Linkage of radiogold to immunoglobulins as candidate isotopes for radioimmunotherapy

D.K. Hazra, V.L. Lahiri, G.K. Chaturvedi, P. Khanna and S. Saran
SN Medical College, Agra, India.

Using selective criteria based on half-life compatible with tumour accumulation time, suitable beta emission and possibility of manufacture in carrier-free form at economic cost, we had previously suggested the use of ^{111}Ag (T1/2 7.45 days) and ^{199}Au (T1/2 3.15 days) as possible candidates for radioimmunotherapy (*Br J Cancer* 1986, **54**, 550–551). ^{111}Ag was prepared from ^{110}Pd by *n*-gamma process and separated by Dowex anion exchange chromatography to yield a carrier free product. This communication describes the preparation of ^{199}Au from ^{198}Pt by *n*-gamma reaction and subsequent purification by solvent extraction method using ethyl acetate as solvent and standardised (in collaboration with Dr RG Deshpande, Isotope Division, BARC, India). Unlike ^{111}Ag which is monovalent and poses problems as regards its linkage with conventional bifunctional chelates, gold has been linked stably to immunoglobulin for *in vitro* use. Its linkage for *in vivo* use has also been reported by Anderson *et al.* (*Nucl Med Biol* 1988, **15**, 293–297). It is proposed to explore both this and other methods of linking gold to immunoglobulin for *in vivo* use.

Antibody guided enzyme activation of amygdalin

G. Rowlinson-Busza, A. Bamias, T. Krausz and A.A. Epenetos
ICRF Oncology Group and Dept of Histopathology, Hammersmith Hospital, London W12 0HS, UK.

The cyanogenic glycoside amygdalin (mandelonitrile-B-D-glucosido-6-B-D-glucoside) has two B-linked glucose residues, which are cleaved by B-glucosidase (EC3.2.1.21) to yield free cyanide. If the amygdalin can be activated specifically at the site of a tumour, then tumour cells will be killed without the systemic toxicity usually associated with chemotherapy. We have linked B-glucosidase from sweet almonds (prunus serotina) to the tumour-associated monoclonal antibody H17E2 against human placental alkaline phosphatase, using the heterobifunctional cross-linker MBS (m-maleimidobenzoyl N-hydroxysuccinimide ester). There was minimal loss of enzyme activity and antibody immunoreactivity following conjugation. The antibody–enzyme conjugate has been tested *in vitro* for its ability to activate amygdalin and produce a cytotoxic effect. The conjugate proved more effective at killing tumour cells in culture in the presence of amygdalin than an unconjugated mixture of antibody and enzyme at the same concentrations.

Quantitative measurement of monoclonal antibody distribution and blood flow using positron emission tomography and ^{124}Iodine in patients with breast cancer

C.B. Wilson, D.E. Snook, B. Dhokia, C.V.J. Taylor, I. Watson, A.A. Lammertsma, R. Lambrecht, J. Waxman, T. Jones and A.A. Epenetos
ICRF Oncology Group, MRC Cyclotron Unit, and Dept of Clinical Oncology, RPMS Hammersmith Hospital, London W12 OHS, UK and King Faisal Hospital Cyclotron Unit, Riyadh, Saudi Arabia.

The uptake and *in vivo* quantitation of monoclonal antibodies have been measured non-invasively using positron emission tomography (PET) and iodine-124 in 9 patients with breast cancer. Blood flow measurements were also made using oxygen-15 labelled water and PET to evaluate antibody delivery. Seven patients with ductal carcinoma were studied with HMFG1 antibody and 2 patients with non-specific antibody. Tumour uptake ranged from 2–7.7 $\times$ 10^{-3} of injected dose per gram of tissue. Values for normal tissues including liver, lung and bone were also obtained. In 2 out of 7 patients there was increased uptake of specific over non-specific antibody. There was no correlation between antibody uptake and blood flow. This report exemplifies the potential of PET for the non-invasive and accurate quantitative assessment of targetted antibody which is a prerequisite to therapy.

Radioimmunotargetting: thiophosphorylation for enhancing the metabolic stability of ^{32}P-labelled conjugates

H. Band, J. Long, J. Welsh and A. Creighton
Cellular Pharmacology Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK.

The hard beta emitting isotope ^{32}P has considerable potential for the therapy of small solid tumours. We have labelled clinically relevant monoclonal antibodies SM3, HMFG1, HMFG2, and AUA1 with ^{32}P (Foxwell procedure), obtaining conjugates of high specific activity with no loss of antibody function.

The LD_{50} of ^{32}P-labelled HMFG2 conjugates was found to be about 90 μCi/25 g mouse and from histological studies the dose limiting toxicity is the bone marrow. This is probably due to the release of ^{32}P-phosphate into the body pool following metabolic breakdown. We are investigating a number of approaches for reducing marrow toxicity. Preliminary studies have shown that autologous bone marrow replacement (within 7 days after treatment) will allow an otherwise lethal dose of 250 μCi/25 g mouse to be administered. Also, by giving excess ^{31}P phosphate in the drinking water, mice were protected against a dose of 180 μCi/25 g mouse. In further studies we have replaced phosphate by the metabolically more stable thiophosphate. In a model system utilising the readily available ^{35}S-thio-ATP we have shown that thiophosphorylated HMFG2 has a much longer beta phase half-life of 77 h compared to 29 h for its phosphorylated counterpart and that once it has been degraded, the ^{35}S-label is cleared through the urine much faster. Localisation of the ^{35}S-label into the bone/marrow is approximately half that of the ^{32}P-orthophosphate.

By using one or more of these approaches, it should be possible to administer potentially therapeutic levels of ^{32}P-labelled Mabs in man.