^{188}Re(V) for transchelation onto the reduced antibody fragment. Rhenium-188 was eluted from a ^{188}W / ^{188}Re generator (purchased from ORNL) with 0.05 M ammonium nitrate and was evaporated to dryness. The residual $^{188}ReO_4^-$ was dissolved in a range of buffers: 0.2 M acetate (pH 4.0 - 5.5), 0.2 M phosphate (pH 5.0 - 6.0) and 0.2 M carbonate (pH 6.5 - 8.5). The $^{188}Re(VII)O_4^-$ was reduced to ^{188}Re(V) with stannous chloride (1 - 5 mg/ml) in the presence of a complexing agent (gluconate or citrate) to stabilise the reduced ^{188}Re. Complexation with citrate (0.5 M) was compared to complexation by gluconate over a range of concentrations (0.1 - 0.5 M).

Once reduced the ^{188}Re complexes were incubated with the DD-3B6/22 Fab' fragments at 37°C. Transchelation of the complexed ^{188}Re(V) from the gluconate complex was 2 times more effective than that of the citrate complex. The effect of temperature, pH and antibody concentration on the amount and rate of transchelation was also evaluated. The final product had a specific activity of 35 mCi/mg with an immunoreactive fraction of 77%. Stability of the product was assessed under various conditions: temperature, presence and absence of an inert atmosphere and presence of ascorbic acid (stabiliser). Best condition for storage were determined using an excess of ascorbic acid (40,000 fold) at 4°C in PBS pH 7.2. Under these conditions 90% of the ^{188}Re was associated with the Fab' fragment after 24 hours.

As the DD-3B6/22 antibody only recognises the cross linked fibrin of humans and primates, a pseudo animal model was established using antigen coated sepharose beads. Pharmacokinetics of the final product was evaluated in balb/c and nude mice transplanted with both D-dimer (+Ve) and Glycine (-Ve) beads. Results show that ^{188}Re DD-3B6/22 Fab' clears rapidly from the blood (α = 2.4 hr, β = 3.5 hr) and is excreted through the renal system. Localisation to subcutaneous antigen beads shows specific uptake to the D-dimer (antigen) beads was achieved within 6 h (0.23% ID) and was maintained for 24 hour post injection. Specificity to antigen implants was 5:1 ($P < 0.001$) when compared to non-specific bead implants. These results correlate well with those obtained for the ^{99m}Tc DD-3B6/22 Fab' in mice. The radiolabelling procedures are congenial for therapeutic levels and hence we believe that the ^{188}Re DD-3B6/22 Fab' has some potential for use in treatment of Ovarian cancer.