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Radioimmunoscintigraphy using an anti-prostate monoclonal antibody (E4): a dosimetric evaluation

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Abstract The aim of this study was to evaluate different strategies to increase the tumour radiation dose for experimental radioimmunotherapy using ^{125}I-labelled monoclonal antibody (MAb) E4 in a nude mice model xenografted with DU-145 tumours. The effects from a single injection of the ^{125}I-labelled MAb E4, the same total amount of radiolabelled MAb E4 divided into three repeated injections, and the effect of pre-targeting with non-labelled MAb E4 for reducing the amount of shed antigen were investigated. Based on repetitive quantitative radioimmunoscintigraphies, calculation of the tumour radiation dose delivered from the ^{125}I-nuclide was performed for each strategy. The single injection strategy without pretargeting rendered the highest mean tumour radiation dose, i.e. 0.23 Gy/MBq. Pretargeting with non-labelled MAb E4 before a single injection of [^{125}I]E4 resulted in a slightly lower mean tumour radiation dose, i.e. 0.19 Gy/MBq, compared to the single injection alone. An even lower mean tumour radiation dose, i.e. 0.14 Gy/MBq, was obtained when the same total administered amount of activity was divided into three separate injections given in 10-day intervals. We concluded that the single injection strategy is the most efficient when using MAb E4 in this tumour model. The tumour radiation doses were not increased by dividing the same amount of activity into three injections or by pretargeting with non-labelled MAb E4.

Key words Monoclonal antibody · Nude mice · Xenografts · Radioimmunoscintigraphy · Tumour dose

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Introduction

MAb E4 with specificity against a tumour-associated surface antigen on prostate cancer cells has demonstrated high efficiency when used for radioimmunolocalisation (RIL) [2, 3, 10]. Intraperitoneal injections of the ^{125}I-labelled MAb E4 have facilitated successful radioimmunoscintigraphy (RIS) of xenografted DU-145 tumours in nude mice [2]. Furthermore, a curatively intended tumour radiation dose has induced marked cellular effects at radioimmunotherapy (RIT) using ^{131}I-labelled mAb E4 in the same experimental model [13].

There are suggestions from these earlier investigations that the MAb E4 has potential for clinical use, and deserves further investigation. Several crucial characteristics of the MAb E4 are yet to be described. Particularly, the kinetics of the radiolabelled MAb E4 are important for the activity accumulation in the tumour. The therapeutic potential, i.e. tumour dose per administered activity (Gy/MBq), has not been tested. Since radiotoxicity, especially concerning bone marrow, limits the amount of activity that can be administered, it is also important to evaluate the radiation doses to non-tumour tissues. Previous experience has demonstrated that different administration strategies for radiolabelled MAbs

can be used to increase the tumour radiation dose in some antigen-antibody systems [11, 14].

Thus, activity administration by single injection of the radiolabelled MAb compared to repeated injections or single injection given after pre-targeting with non-labelled antibody has been shown to influence the radiation dose to the tumour [17].

The aims of the present experimental study were to further elucidate the kinetics and efficacy of the ^{125}I-labelled MAb E4 in RIS of prostate cancer. The aim was also to investigate the effects of different activity administration strategies of the radiolabelled MAb E4 in order to find the technique that will give the optimal tumour radiation dose.

Materials and methods

Animal model

Forty nude mice (Bomholt Gaard nu/nu, 9 weeks old) were injected subcutaneously in front of each right hindleg with approximately 2.5×10^6 DU-145 cells from the human PCa cell line DU-145 [16]. The tumour diameter varied between 3.9 mm and 10 mm with a mean value of 5.8 mm after a period of 14 days. The animals were randomly placed into four groups, and uniformly mixed in seven different cages. Group A (seven animals) served as the untreated control group. Group B (ten animals) received a single injection of ^{125}I-labelled MAb E4. Group C (11 animals) received a single injection of ^{125}I-labelled MAb E4 after a pretargeting dose with non-labelled MAb E4. Group D (12 animals) received three consecutive injections of ^{125}I-labelled MAb E4 with an interval of 10 days between the injections. The doses were prepared for injection according to the description below. The weight of the animals was registered every second day during the observation period of 30 days, and the size of the tumours was measured at the same intervals using a slide calliper. The tumour volume was calculated using the formula for an ellipsoid ($\pi/6 \times \text{length} \times \text{width}^2$), and was used as a measure of the tumour mass, assuming that the tumour density was equal to water. The animals had free access to pellets and water containing 1 g KI/1000 ml.

On day 30 all animals were sacrificed. The tumours were dissected and their radioactivity registered using a calibrated thin sodium iodide crystal detector (Harshaw, Netherlands). Following radioactivity measurements, the tumours were weighed and divided into two halves, one of which was used for autoradiography, and the other for histological examination. After dissection, separate measurements of radioactivity in heart, lungs, liver, spleen, kidneys, gut, and blood were performed in the sodium iodide crystal detector.

The Ethics Committee and Radiation Protection Committee of Umeå University approved the project.

Antibody and radiolabelling

The MAb E4 was, as in earlier studies, labelled with ^{125}I (IMS 30; Amersham, Little Chalfont, UK) using the Chloramine *T*-method [1, 8]. Each single intraperitoneal injection consisted of 100 μg radiolabelled MAb E4 and an average activity of 20 MBq/animal. The radiolabelled MAb E4 was diluted in phosphate-buffered saline (PBS) to a volume of 40 μl. Pretargeting was performed using 100 μg non-labelled MAb E4 diluted in PBS to a volume of 40 μl. The repeated injections consisted of 33 μg MAb E4, radiolabelled with ^{125}I to an average activity of 7 MBq/animal and diluted in PBS to 40 μl each. Separate radiolabellings were performed for each repeated injection. All radiolabellings were performed on the same day as the injections.

Scintigraphy

The animals were anaesthetised for each scintigraphy using an intraperitoneal injection of 0.1–0.2 ml of 1:1:2 mixture of Dormicum (Midazolam 5 mg/ml; Roche, Basel, Switzerland), Hypnorm (Fluanisonum 10 mg/ml and Fentanylum 0.02 mg/ml; Janssen Pharmaceutica, Titusville, N.J., USA), and sterile water. Acquisitions were performed using a GE 400 Maxi gamma camera (General Electric, Milwaukee, Wis., USA) equipped with a pin hole collimator. The gamma camera system was calibrated using a defined activity of ^{125}I (IMS 30; Amersham, Little Chalfont, UK). A special holder was used in order to reproduce the geometry at each scintigraphic recording. The holder kept the anaesthetised mouse at a distance of 10 cm from the pinhole collimator. Each acquisition was performed using a 128×128 matrix with a pre-set count of 200,000 scintillations. In case the activity was low and the examination therefore lasted too long, the acquisition was interrupted manually when the animal showed signs of waking up, which usually occurred after 20–25 min.

Dosimetry

The dosimetry was based on repeated quantitative scintigraphies. Irregular regions of interest (ROI) were drawn manually around each tumour, and a mirrored ROI was set on the contralateral side of the image for background subtraction (Fig. 1). The counts in each ROI were then registered. When performing the background subtraction, the volume of the contralateral mirrored ROI was first reduced with the calculated volume of the tumour [9]. Based on the repeated scintigraphies, the total number of decays in the tumour was estimated. Using these results in combination with the measurements of the tumour size, the radiation dose delivered to the tumour was calculated. In the group receiving repeated [^{125}I]E4

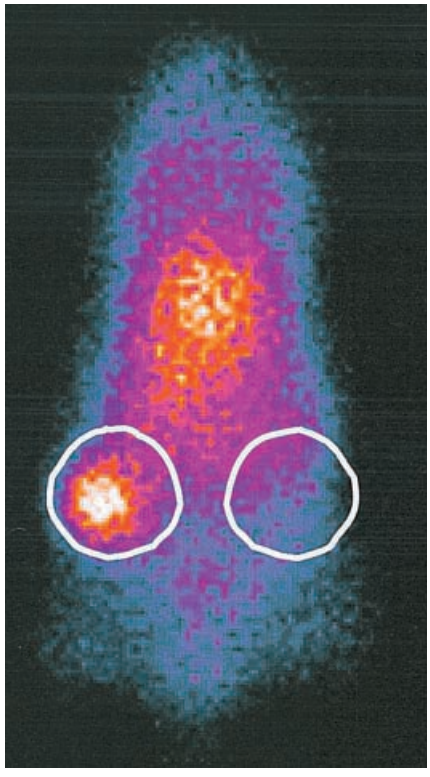

Fig. 1 Radioimmunoscintigraphic image of a single mouse after injection of [^{125}I]E4. Regions of interest are drawn around tumour (*left*) and background (*right*)

injections the animals had to be sacrificed at the same time as the other groups for ethical reasons because the tumours had grown too large. Therefore the activity administrated in the second and third injections in these groups could only irradiate the mice for 20 and 10 days, respectively, compared to 30 days for the first injection. The tumour dose for the mice in the group receiving repeated [^{125}I]E4 injections was therefore calculated too low based on the scintigtraphic recordings. This was adjusted by adding the extrapolated radiation effect from the second and third injection which would have occurred during 10 and 20 days, respectively, from the end of the experiment if the animals had been kept alive. Assuming a spherically shaped tumour with a homogeneously distributed radioactivity, an "*S*-value" (Gy/MBq-d) was calculated by using the nodule module of the MIRDOSE 3 program [15]. The size of a tumour is changing with time, usually increasing, and since the S-value depends on the tumour mass it is also varying with time. The activity in normal tissues gives a small contribution to the tumour dose, but it was not considered relevant to include this for the evaluation of the different methods to administer the antibody. Based on the fraction of energy emitted that is carried by photons this share can be estimated to approximately 68% of the mean absorbed dose in normal tissues.

It may also be noted that 18% of the energy emitted in the decay of ^{125}I is associated with Auger electrons, and, since the [^{125}I]E4 is mainly deposited on the tumour cell surface, this radiation should not contribute to the therapeutic effect.

Histology

One-half of each tumour was immediately fixed in 4% formaldehyde, dehydrated and embedded in paraffin. Six-micrometer-thick slices were cut and stained with H&E before examination by light microscopy.

Autoradiography

The other half of each tumour was fixed in Bouin's solution (15 ml 1.2% picric acid, 5 ml 40% formaldehyde, and 1 ml concentrated acetic acid). The fixed tumours were embedded in paraffin and sectioned in 20-μm-thick slices. After removal of paraffin the slices were mounted on gelatinised glass slides and dipped in the MTB2-emulsion (Kodak, Rochester, N.Y., USA) at a temperature of 40 °C. The slides were exposed in the dark at 4 °C for 4 to 10 weeks, followed by development in D19B-solution (Kodak), and fixation in F-24-solution (Kodak). The slides were then examined by light microscopy.

Statistics

Growth curves demonstrating mean relative change in tumour volume were constructed (Fig. 2). Mean values of measurements from days 0, 2 and 4 were used as the start volume. Kruskall-Wallis test was used to test differences in tumour size between the groups. Differences were considered significant if the *P*-value was less than 0.05.

Results

The animals were in good health, gained weight and retained good general condition during the entire experiment. Tumour growth was rapid in all groups with an average doubling time of approximately 14 days. No significant difference in growth rate was registered between the groups ($P = 0.5$; see Fig. 2).

A very low activity was registered in six mice, three from group B and three from group C, following the injection of radiolabelled mAb E4. This was probably due to accidental injections into gut, and the animals were therefore excluded from the analysis.

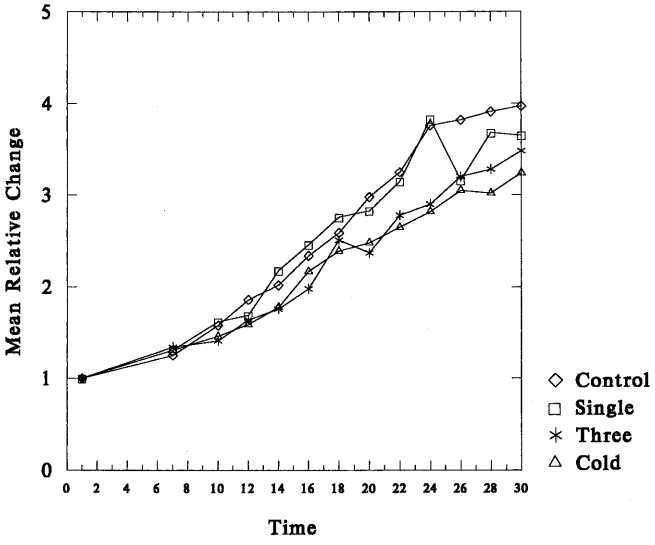

Fig. 2 Growth diagram showing mean tumour growth in the four different animal groups during the experiment (□ single injection of [^{125}I]E4, △ preload with non-labelled E4, ∗ three injections of [^{125}I]E4, ◇ untreated control)

A tumour dose of 0.23 Gy/MBq was calculated in the single [^{125}I]E4 injection group and 0.19 Gy/MBq in the group pretargeted with non-labelled E4, while 0.14 Gy/MBq was registered in the group that received repeated injections of [^{125}I]E4. The tumour uptake reached a peak value of 1.7% of the injected dose (I.D.) in the single [^{125}I]E4 injection group, 0.8% of I.D. in the group pretargeted with non-labelled E4, and 1.5% of I.D. in the repeated [^{125}I]E4 injection group. The peak tumour uptake was registered 4 to 5 days after the injections. The tumour-non tumour dose ratio, measured after dissection of the sacrificed animals on day 30, was higher in the group with repeated injections compared to the single [^{125}I]E4 injection group as well as the group pretargeted with non-labelled mAb E4. In the group receiving repeated [^{125}I]E4 injections the maximal non-tumour activity concentration was registered in blood (1.4% of I.D. per g tissue). The tumour activity concentration was 2.3% of I.D. per g tissue, while the liver-, spleen- and kidney activity concentrations were less than one-third compared to the tumour. In the other groups treated with [^{125}I]E4, the tumour activity concentration was almost equal to blood (about 1% of I.D. per g tissue).

The microscopic examination of stained sections from the tumours showed typical poorly differentiated DU-145 PCa tumours with no signs of radiation effects from the injected mAb E4 (Fig. 3). There was no significant difference in morphology observed between the four experimental groups.

Autoradiographies of sections from the tumours showed that the radioactivity accumulated primarily in vital tumour tissue and less in the necrotic parts or

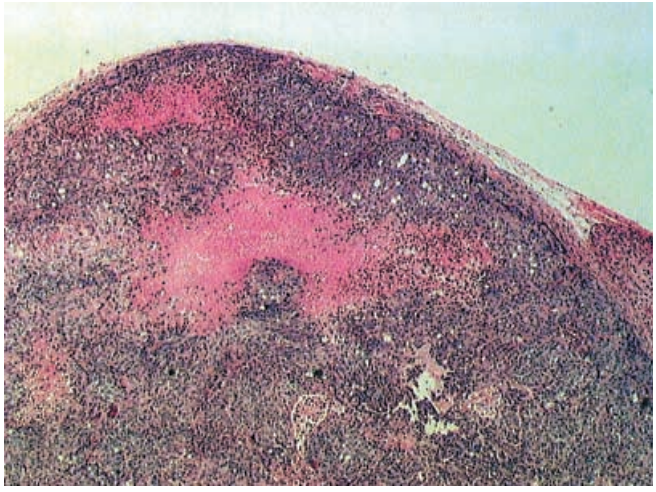

Fig. 3 Tumour section. Note necrotic areas, stained in *pink*, surrounded by viable tumour tissue. ×25

adjacent non-tumour tissues. However, the accumulation of activity was slightly heterogeneously distributed through all the vital tumour tissue, not only adjacent to the blood vessels (Fig. 4).

Discussion

In experimental RIS, ^{125}I is not suitable for therapeutic use as 20% of the energy released in the decay is emitted as Auger electrons. The short range of these Auger electrons will not induce cell damage unless they are internalised into the cell nuclei. However, we have found significant advantages in using ^{125}I for studies of antibody kinetics and dosimetry, due to its long physical half-life and significant imaging properties.

Clinical RIL is characterised by low radiation dose. The dose rate in the tumour is usually 0.1–0.2 Gy/h [5].

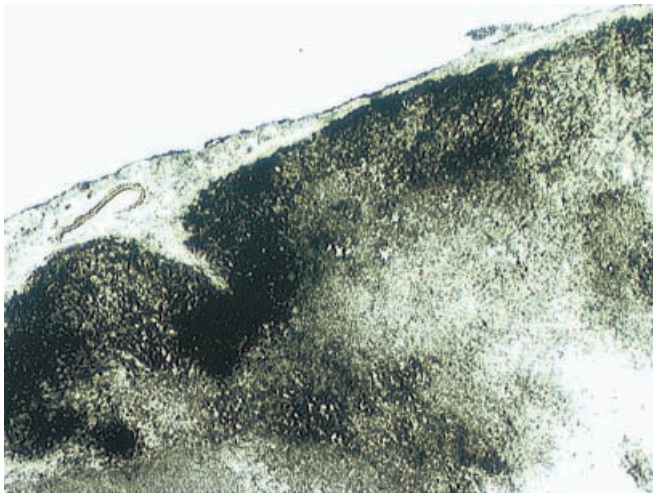

Fig. 4 Autoradiography from tumour section. The activity is higher in viable tumour tissue (*darker areas*) than in necrotic areas. ×25

Radiotoxicity to other organs, especially the bone marrow, is the major limitation for the amount of radioactivity that can be administered in order to achieve therapeutic tumour doses [4, 18]. Many strategies to improve tumour targeting and to reduce the radiation dose to non-tumour tissues have therefore been proposed [7].

By pretargeting with non-labelled antibody, it has been possible to increase the tumour-nontumour dose ratio two- to fourfold. The proposed mechanism is a reduction in the amount of circulating tumour antigen [6].

In the present experiment, no increase in the tumour uptake or reduction in the non-tumour radiation dose was observed following pretargeting with unlabelled MAb E4. The only effect noted was a slightly reduced absorbed tumour radiation dose per administered amount of activity compared to the single injection strategy. This is probably due to the characteristics of the specific cell surface antigen for MAb E4. Presumably, the amount of circulating MAb E4 antigen in this experimental tumour model is low, and therefore the pre-injected non-labelled MAb E4 is occupying the antigen on the tumour cell surface.

The difference in tumour vs. non-tumour dose ratio between the group with repeated injections and the other groups is probably due to a shorter interval between the last injection and the measurement in the group with repeated injection.

Higher total amount of activity can be reached by repeated injections of radiolabelled MAb, which in earlier experiments has been shown to significantly increase the specific tumour dose. By dividing the radiolabelled anti-tumour MAb B72.3 IgG into several consecutive injections, equal or higher tumour doses have been achieved compared to a single injection of the same amount of activity in nude mice experiments using xenografted human colon adenocarcinoma [11, 14]. In a later study using a radiolabelled anticytokeratin MAb (TS1) in a nude mouse HeLa cell tumour model, however, significantly lower tumour doses and tumour-to-nontumour ratios were achieved following repetitive injections of radiolabelled MAb compared to the same amount of activity given in a single injection [17]. In the present study we were not able to demonstrate any improvement of the tumour dose following preloading with non-labelled antibody or by giving an equal total amount of activity in repetitive injections compared to the dose achieved by single injection. In accordance with our previous experience, MAb E4 demonstrated significant tumour localisation properties in this experiment. Maximal tumour uptake was achieved 4 to 5 days after injection with ^{125}I-labelled MAb E4 in the animals that received a single dose.

The fact that maximal tumour radiation dose from MAb E4 seems to be achieved by a single injection could be advantageous in a clinical situation, as use of MAbs in clinical practice might initiate the development of human anti-mouse antibodies (HAMA). An increased use of mouse-derived antibodies for both diagnostic and

therapeutic use will increase the adverse reactions against HAMA. The risk of development of HAMAs from a single dose should be lower compared to repeated doses. This advantage can be reinforced by development of humanised MAbs with less antigenic properties. This can also facilitate the use of repeated doses, without reduction of radiotracing capacity.

Conclusions

When using MAb E4 for RIL in a nude mice model with xenografted DU-145 PCa tumours, a single injection strategy without pretargeting rendered the highest mean tumour radiation doses. The tumour radiation doses were not increased by dividing the same amount of activity into three injections or by pretargeting with non-labelled MAb E4.

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