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Application Notes

In vivo quantitation of a ^{125}I -benzamide derivative uptake within B16 melanoma subcutaneously implanted in mice. Correlation with quantitative autoradiographic method

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Goal:

In the course of our investigations aimed at improving the biological characteristics of compounds having a melanoma specific affinity (benzamide series), many derivatives have been designed and assessed on the basis of their biodistribution properties in B16 melanoma bearing mice.

The objective of the present study was to quantify *in vivo* the melanoma uptake (expressed in % of injected dose/g of tumor) of a new derivative (called U4841) radiolabelled with ^{125}I . We therefore compared tumoral uptake values determined using the γ IMAGER with those determined using whole body quantitative autoradiographic method.

Material and methods:

The tumoral uptake of ^{125}I -U4841 was assessed in B16 melanoma bearing mice, using *in vivo* scintigraphic imaging and digital autoradiography. *In vivo* radionuclide imaging was performed with the γ IMAGER (Biospace Lab, Paris) especially dedicated to *in vivo* scintigraphy of small animal, a scintigraphic camera with a continuous 4 mm thick x 120 mm diameter CsI(Na) crystal and a position-sensitive Hamamatsu R3292 photomultiplier, leading to an intrinsic resolution of 2 mm and a 10 cm field of view.

C57BL6J mice bearing a B16-melanoma subcutaneously implanted on the right flank (mean diameter: 8 –10 mm) were injected intravenously with 3.7 MBq (100 μCi) ^{125}I U4841.

The activity injected to each mouse was determined (i) by counting an aliquot of each syringe and (ii) by scintigraphic acquisition of the syringe using the γ IMAGER before and after injection.

Using the γ IMAGER, B16 melanoma uptake was assessed by repeated quantitative measurements of the activity distribution in the same animals at 1h, 3h, 6h, 24h, 48h and 72h after injection. A 10-minute duration image was acquired on anesthetized mice placed in anterior position over the parallel-hole collimator 1.8 / 0.2 / 20 (Hole diameter / septum thickness / height in mm) of the γ IMAGER. A standard with known activity (15% of injected dose) was placed systematically close to the mouse.

B16 bearing mice injected with ^{125}I -U4841 were randomized into 3 experimental groups:

Group A: each animal underwent 6 scintigraphic examinations at 1h, 3h, 6h, 24h, 48h and 72h after injection.

Group B: At the end of scintigraphic acquisition at selected time-points, mice were sacrificed, tumor was removed and weighed.

Group C: a conventional biodistribution study was performed using digital autoradiographic analysis of whole-body sections of B16-melanoma bearing mice sacrificed at the time-points selected.

Using the Biospace measures γ VISION + software, tumor radioactivity was quantified in regions of interest (ROIs): For each animal, ROIs were drawn around B16 melanoma, saved and re-used systematically for longitudinal follow-up. After radioactive decay correction, tumor uptake was expressed as % of injected dose for group A and expressed in % of the injected dose / g of tumor for group B.

Tumor uptake values determined by scintigraphic method were compared to those determined by quantitative autoradiographic technique.



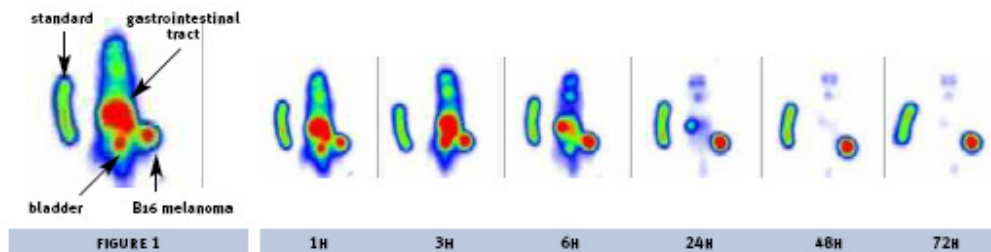
Results :

Qualitative analysis of scintigraphic images

The distribution of ^{125}I -U4841 in the mouse at 1h after injection is shown in figure 1: B16 melanoma could already be visualized 1 hour after injection. Radioactivity was also distributed within the gastrointestinal tract and bladder.

Images acquired at different time-points after injection in the same animal clearly evidenced in vivo the changes in radioactivity distribution from 1h to 72 hours after iv injection of ^{125}I -U4841. Although ^{125}I -U4841 was rapidly cleared from the body, a high retention within the tumor could be observed on scintigraphic images from 1h to 72h after injection. From 24h after injection, radioactivity was also clearly retained within the eyes and thyroid of the mice, as illustrated by imaging that allowed delineation of these organs.

Quantitative analysis of scintigraphic images

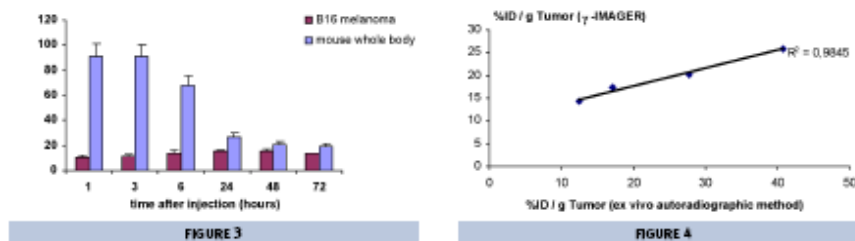


The reproducibility of ^{125}I isotope scintigraphic acquisition was assessed by calculating the coefficient of variation ($\text{CV} = 100 \times \text{SD}/\text{mean}$) of radioactivity quantitation of the standard that was scanned 25 times over 72 hours: CV was 1.05%, therefore reflecting a reproducibility of 98.9% in ^{125}I isotope acquisition.

Figure 3 compares the evolution of ^{125}I -U4841 distribution within whole body of the mouse and within B16 tumor (in % injected dose) for 72 hours:

Although the radiolabelled compound was rapidly cleared from the body (the main clearance was observed to occur between 6 h and 24 hours), a high retention within the tumor was observed (about 15% of injected dose at 72 hours).

Figure 4 shows a good correlation between the tumor uptake of ^{125}I -U4841, expressed in % injected dose / g of tissue determined by in vivo scintigraphic imaging using the γ IMAGER and those determined by quantitative autoradiography.



For this study, using a molecule that selectively distributed with a high affinity within B16 melanoma, the correlation coefficient between the two methods was 98,45%.

Conclusion :

The use of the γ IMAGER allowed to assess the biodistribution of ^{125}I -U4841 within subcutaneously implanted B16 melanoma and to monitor its pharmacokinetics in the same animal for 72 hours.

Considering the determination of tumor uptake of ^{125}I -U4841 in term of % injected dose / g of tissue, a high correlation coefficient (98%) was found between the γ IMAGER method and quantitative autoradiography.

The improvement of such a preliminary study would require that tumor weight could be estimated in vivo from calliper measurements. This is currently under investigation in our lab.

Until recently, biodistribution studies used a large number of animals being sacrificed at a range of selected time-points. Such an in vivo method using the γ IMAGER allows longitudinal studies in the same animal therefore decreasing sources of interindividual variations and thus being also interesting in term of animal costs and ethical practises.

Considering the advantages of ^{125}I radiolabelling in experimental studies (flexibility of labelling due to a wide variety of probes, and human application with ^{123}I for SPECT, and ^{131}I for therapy), such an accurate in vivo method using the γ IMAGER for determining uptake of ^{125}I -labelled molecules in experimental subcutaneously implanted tumors would accelerate the screening and the development of new diagnostic and therapeutic agents in pre-clinical cancer research.



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