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Blood and tissue kinetics of radiolabelled anti-CEA monoclonal antibody and F(ab)$_2$ and Fab fragments in nude mice with human tumour xenografts: implications for tumour imaging and radioimmunotherapy

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Summary

The kinetics of a radiolabelled anti-CEA monoclonal antibody and its F(ab)$_2$ and Fab fragments have been examined in mice including those with human tumour xenografts reactive with this antibody. With intact antibody the blood α phase half time was 9.9 h, the β catabolic phase 128 h, with 55% of the remaining antibody in the intravascular compartment. Tumour localization was slow, peak levels of 22% of the dose g^{-1} being attained by 24 h, but remaining constant to at least 48 h. F(ab)$_2$ fragments α and β half times were 2.95 and 7.8 h, with an intravascular fraction of only 11%. Peak levels of tumour localization were seen within 7 h of injection but reached only 10% of the dose g^{-1} and then declined. Fab fragment half times were only 0.31 and 5.4 h, with 9% remaining intravascularly. Tumour localization was seen early but at only 2.5% of the dose g^{-1}, and then declined. Tumour to blood ratios increased over time with all preparations, but reached only 2.5:1 with intact antibody compared with 15:1 and 19:1 with F(ab)$_2$ and Fab respectively. These studies emphasize that the improved tumour: normal tissue ratios obtained with antibody fragments are the result of both faster and greater extravasation of fragments and their faster overall catabolism.

Introduction

Imaging of tumours with radiolabelled monoclonal antibodies has been extensively described both in experimental and clinical studies. Fragments of monoclonal antibodies, particularly F(ab')$_2$ and Fab fragments are generally regarded as showing

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improved tumour imaging [1–8]. This is possibly due to the faster rate of extravasation of fragments, allowing quicker tumour localization. In addition fragments are catabolized more quickly than antibody, causing accelerated reduction of blood and normal tissue levels of radiolabel. Thus increased discrimination between tumours and normal tissues seen with fragments is probably a result of two factors, increased extravasation and increased catabolism. In the present study with an anti-CEA monoclonal antibody these factors have been examined by measuring the blood kinetics and intravascular–extravascular ratios with intact antibody, $F(ab)_2$ and Fab. These have been related to the kinetics of localization of the antibody and fragments in tumour xenografts in nude mice.

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Materials and methods

Antibody and fragments

The anti-CEA antibody designated 198 produced in this laboratory and its $F(ab)_2$ and Fab fragments were used. The preparation and purification of these have been described previously [8]. The bivalent fragment is designated $F(ab)_2$ not $F(ab')_2$ since it was made by papain digestion of the antibody rather than conventional pepsin digestion which produces fragments designated $F(ab')_2$. The affinities of the intact antibody and its $F(ab)_2$ and Fab fragments in reaction against cell surface CEA antigen on MKN45 gastric carcinoma cells have been previously determined by the method of Roe *et al.* [9] to be 3.75×10^{-8} l mole^{-1}, 4.79×10^{8} l mole^{-1} and 1.74×10^{8} l mole^{-1} respectively [10].

Radiolabelling

Antibody and fragments were labelled with ^{125}I or ^{131}I using Na ^{125}I and Na ^{131}I (Amersham International, UK) and Iodogen [11] to specific activities of about 37 MBq mg^{-1}. The immunoreactive fractions of radioiodine labelled preparations were determined in a quantitative binding assay against *in vitro* culture-derived cells of the CEA producing gastric carcinoma cell line MKN45, and the CEA negative osteosarcoma 791T as previously described [8].

Blood kinetics

Groups of 3–4 BALB/c mice (Bantin and Kingman, Hull, UK) were injected i.v. via a lateral tail vein with 10–20 μg of labelled preparation. Subsequently blood samples were taken periodically from the tail vein into 10 μl microcapillary pipettes (Drummond Microcaps, Drummond, USA). Blood samples were assayed for radioiodine. Count rates were plotted against time in a semi-logarithmic plot. The clearance curves for all three preparations were essentially bi-phasic. Least squares regression analysis was performed on logarithmic plots of the data from individual mice and half times of each component were calculated. The data in both the first (α) and second (β) phases were extrapolated using the method of Sterling [12] to give respectively: (i) the count rate in the blood at time zero before extravasation had taken place, (ii) the hypothetical blood count rate at time zero after equilibration with the extravascular component. From this and the extrapolated data from the phase, the relative volumes of intra- and extravascular distribution components were calculated.

Tumour xenografts

A tumour xenograft line was established in athymic nude mice (Harlan, Olac, Oxon, UK) of th

CEA producing gastric carcinoma MKN45 [8, 13]. The tumours were routinely passaged by subcutaneous transplantation.

Tumour localization studies

Groups of two mice with MKN45 or 791T xenografts were injected with mixtures of ^{131}I-labelled 198 antibody and either ^{125}I-labelled F(ab)$_2$ or Fab fragment (2–3 μg of each). At times after injection groups of two mice were killed and dissected. Radioiodines were measured in weighed samples of blood, tumour and other organs. Levels of radioiodine were expressed as: (i) the proportion of the injected dose g^{-1} of tissue; (ii) a tumour to blood ratio = count rate of radioiodine g^{-1} tissue/count rate of radioiodine g^{-1} blood.

Results

IMMUNOREACTIVE FRACTIONS

The immunoreactive fractions of intact ^{125}I-labelled 198 and ^{131}I-labelled F(ab)$_2$ and Fab fragments were virtually identical. Thus at the plateaux of titrations 48–52% of the radiolabel bound to MKN45 target cells but there was minimal (< 5%) binding against 791T control cells.

BIODISTRIBUTION AND TUMOUR LOCALIZATION

Intact antibody

Blood clearance studies of intact antibody showed an initial clearance/equilibrium phase with a mean half time for four mice of 9.9 h, followed by a slow catabolic phase with a mean half time of 128 h. The intravascular fraction was 55%. Blood, tumour and normal tissue kinetics in mice with MKN45 xenografts showed peak levels of antibody in tumour (20% of dose g^{-1}) 24 h after injection and this was maintained to the 48 h time point (Fig. 1a). Levels of radiolabel in liver, kidney, spleen and stomach were lower than those in tumour at all three time points. That of blood was higher at the 7 h time point, but lower at 24 and 48 h. The changes in tumour and blood levels caused a progressive increase in tumour to blood ratio (Fig. 2), although this reached a mean of only 2.5:1 by 2 days.

F(ab)$_2$ fragment

The blood clearance profile of F(ab)$_2$ was different from that of intact antibody. The initial phase had a mean half time, in the three mice, of 2.95 h, and the second phase a half time of 7.8 h. The intravascular fraction was 11%.

In mice with MKN45 xenografts, tumour levels of F(ab)$_2$ at 7 h after injection were similar to those of intact antibody, at about 10% of the dose g^{-1} and similar to that in the blood. However, kidney and stomach at 15% and 22% had the highest levels. Subsequently levels of F(ab)$_2$ declined in all tissues, although that in tumour declined more slowly than other organs (Fig. 1b). Expressed as a tumour to blood ratio, tumour levels rose rapidly over the two day observation period to reach 15:1 (Fig. 2).

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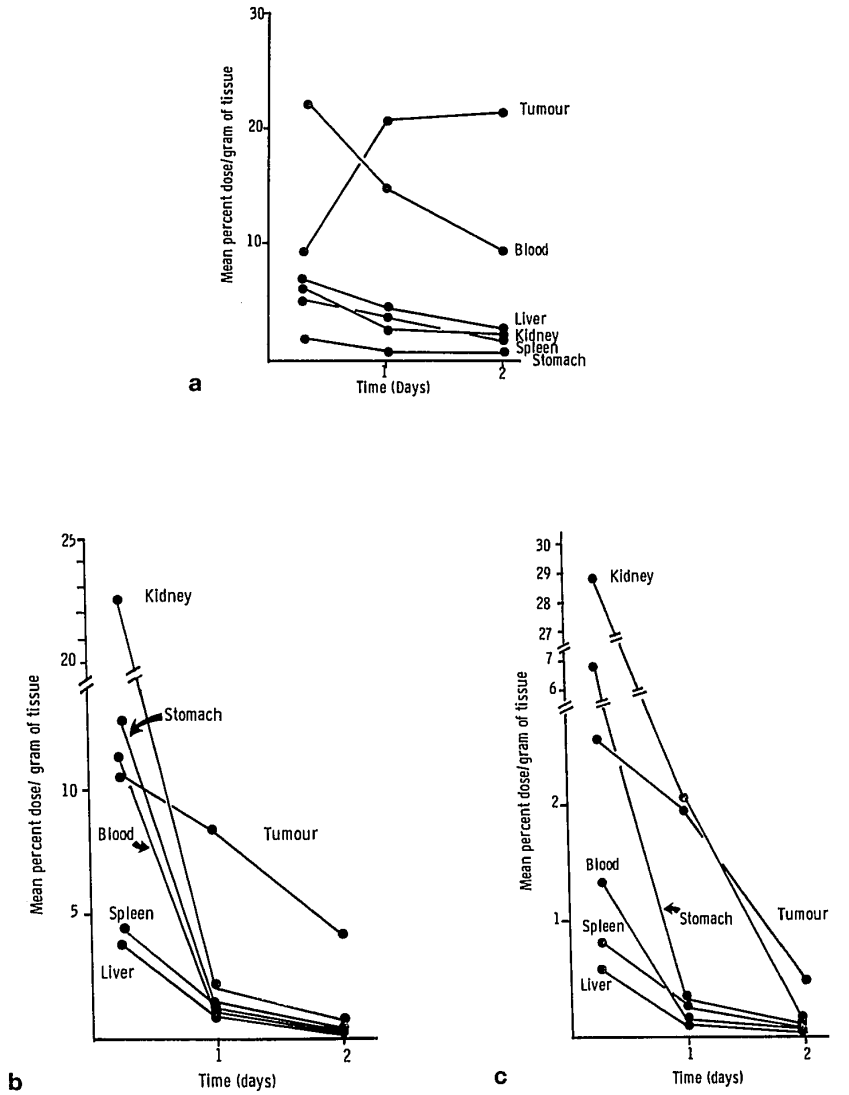


Fig. 1. Blood, tumour and tissue kinetics of ^{125}I-labelled 198 antibody injected together with ^{131}I-labelled F(ab)$_2$ or ^{131}I-Fab fragments in mice with xenografts of gastric carcinoma MKN45. Groups of two mice were killed at each time point, thus each time point has four mice for intact antibody and two for fragments. Tumours weighed between 0.14 and 0.92 g (mean = 0.35 g). Note different vertical axis scales. (a) Intact antibody; (b) F(ab)$_2$; (c) Fab.

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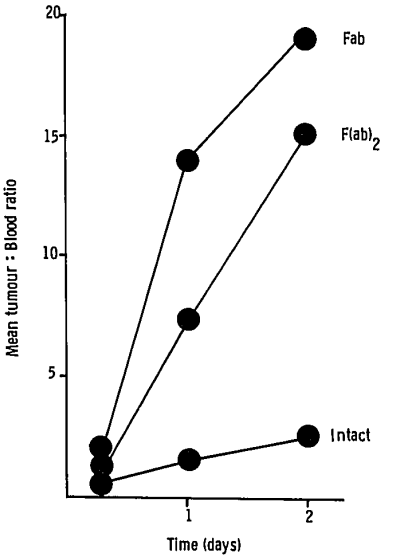


Fig. 2. Mean tumour to blood ratios with ^{125}I-labelled 198 antibody and ^{131}I-labelled $F(ab)_2$ and Fab.

Fab fragment

The blood clearance of the Fab fragment was even more rapid than that of the $F(ab)_2$, with a mean initial half time in the three mice of 0.31 h, followed by 5.4 h. The intravascular fraction was 9%. In mice with MKN45 xenografts, kidney and stomach had the highest levels of radioiodine 7 h after injection. That of tumour was only 2.5% (Fig. 1c). Levels in all tissues then declined. Tumour to normal tissue discrimination increased to the 24 h point for all organs, although after this the relative levels of radioiodine in kidney and liver were similar. The tumour to blood ratio increased throughout to 19:1 at 2 days (Fig. 2).

Discussion

These studies confirm the improved tumour discrimination which has been extensively described with fragments of anti-tumour antibodies compared with intact antibody [1–8, 10]. Thus, when data were expressed as a tumour to blood ratio, fragments were about 6–8 fold better than intact antibody. Imaging of tumours with fragments would therefore be expected to be improved, and indeed earlier studies with the 198 antibody and its fragments labelled with ^{131}I did show improved tumour discrimination [14].

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It has been suggested that characteristics of antibodies such as their immunoreactive fractions and affinities may influence tumour localization [15–17]. The 198 antibody and its fragments have very similar affinities for cell surface CEA and the radiolabelled preparations used in the present study had very similar immunoreactive fractions, but the preparations had quite different biodistribution and tumour localization characteristics. Firstly it was found that fragments of the 198 antibody are catabolized more quickly than intact antibody, catabolic phase half times of $F(ab)_2$ and Fab were 7.9 and 5.4 h respectively, dramatically shorter than the catabolic phase half time of 128 h with intact antibody. It is known that a major site of fragment catabolism in mice is the kidneys [18] and this organ had the highest levels of radioiodine in mice given antibody fragments. The radioiodine in the kidneys probably represents partly fragments undergoing catabolism there and partly radioiodine being excreted following catabolism elsewhere, probably liver and intestine [18]. The high stomach levels of radioiodine are no doubt due to gastric secretion of radioiodine following catabolism elsewhere. The increased rate of catabolism of fragments is no doubt a major factor producing lower absolute levels of fragment in tumour tissue compared with intact antibody. Thus although peak levels of $F(ab)_2$ fragment in tumour, at about 10% of the dose g^{-1} 7 h after injection, were similar to that with antibody, the level of fragments thus declined while intact antibody increased. The 7 h levels of Fab at 2.5% of the dose g^{-1} were only about a quarter of those with intact antibody and then declined.

In addition to increased rate of catabolism of fragments compared with antibody, as shown by shorter β-phase half times, the second finding from the present study was the increased rate and extent of extravasation of the antibody fragments. The extravasation phase half time of intact antibody was 9.9 h, but that of $F(ab)_2$ and Fab was 2.95 and 0.31 h respectively. The intravascular fraction of intact antibody was 55%, quite in keeping with the distribution pattern of IgG in animals and man. $F(ab)_2$ and Fab had intravascular fractions of only 11% and 9% respectively. The availability of a higher proportion of the remaining material in the extravascular compartment no doubt facilitates accretion of antibody into tumour tissue.

A third marked difference in the tumour localization of intact antibody compared with fragments is evident from these studies. With intact antibody, tumour levels rise and then remain stable (as a proportion of the dose per unit weight), often for several days [3, 7, 8, 19–21]. With fragments, after the initial localization, the levels decline (Fig. 1) [3, 7]. This might represent removal of fragments from tumour to maintain equilibrium with blood, although in fact tumour levels decline more slowly than blood levels (e.g. Fig. 1a) [3] with a concomitant rise in tumour : blood ratios. Almost certainly tumours catabolize antibodies localized in them by immune fixation. This is evident from a number of reports, including one with the 198 antibody used in the present study that mice with tumours showing antibody localization show an overall increased catabolic rate for antibody [22, 23]. There is little information on the mechanism of intratumour catabolism of antibody, although Endo *et al.* [21] analysed detergent extracts of homogenates of lung tumour xenografts from mice injected

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several days before with monoclonal antibody and found that antibody had been degraded to $F(ab')_2$ size molecules. It is possible, perhaps, that intratumour catabolism of fragments proceeds more quickly than catabolism of intact antibody. This could be assessed experimentally by studying the whole body catabolic rate of fragments in animals with tumours localizing the antibody compared with control mice.

These studies show that the improved discrimination between tumour and normal tissue is due to a combination of greater and faster extravasation of fragments and increased rates of catabolism. This improved discrimination is obtained, however, at the expense of the absolute levels of preparation localizing in tumours which is much lower with antibody fragments. These factors need to be considered in the use of radiolabelled antibodies for tumour imaging and radioimmunotherapy. In the latter situation particularly radiation doses to tumour will be reduced by the use of fragments both because of lower levels of localization initially and by faster clearance from tumour sites. In addition other tissues, particularly kidneys, bladder and stomach, can have much higher levels of radiolabel than tumour, at least transiently, and radiation doses to these organs might be higher than those to tumour itself. The present study was carried out with radioiodine as the tracer and this undergoes rapid clearance from tissues following antibody catabolism and then urinary excretion. Other radiolabels, particularly radiometals, tend to be retained in tissues following antibody or fragment catabolism. For example, earlier imaging studies with fragments of the 198 antibody labelled with ^{111}In showed that this tracer is retained for prolonged periods in the kidneys [14]. This needs to be borne in mind in considering for radioimmunotherapy other radiometals, such as ^{90}Y [24]. Overall these studies emphasize that in both diagnostic and therapeutic applications of radiolabelled monoclonal antibodies careful consideration needs to be given to the multiple choices of intact antibody, Fab or $F(ab)_2$ fragments in various combinations with radiohalogens or radiometals, because the biological handling of the radionuclide can be influenced by the type of antibody or fragment. In addition to Fab and $F(ab)_2$ fragments, consideration should perhaps be given to other fragments, such as Fab/c, which can give faster and greater extravasation than intact antibody [25] but which are not metabolized as quickly as Fab or $F(ab)_2$ and do not show rapid renal clearance and catabolism.

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