EUROPEAN JOURNAL OF CANCER 45 (2009) 3098–3103

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journal homepage: www.ejconline.com

The ratio of maximum percent tumour accumulations of the pretargeting agent and the radiolabelled effector is independent of tumour size

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ARTICLE INFO

Article history:
Received 11 June 2009
Received in revised form 2 September 2009
Accepted 7 September 2009
Available online 5 October 2009

Keywords:
Tumour accumulation
Tumour size
Pretargeting
Effector
Optimisation

ABSTRACT

Our previous studies have indicated that the optimal dosage ratio of pretargeting antibody to effector is proportional to their maximum percent tumour accumulations (MPTAs). This study quantitatively describes how both MPTAs and their ratio change with tumour size, to simplify pretargeting optimisation when tumour size varies. The CC49 antibody dosages below saturation of the tumour antigen level were first examined for the LS174T tumour mouse model. Then the MPTAs of the antibody in mice bearing tumours of different sizes were determined, always at antibody dosages below antigen saturation. Historical data from this laboratory were used to collect the MPTAs of the ^{99m}Tc-cMORF effector for different tumour sizes, always at effector dosages below that required to saturate the MORF in tumour. The MPTAs versus tumour sizes for both the antibody and the effector were fitted non-linearly. The best fit of the antibody MPTA ($Y_{antibody}$) with tumour size (x) in grams was $Y_{antibody} = 19.00\ x^{-0.65}$ while that for the effector was $Y_{effector} = 4.51x^{-0.66}$. Thus, even though the MPTAs of both vary with tumour size, the ratio ($Y_{antibody}/Y_{effector}$) is a constant at 4.21. In conclusion, the MPTA ratio of the antibody to the effector was found to be constant with tumour size, an observation that will simplify pretargeting optimisation because remeasurement of the optimum dosage ratio for different tumour sizes can be avoided. Theoretical considerations also suggest that this relationship may be universal for alternative antibody/effector pairs and for different target models, but this must be experimentally confirmed.

1. Introduction

That tumour size has an influence on tumour accumulation of antibodies has been recognised for more than 20 years.[1] Although tumour accumulation in %ID/g usually decreases with increasing size,[1,2] this relationship is not without exceptions.[3,4] The tumour size influence on the accumulation of tumour targeting agent is very important both pre-clinically and clinically, because tumour size differences are common in animal tumour models and especially in patients.

Unlike conventional tumour direct-targeting with radiolabelled antibodies, optimisation of dosage and timing is more complicated in tumour pretargeting and the influence of tumour size must be considered. In conventional direct-targeting, as long as the antibody dosage does not exceed that required to saturate the tumour antigens and is nontoxic,

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2009.09.007

any dosage may be used.[5–8] Instead of only one dosage to consider, pretargeting requires selecting two dosages and one interval (even more if imaging or clearing agents are involved). Optimisation requires that the dosages of antibody and effector be selected to provide the maximum percent tumour accumulation (MPTA) of the effector and the highest tumour/non-tumour ratios of the effector.[9]

Previously, we established experimentally that optimal pretargeting will be achieved when the dosage of antibody ($D_{antibody}$) is equal to or below that required to saturate the antigen levels in tumour and the dosage of the effector ($D_{effector}$) is just equal to that required to saturate the pretargeting antibody in tumour.[9] Under these conditions, both the antibody and effector are at their MPTAs and the tumour to normal tissue ratios are at their maximum values. For any given tumour, the optimal dosages and their MPTAs are related by the equation below, which represents the quantitative relationship under the conditions mentioned above[9] and shows that the optimal dosage ratio is proportional to the ratio of the MPTAs of the antibody to the effector.

$$\text{Optimal}\frac{D_{effector}}{D_{antibody}} = \frac{MW_{effector} \times \text{gpm} \times \text{accessibility}}{MW_{antibody}} \times \frac{MPTA_{antibody}}{MPTA_{effector}}$$

where $MW_{antibody}$ and $MW_{effector}$ are the molecular weights of the antibody and effector; gpm is the average number of the effector-binding groups on each antibody; and the accessibility is the fraction of the antibody in tumour still accessible to the effector at the time of effector administration. As the equation makes clear, the optimum dosage ratio depends upon the ratio of the MPTAs of antibody and effector. Since the two MPTAs vary differently with tumour size, selecting the optimum dosage ratio will be difficult unless it can be shown that the ratio of the MPTAs is a constant. If so, optimisation of the dosage ratio will be simplified since the optimal dosage ratio obtained from one tumour size will then be applicable to all others.

We have now examined how both the MPTAs of the MORF-CC49 antibody and labelled cMORF effector as well as their ratio vary with the size of LS174T tumours in nude mice. The pharmacokinetics of the CC49 antibody was reexamined (by assuming that the biodistribution of ^{111}In-DTPA-CC49 is sufficiently similar to that of native CC49 and MORF-CC49) to select a time post administration when tumour accumulation was essentially completed. The antibody dosage was then varied within a large range and the tumour accumulation measured at the selected time (48 h) post administration. By demonstrating a linear increase in absolute tumour accumulation with increasing antibody dosage, it was possible to select with confidence a dosage that was greatly below that required to saturate the antigen level in the tumour. Thereafter, the antibody MPTAs were all measured for dosage below saturation in mice with different size tumours. In the case of the cMORF effector, the results of multiple historical pretargeting studies from this laboratory were used to provide a series of effector MPTAs and tumour sizes at sacrifice. These data, both published and unpublished (listed in Table 1) were obtained in the same LS174T tumour mouse model administered either the MORF-CC49 or MORF-MN14 antibody. In all cases, the effector dosages were below the MORF saturating dosage established earlier. Thereafter, both the MPTAs of the antibody and effector versus tumour size were fitted and their MPTA ratio calculated.

2. Material and methods

The CC49 antibody was produced by Strategic Biosolutions (Ramona, CA) from the CC49 hybridoma. Labelling of the antibody with ^{111}In was as previously described.[4,10] The base sequences of MORF and its complement (cMORF) were as previously described.[11] The p-SCN-Benzyl-DTPA was from Macrocyclics (Dallas, TX). The P-4 resin (Bio-Gel P-4 Gel, medium) was purchased from Bio-Rad Laboratories (Hercules, CA) and the Sephadex G-100 resin was from Pharmacia Biotech (Uppsala, Sweden). The $^{111}InCl_3$ was from Perkin Elmer Life Science Inc. (Boston, MA). All other chemicals were of reagent grade and were used without purification.

2.1. *Biodistribution and tumour accumulation of ^{111}In labelled CC49*

All animal studies were performed with the approval of the Institutional Animal Care and Use Committee of UMass Medical School. For tumour induction, 10^6 LS174T colon cancer cells were injected into the left thigh of each Swiss NIH nude mouse (Taconic Farms, Germantown, NY). After injection of radiolabelled antibody, the mice were sacrificed by exsanguination via heart puncture under halothane anaesthesia. For biodistribution, samples of blood and other organs were removed, weighed, and counted in a NaI(Tl) well counter (Cobra II automatic gamma counter, Packard Instrument Company, CT) along with a standard of the injectate as previously described.[11] Blood was assumed to constitute 7% of body weight.

Three animal studies were performed. Firstly, the pharmacokinetics of ^{111}In labelled CC49 was examined in five groups ($N = 4$) of LS174T tumoured mice with tumours implanted 9 d earlier. Each animal received 30 μg (17 μCi) of ^{111}In labelled CC49 and were sacrificed at 11, 24, 48, 72 or 96 h. Secondly, the influence of antibody dosage on tumour accumulation was examined in six groups ($N = 4$) with tumours implanted 13 d earlier. Each animal received 20, 40, 80, 120, 160, or 200 μg of ^{111}In labelled CC49 (12 μCi) and was sacrificed at 48 h. Finally, the influence of tumour size on the antibody MPTA was examined in 20 mice. From day 9 to day 13 post tumour implantation, four mice per day were each administered 30 μg of ^{111}In labelled CC49 (24 μCi at day 9) and each mouse was sacrificed 48 h after injection. The antibody MPTAs were plotted individually against the tumour weight and curve fitted into a power function. As will be shown, the 200 μg dosage of CC49 did not saturate the tumours. Therefore, the convenient 30 μg dosage was selected with assurance that the tumour would not be saturated at all tumour size.

2.2. *The MPTAs of the ^{99m}Tc-cMORF effector*

In the course of multiple pretargeting studies, we have accumulated numerous pretargeting data, both published and unpublished. Only those MPTAs of the ^{99m}Tc-cMORF effector