Preliminary observations on the microdistribution of labelled antibodies in human colonic adenocarcinoma xenografts: relevance to microdosimetry

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Summary Autoradiography with $^{125}$I-labelled antibodies 17-1-A and 11-285-14 (anti-carcinoembryonic antigen) injected singly or together into nude mice carrying two distinct human colorectal cancer xenografts delineates marked changes in distribution and retention of isotope over 72 h, which are relevant to microdosimetry. The antibodies localise independently at low concentrations. Slow accumulation and retention predominantly in membranes of glands and necrotic areas suggest that therapy will succeed best with isotopes whose range, half-life and/or mode of delivery can exploit optimally the greater selectivity of the late retention.

Material and Methods

Dosimetry in patients with colorectal cancer using $^{131}$I-radiolabelled antibodies to carcinoembryonic antigen predicts disappointingly low doses of radiation to tumours before limiting bone marrow toxicity is approached, but partial response has been seen, even at an estimated tumour dose of 306 cGy (Begent et al., 1989). Calculations of dose to tumour based on the assumption of a uniform distribution therein may well underestimate the cytotoxicity to individual cells within the mass. It has been suggested that radiolabelled antibodies to membrane-bound antigens on cells in vitro have a greater potential for cell kill (Kozak et al., 1986), while released antigen may adversely affect antibody localisation in xenografts (Pedley et al., 1989). A comparative study of the distribution and retention of 17-1-A (Herlyn et al., 1983, 1986) and anti-carcinoembryonic antigen 11-285-14 antibodies (Lewis et al., 1984) over time by autoradiography in two distinct human colorectal cancer xenografts MAWI and TAF (Lewis et al., 1983) serves to demonstrate how the inherent temporal and spatial heterogeneity of isotope localisation in the tumours might be exploited.

Results

MAWI xenograft: localisation of 17-1-A and 11-285-14 antibodies

In the MAWI xenograft, 17-1-A was initially distributed within fibrovascular cores and along basement membranes of the tumour. By 72 h the association with blood vessels had significantly diminished, but there was still accumulation of grains on the basement membranes in addition to localisation on cell surfaces and luminal aspects (Figure 1). Likewise the direct relationship of the 11-285-14 antibody with vascularity diminished over 72 h, with an increasing number of grains appearing over isolated tumour cells and on the luminal surfaces of poorly formed malignant glands, albeit mainly in peripheral areas of tumour (Figure 2).

Auroradiographs derived from the administration of mixed antibodies demonstrated the superimposition of 11-285-14 distribution on the pattern of 17-1-A localisation. Luminal and basement membrane aspects of the malignant glands were labelled, as were single tumour cells within the mucinous areas. The overall intensity of grains which, by comparison with single administration, could be attributed to 17-1-A deposition in the mixture, was diminished in accordance with the reduced amount of antibody given. Both antibodies appeared to maintain their individual distribution patterns, with no augmentation of one upon the other (Figure 3).

TAF xenograft: localisation of 17-1-A and 11-285-14 antibodies

In the TAF xenograft the antibodies also showed changes in accumulation (seen in grain density and distribution) with time. At 24 h, the grains associated with 17-1-A antibody were mainly confined to areas of tumour close to blood vessels and fibrovascular stroma. By 48 h there was a light...
distribution of grains over tumour cells, but no association with glandular lumina. A few grains did remain in the blood vessels. In general the necrotic areas appeared to be less granular than the viable areas of the tumour. By 72 h there was heavy accumulation over the tumour cell surfaces, especially the luminal surfaces of acini. The blood vessels had cleared, and there was diffuse granularity throughout the tumour with small foci of heavy staining in areas tending towards glandular differentiation.

With 11-285-14 the antibody initially concentrated within or adjacent to blood vessels (Figure 4, 24 h) but was later found over the tumour cell surfaces and to a reduced extent in the fibrovascular cores (Figure 5, 72 h). The distribution was noticeably more focal than the distribution seen in the MAWI model, giving the impression of antibody molecules seeping out of the blood vessels, diffusing across the tumour tissue, being held both at specific binding sites and in cul-de-sacs of disaggregated or necrotic tissue where mechanical drainage may be poor.

The mixture of the two antibodies gave a diffuse grain distribution throughout the tumour with occasional small focal collections, resembling an additive picture of the two antibodies given singly.

As a general impression, the overall localisation of both antibodies was faster in the TAF model than in the MAWI. This may be attributable to greater vascularity and less necrosis in the former. Within the TAF tumour, the distribution of 11-285-14 antibody seemed slower than that of the 17-1-A. Whether this was due to impedance by a small quantity of released carcinoembryonic antigen remains speculative, but the focal retention of the 11-285-14 corresponded with local concentrations of CEA in acini, demonstrated by immunohistochemistry. However, when immunoperoxidase (indirect staining) was used to demonstrate CEA in sections from MAWI xenografts which had received 11-285-14 antibodies 72 h previously, only a few of the areas of weak focal reactivity were associated with significant accumulation of grains.

Discussion

Two human colorectal cancer xenografts, with similar gross accumulation of 125I-labelled anti-CEA and 17-1-A antibodies over 72 h (data after Pressman et al., 1957, not shown) and with similar expression of CEA (TAF 1.5–6.2 μg g−1; MAWI 34–59 μg g−1; Lewis et al., 1983), show uneven distribution of antibodies over time. While this might have been expected...
for the moderately differentiated MAWI tumour, the TAF
tumour, with apparently more consistent undifferentiated
morphology, shows isolated foci of reactivity. The antibodies
take a significant time, in relation to the half-life of 125I, to
traverse the tumours after extravasation from the blood and
their ultimate retention is probably as much determined by
physiological factors as by distribution of antigen. Superim-
posed on the antibody passage through tumour tissue in
accordance with Stokes' Law, antigen-targeted antibodies
develop from the classical diffusion and convection transport
equations as they are held back, producing spatial and tem-
poral heterogeneities which will have severe implications on
the survival prognosis of individual cells. The simple linear
quadratic equation between cell survival and tumour dose is
not expected to hold true in radioimmunotherapy.

Well-oxygenated cells neighbouring the bloodstream will
initially receive the full impact from circulating radiolabelled
antibodies as well as any specific dose enhancement due to
retention. This is clearly demonstrated by the 24 h
autoradiographs in either model. Late retention occurs
markedly in necrotic areas and is best exploited for therapy
by isotopes with sufficient range to stabilise dispersed cells
within these spaces as well as surrounding viable tumour
cells.

Evidence is accumulating that an assessment of tumour
sterilisation on a single absorbed dose value for the whole
tumour may possess serious shortcomings. Sizeable fluctua-
tions (at least by a factor of 4) in the local dose have been
shown by implanting micro-thermoluminescent dosimeters in
tumours (Griffith et al., 1988). In vitro survival curves with
212Bi-labeled membrane specific and non-specific antibodies
have demonstrated marked high efficiencies in cell killing due
to antibody binding (Kozak et al., 1986). Calculations of the
energy deposited in the cell nuclei resulting from antibody
binding have shown significant departures from the mean
energy deposition resulting from a uniform distribution of
the label, an implicit assumption of the conventional MIRD
procedure. For example, the ratio of the mean dose per
tumour cell nucleus from 125I-labeled antibodies bound to
cell surface antigen versus a uniform distribution of the
antibody, for a cell separation of 40 μm, can be greater than
2. The magnitude of the dose enhancement is strongly depen-
dent on the radionuclide emission range, the tumour his-
tology and the uniformity of antibody binding. For colonic
tumours of the TAF type where antibody localises so focally,
short range emissions must be ineffective for therapy. For an
improved appraisal of the efficacy of radioimmunootherapy, it
is essential that more detailed studies relating antibody reten-
tion to the prevalence of stem cells in three-dimensional
architecture in tumours should be performed.

Calculations from direct antibody deposition of activity
should not deter exploration of systems which incorporate a
time delay. For example, one could attempt to generate a
short-lived radioisotope in situ from a less destructive isotope
targeted to the tumour, such as a soft beta parent generating a
hard beta or alpha particle emitting daughter in situ. Gansow (personal communication) has already suggested the
217Pb—212Bi alpha generator system. If, as suggested by our
results, the major location of the antibodies is close to the
blood vessels at 24 h, the peak activity in this system (2.8 h)
occurs too early to be readily exploited for therapy. It is
doubtful whether even Fab' fragments would disseminate
sufficiently quickly into the centre of the tumours. Alterna-
tively, one could design a two-phase system in which a bound
antitumour—antihapten antibody, having had time to
traverse the tumour and clear the bloodstream, will capture a
readily diffusible labelled hapten, sent in sequentially, 72 h
after antibody administration. Simple chelating agents may
clear too rapidly to be trapped, and molecular size and charge
would have to be optimised. Since the bulk of the
hapten would clear more rapidly than an intact antibody, it
might be possible to consider radioactive isotopes which have
been discounted previously on the grounds of their predicted
toxicities when directly attached to antibodies.

Where heterogeneity of antigen expression presents severe
restrictions to the progress of radioimmunotherapy, the
results of this study provide the basis for some optimism that
these problems can in part be overcome by the administra-
tion of cocktails of monoclonal antibodies directed against
multiple non-overlapping antigens. The regimen devised
should incorporate mixtures of carrier and isotope such that
all positions and times of retention are best matched to
destroy cells which would otherwise be capable of being
recruited into division after the preliminary response has
deprecated their neighbours.

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