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Letter to the Editor

Dose Dependent Localisation of a Monoclonal F(ab')2 Fragment against CEA in the Mouse Xenograft Model

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THE POSSIBILITY of using radiolabelled anti-tumour localising antibodies for the systemic treatment of disseminated cancer [1] has prompted model studies aimed at maximising dose levels. One of the major limitations of using intact immunoglobulin for this purpose is the ability to react with human Fc-receptors. This may lead to non-specific accumulation of antibody in the liver, lung and spleen where large populations of Fc-receptor positive cells reside [2]. This in turn would limit the effective therapeutic dose of antibody that could be administered without inducing an excessive toxicity in normal organs.

Antibody fragments have been suggested as possible localising and targeting agents to overcome these problems [2–4]. However, the overall concentration, and effective radiation dose, at the tumour site would be reduced since fragments are known to reside at this site for shorter periods than the corresponding intact antibody [5]. The aim of the present investigation, reported here in preliminary form, was to determine whether an increased administered dose of an F(ab')2-antibody fragment in a human tumour xenograft model, could compensate for the lower overall concentration at the tumour site. The results obtained, however, show that increasing doses have an adverse effect on the tumour to normal tissue discrimination and this may have a direct bearing on the utilisation of antibody fragments for attempted therapy in humans.

F(ab')2 fragments were prepared from an IgG2a monoclonal antibody (1C12) directed against CEA and shown to be homogenous as described previously [5]. Radio-labelling with 125-I, to a specific activity of 0.5 μCi/μg, was carried out by the chloramine T method without significant loss of immunological activity. The distribution studies were carried out in nude mice bearing the human tumour xenograft "MAWI" [6]. Tumour weights were between 14 and 385 mg. Groups of mice were given intraperitoneal injections of the radiolabelled antibody fragment in doses of 4, 58, 117, 234 and 380 μg and the groups were sacrificed for tissue counting at 4, 24 and 48 hr after injection. The tissues examined were blood, liver, spleen, kidney, lung, muscle, colon and tumour. Tumour to normal tissue ratios (T : N) were calculated as the ratio of counts/g of tumour divided by the counts/g of the normal tissue.

The effect of increasing the administered dose on the magnitude of the T : N ratios, at 24 and 48 hr after injection, is shown in Table 1. At 24 hr, and with the highest dose (380 μg), a decrease in the T : N ratio of between 59 and 70% of that obtained with the 4 μg dose, was observed. These effects, although less marked, were also seen at 4 hr after injection (data not shown). At 48 hr after injection, however, a significant fall in the T : N ratios was observed with the highest dose only. As expected from previous studies [2–5], the ratios for each

Table 1. *The effect of increasing dose* (4–380 ug) *on the tumour to normal tissue ratio at* 24 *and* 48 hr *after injection of F(ab')2-1C12 into MAWI xenograft-bearing nude mice. Variation between mice (four animals per group), omitted for clarity, was within* 12%

Tissue	Time (hr)	4 μg	58 μg	117 μg	234 μg	380 μg
Blood	24	4.4	4.2	2.8	1.9	1.7
	48	13.0	15.3	—	13.6	6.2
Liver	24	9.2	10.1	7.7	4.5	3.8
	48	24.0	30.0	—	25.0	8.1
Spleen	24	9.2	10.6	6.9	4.4	4.0
	48	19.2	20.1	—	24.0	12.7
Kidney	24	4.1	3.7	2.2	1.5	1.2
	48	8.5	8.6	—	7.8	3.8
Lung	24	5.2	5.9	3.7	2.6	2.1
	48	12.2	17.2	—	17.0	7.7
Muscle	24	19.0	20.1	16.3	10.5	7.9
	48	50.0	66.0	—	69.0	19.5
Colon	24	13.0	11.6	7.5	5.3	4.1
	48	36.0	36.4	—	49.0	14.0

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Table 2. Relationship of administered dose of 125-*I-F(ab')*2-1C12 *to the uptake in MAWI tumour xenograft measured as percentage injected dose/g and absolute concentration in* ng/g *of tumour. The uptake ratio was calculated by dividing the absolute concentration at each dose level by the concentration at the lowest dose of* 4 μg.

Dose μg	% injected dose/g	Absolute concentration ng/g	Uptake ratio
4	4.1	160	1
58	1.5	870	5.4
117	0.96	1123	7.2
234	0.70	1638	10.2
380	0.65	2470	15.4

tissue increased significantly with time and this was maintained for each dose administered (Table 1).

The recovery of the T : N ratios, for all but the highest dose, by 48 hr after injection is consistent with the fast clearance of F(ab')2 fragments from normal tissues as shown in our previous study [5]. However, the present investigation suggests that, when the antibody is administered as a bolus, the magnitude of the dose could be a critical limiting factor in achieving high tumour to normal tissue discrimination at time points up to 48 hr. This could be particularly relevant when using therapeutic antibody-isotope conjugates with relatively short physical half-lives.

The absolute amount of fragment localising at the tumour site at 24 hr, shown in Table 2, was found to be limiting at the higher doses. One may speculate on two possible factors. First, as antibody binding sites become occupied, depending on the size of the xenograft, steric hindrance may reduce the binding affinity causing a saturation effect which may lead to premature loss of fragment from the tumour site. Secondly, de-iodination of the antibody fragment may increase with the higher doses, resulting in correspondingly less radiolabelled antibody fragment in the blood pool with a reduced uptake of label in the tumour. These factors would, of course, effect the T : N ratios discussed above.

Our previous studies [5] have shown that a net loss of F(ab')2-1C12 fragments occurs at the tumour site at 48 hr after injection. Here we have shown a recovery in T : N ratios by this time, thus emphasising the importance of time-dependent excretory mechanisms in the pharmacokinetic behaviour of escalating doses of F(ab')2–1C12 fragments.

The main conclusion from this study is that increasing doses of F(ab')2–1C12 fragments impose an increased demand on the mouse antibody clearance mechanisms resulting in an increased level of fragment in normal tissues. There is a concomitant decrease in the T : N ratios but, by the time these have improved at 48 hr after injection, the concentration at the tumour site would have started to decline.

The implications of our findings, if they prove to be general and pertinent to potential therapy in man, will clearly depend on whether the residence time of F(ab')2 fragments at the tumour site can be extended to compensate for an increased burden on normal tissue excretion.

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