

¹¹¹In-labelled somatostatin analogues in a rat tumour model: somatostatin receptor status and effects of peptide receptor radionuclide therapy

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Abstract. *Purpose:* Peptide receptor scintigraphy with the radioactive somatostatin analogue ¹¹¹In-DTPA-octreotide is a sensitive and specific technique to show in vivo the presence of somatostatin receptors on various tumours. Since ¹¹¹In emits not only gamma rays but also therapeutic Auger and internal conversion electrons with a medium to short tissue penetration (0.02–10 μm and 200–550 μm, respectively), ¹¹¹In-DTPA-octreotide is also being used for peptide receptor radionuclide therapy (PRRT). In this study we investigated the therapeutic effects of ¹¹¹In-DTPA-octreotide in tumours of various sizes. Regrowth of a tumour despite PRRT with ¹¹¹In-DTPA-octreotide may be due to the lack of crossfire from ¹¹¹In, whereby any possible receptor-negative tumour cell can multiply. We therefore also investigated the somatostatin receptor status of the tumour before and after PRRT.

Methods: The radiotherapeutic effects of different doses of ¹¹¹In-DTPA-octreotide in vivo were investigated in Lewis rats bearing small (≤1 cm²) or large (≥8 cm²) somatostatin receptor-positive rat pancreatic CA20948 tumours expressing the somatostatin receptor subtype 2 (sst₂). In addition, the somatostatin receptor density on the tumour after injection of a therapeutic labelled somatostatin analogue was investigated when the tumour was either declining in size or regrowing after an initial reduction in size. To initiate a partial response of the tumour (so that regrowth would follow) and not a complete response, a relatively low dose was administered.

Results: Impressive radiotherapeutic effects of ¹¹¹In-labelled octreotide were observed in this rat tumour model. Complete responses (up to 50%) were found in the animals bearing small (≤1 cm²) tumours after at least three injections of 111 MBq or a single injection of 370

MBq ¹¹¹In-DTPA-octreotide, leading to a dose of 6.3–7.8 mGy/MBq (1–10 g tumour). In the rats bearing the larger (≥8 cm²) tumours, the effects were much less pronounced and only partial responses were achieved in these groups. Clear sst₂ expression was found in the control as well as in the treated tumours. A significantly higher tumour receptor density ($p < 0.001$) was found when the tumours regrew after an initial decline in size after low-dose PRRT in comparison with the untreated tumours.

Conclusion: Therapy with ¹¹¹In-labelled somatostatin analogues is feasible but should preferably start as early as possible during tumour development. One might also consider the use of radiolabelled somatostatin analogues in an adjuvant setting after surgery of somatostatin receptor-positive tumours in order to eradicate occult metastases. We showed that PRRT led to an increase in the density of somatostatin receptors when the tumours regrew after an initial decline in size because of PRRT. Upregulation of the somatostatin receptor may lead to higher uptake of radiolabelled peptides in therapeutic applications, which would probably make repeated injections of radiolabelled peptides more effective.

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Introduction

A variety of receptor proteins with high affinity for regulatory peptides, including somatostatin, are expressed on cellular membranes. Somatostatin receptors are integral membrane glycoproteins, and at present five somatostatin receptor subtypes (sst_{1–5}) have been cloned. All subtypes bind somatostatin with high affinity, while the more stable analogue octreotide binds with high affinity to the somatostatin receptor subtype 2 (sst₂) and with lower affinity to sst₃ and sst₅. It shows no binding to sst₁ or sst₄ [1–5]. Continuing research resulted in the development of the

somatostatin analogues Tyr³-octreotide and Tyr³-octreotate; in the latter, the alcohol Thr(ol) at the C-terminus as used in octreotide is replaced with the natural amino acid Thr. This analogue was found to have very high affinity for sst₂ and showed the highest uptake in the rat pancreatic CA20948 tumour in a biodistribution study in rats using different ¹¹¹In-labelled somatostatin analogues [6].

Many tumours, and particularly those of neuroendocrine origin, express an increased number of somatostatin receptors, which can be visualised by imaging techniques. These somatostatin receptor-positive tumours can be visualised with, for example, ¹¹¹In-DTPA-octreotide. ¹¹¹In-DTPA-octreotide scintigraphy is nowadays recognised to be the imaging technique of choice for the localisation and staging of somatostatin receptor-positive neuroendocrine tumours [7, 8].

Another application of this ¹¹¹In-labelled somatostatin analogue is for peptide receptor radionuclide therapy (PRRT). Since ¹¹¹In emits not only gamma rays, which are visualised during scintigraphy, but also Auger and conversion electrons, an effect on tumour cell proliferation could be expected. These electrons have a tissue penetration of 0.02–10 µm and 200–550 µm respectively, so especially the radiotoxicity of Auger electrons is very high if the DNA of the cell is within their particle range [9, 10]. In a previous study we showed that ¹¹¹In-DTPA-octreotide is able to control tumour growth in a single cell in vitro system [11]. In this in vitro system it was demonstrated that the Auger electrons emitted by ¹¹¹In, and not the conversion electrons, are responsible for the anti-tumour response [11].

We reported a biological half-life for ¹¹¹In of >700 h in tumour tissue in vivo in patients [7, 8]; therefore, ¹¹¹In-labelled DTPA-octreotide has an appropriate distribution profile in humans for scintigraphy and radionuclide therapy. In clinical studies evidence of tumour response to treatment has indeed been demonstrated [12–14]. We also reported in an animal model [15] that high radioactive doses of ¹¹¹In-DTPA-octreotide could inhibit the growth of somatostatin receptor-positive liver metastases, the therapeutic effect being dependent on the presence of somatostatin receptors.

The aim of this study was to investigate the therapeutic effects of the ¹¹¹In-labelled somatostatin analogue octreotide in tumours of various sizes. Some tumours, especially larger ones, relapse after PRRT with ¹¹¹In-DTPA-octreotide. A possible explanation is that larger tumours probably display greater tumour heterogeneity owing to dedifferentiation of the tumour. One consequence could be that certain tumour cells lose the expression of the somatostatin receptor, leading to a heterogeneous receptor distribution. This might result in a reduction in the anti-tumour effect of PRRT due to the lack of crossfire from ¹¹¹In. It is therefore especially important to know the effect of radionuclide therapy on the receptor expression of the tumour at various stages of tumour development. To address this question, we investigated sst₂ expression in tumours after therapy with a ¹¹¹In-labelled somatostatin analogue.

The sst₂ expression was determined when tumours were either declining in size or regrowing after low-dose PRRT (see Fig. 3). The receptor expression was compared with that in tumours in control animals, not receiving PRRT.

Materials and methods

Labelled peptides

DTPA-octreotide (DTPA=diethylene triamine penta-acetic acid) and ¹¹¹InCl₃ (DRN 4901, 370 MBq/ml in HCl, pH 1.5–1.9) were obtained from Mallinckrodt Medical BV (Petten, The Netherlands). DTPA-octreotide was labelled with ¹¹¹InCl₃ as described previously [16]. DOTA-Tyr³-octreotate (DOTA=tetraazacyclododecane tetra-acetic acid) was labelled with ¹¹¹InCl₃ as described previously [6].

Radionuclide therapy experiments using radiolabelled somatostatin analogues

Tumour model

CA20948 pancreatic tumours were grown in the flank of male Lewis rats (Harlan, The Netherlands; 250–300 g). For this purpose, 500 µl of a cell suspension of CA20948 tumour, prepared from 5 g crude tumour tissue in 100 ml saline, was injected subcutaneously into the flank.

Tumour growth, animal condition and body weight were determined regularly. Loss of more than 10% of original body weight and tumour growth beyond about 15 cm² were indications to euthanise the animal.

Responses were recorded according to the criteria of the South-west Oncology Group [17] with some modifications. Partial response (PR) was defined as at least a 50% reduction in the product of the two largest perpendicular tumour diameters measured at onset of therapy, whereas complete response (CR) was defined as 100% reduction in this product lasting for at least 150 days.

Therapy (low and high doses) in small and large tumours with ¹¹¹In-DTPA-octreotide

At the start of therapy, about 10 days after inoculation, 50% of the rats, all bearing tumours ≤1 cm², were anaesthetised and the radiolabelled peptides were injected into the dorsal vein of the penis. The therapeutic groups received either a single intravenous injection or three injections (1 week apart) of specified amounts of ¹¹¹In-DTPA-octreotide. The specific activity of ¹¹¹In-DTPA-octreotide was 370 MBq/0.5 µg peptide. Groups comprised six to nine rats, a typical group having eight rats. The control group received no injection; earlier studies had shown that the injection of unlabelled peptide did not have an effect on the tumour growth. One week later, in the remaining 50% of the rats, bearing tumours ≥8 cm², therapy was started using the same doses of radiolabelled peptides as were used in the first group.

Low-dose therapy to determine receptor expression

The somatostatin receptor density in CA20948 tumours was determined after injection of 185 MBq (4 µg) ¹¹¹In-DOTA-Tyr³-octreotate in tumour-bearing rats. Therapy started 15 days after

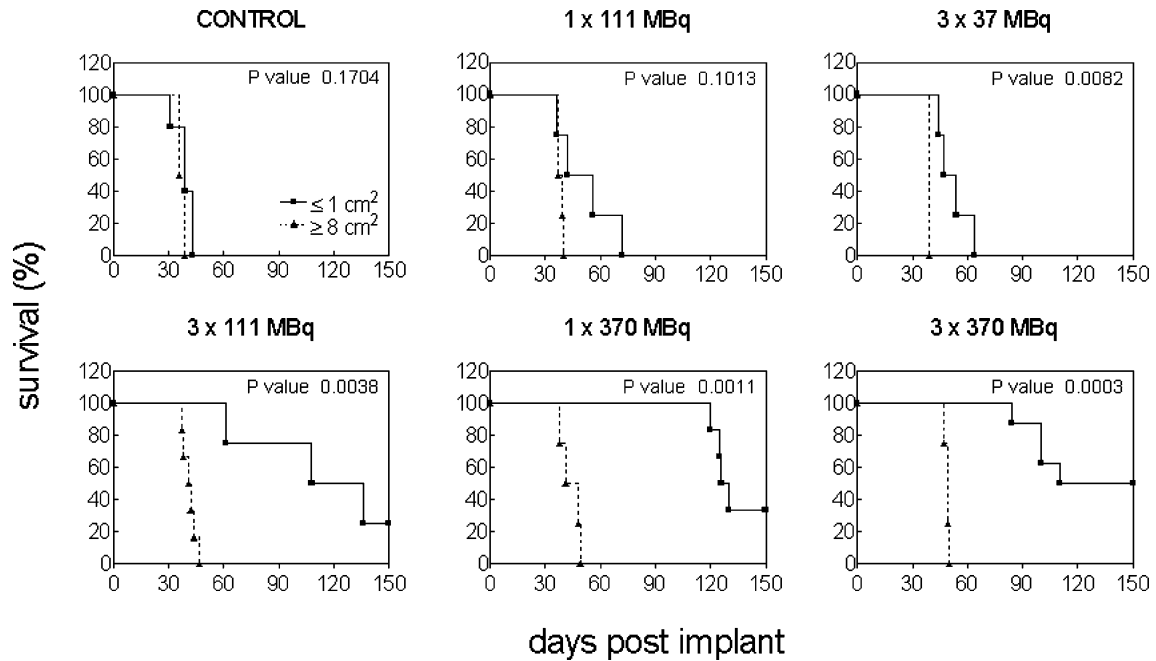


Fig. 1. Survival curves of groups of rats bearing either small ($\leq 1 \text{ cm}^2$) (solid line) or large ($\geq 8 \text{ cm}^2$) (dashed line) CA20948 tumours after the indicated doses of ^{111}In -DTPA-octreotide. For all doses the sig-

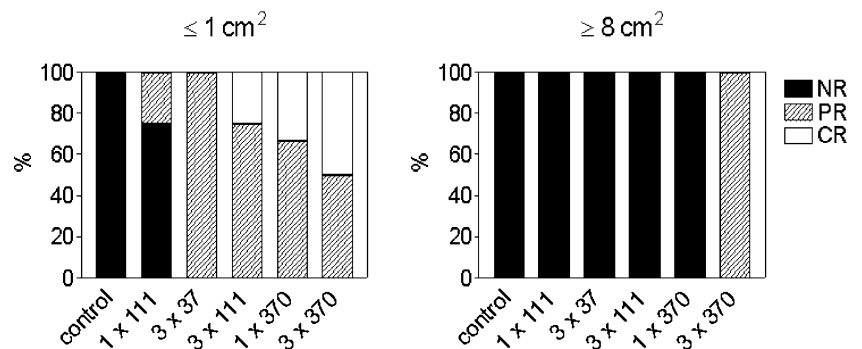
nificance of the difference in survival between rats bearing small and those bearing large tumours was calculated

inoculation. This relatively low dose was administered to initiate a partial tumour response (decline in size, regression) followed by tumour regrowth. The somatostatin receptor status on the tumours was determined at two time points after therapy: (1) in some animals when the tumour size had declined (by at least 50%) and (2) in other animals when the tumour was regrowing after the initial reduction in tumour size. Receptor status using autoradiography with DOTA- ^{125}I -Tyr 3 -octreotate was determined after isolation of the tumour (described below). Only the "vital" parts of the tumour were taken into account for receptor status determination; "vital" parts were discriminated from "necrotic" parts in histological sections.

Statistical analysis

GraphPad Prism (PraphPad Prism Software, Inc; San Diego, USA) was used to design and comparing survival curves for the different groups and to calculate the median survival, this being the time at which half the rats from a therapeutic group had been euthanised. For comparison of survival curves the logrank test (Mantel-Haenszel test) was used.

Fig. 2. Tumour responses in groups of rats bearing either small ($\leq 1 \text{ cm}^2$) or large ($\geq 8 \text{ cm}^2$) CA20948 tumours after the indicated doses of ^{111}In -DTPA-octreotide. NR no response, PR partial response, CR complete response



Autoradiography

The receptor density in CA20948 tumours was determined using autoradiography after therapy with 185 MBq ^{111}In -Tyr 3 -octreotate in tumour-bearing rats. The radioactivity due to PRRT with ^{111}In had decayed by the time of receptor determination by autoradiography. The tumours of the treated groups and of the controls were embedded in TissueTek (Saquira, Zoeterwoude, The Netherlands) after isolation and quickly frozen. Tissue sections ($10 \mu\text{m}$) were mounted on glass slides and stored at -20°C for at least 1 day to improve adhesion of the tissue to the slide. Several slides were used for autoradiography, whereas adjacent sections were haematoxylin-eosin (HE) stained. Sections were air-dried, pre-incubated in 170 mM Tris-HCl buffer, pH 7.6, for 10 min at room temperature (RT) and then incubated for 60 min at RT with 10^{-10}M DOTA- ^{125}I -Tyr 3 -octreotate. In order to determine non-specific binding of the radiopharmaceutical, adjacent slides were co-incubated with 10^{-6}M octreotide. The incubation solution was 170 mM Tris-HCl buffer, pH 7.6, containing 1% (w/v) BSA, 1 mg bacitracin and 5 mM MgCl_2 . After incubation, the sections were washed twice for 5 min in cold incubation buffer including 0.25% BSA, subsequently in buffer alone and once with

cold MilliQ; finally the sections were dried quickly. The sections were exposed to phosphor imaging screens (Packard Instruments Co., Meriden, USA) in X-ray cassettes. The screens were analysed using a Cyclone phosphor imager and a computer-assisted OptiQuant 03.00 image processing system (Packard Instruments Co, Groningen, The Netherlands). OptiQuant was used for quantification of the receptor density expressed in % density light units (DLU) per mm² relative to the control. Depending on the size of the tumour slide, different regions of interest were drawn.

Dosimetry

Estimation of tumour dose was based on biodistribution studies in this rat model as published previously [6]. The dose to the rat tumours in Gy was calculated assuming a uniform distribution of radioactivity in a spherical mass. Tumour-to-tumour dose was taken into account only, and S-values (mean absorbed dose per unit cumulated activity) for ¹¹¹In in spheres of 1 and 10 g were used as described elsewhere [18, 19].

Results

Therapy (low and high doses) in small (≤ 1 cm²) and large (≥ 8 cm²) tumours with ¹¹¹In-DTPA-octreotide

The tumours of the rats in the control group grew excessively; spontaneous growth inhibition was not observed. Survival curves for the control and therapeutic groups are shown in Fig. 1. The treatment with a single dose of 111 MBq ¹¹¹In-DTPA-octreotide, leading to a dose of 0.70–0.87 Gy in the tumour [for the relative biological effectiveness (RBE) of the Auger electrons a factor of 1 was used], did not induce significant differences between the survival curves of the animals bearing the small tumours and those of the animals bearing large tumours, as in both groups the onset of euthanasia was at the same time. For the groups that received the fractionated dose, i.e. three injections of 37 MBq, the survival curves of animals bearing small and those bearing large tumours were significantly different (Fig. 1). Furthermore, in this 3×37 MBq dose group, 100% PR was found for the animals bearing small tumours, whereas a PR of only 25% was observed for the animals with small tumours in the single 111 MBq dose group (Fig. 2).

With increased doses of ¹¹¹In-labelled octreotide, median survival (the time at which half the rats from a group had been euthanised) increased as well (Table 1). This held true both for the rats bearing small tumours and for those bearing large tumours. The effects, however, were far more pronounced in rats bearing small tumours, as also shown by the increase in ratio between the median survival data for small and large tumour-bearing rats with increasing dose (Table 1). For all doses but 111 MBq, a significant difference was found with regard to survival between rats bearing small and rats bearing large tumours (Fig. 1).

Figure 2 shows that after administration of at least 3×111 MBq (2.1–2.6 Gy) or the equivalent single dose, CR occurred in some animals, but only in those bearing small tumours. The CR rose to 50% in rats bearing small tumours

Table 1. Median survival data for groups of rats bearing either small (≤ 1 cm²) or large (≥ 8 cm²) CA20948 tumours after indicated doses of ¹¹¹In-DTPA-octreotide and the ratio between the median survival of the small and the large tumour-bearing rats

MBq	≤ 1 cm ²	≥ 8 cm ²	Ratio
0	39	38	1.04
1×111	49	38	1.29
3×37	51	39	1.30
3×111	122	42	2.94
1×370	128	45	2.88
3×370	130	49	2.65

that received the highest dose, i.e. 3×370 MBq (7.0–8.7 Gy). When therapy was initiated at a later stage (≥ 8 cm² tumours), results were much less pronounced: only PR (i.e. no CR) was reached, and then only after administration of the highest dose (Fig. 2).

Low-dose therapy to determine receptor expression

The radiation dose of 185 MBq ¹¹¹In-labelled somatostatin analogue Tyr³-octreotate led to a tumour dose of 3.3–4.1 Gy (tumour size of 1–10 g; as stated above, for the RBE of the Auger electrons a factor of 1 was used). The mean tumour size at the start of the therapy was 4.8 cm², with a standard deviation of 1.4 cm². As was intended with this dose, most tumours indeed initially responded to the given therapy, but after a certain time all tumours regrew. Figure 3 shows the tumour growth of the rats treated with a single dose of 185 MBq ¹¹¹In-labelled somatostatin analogue. At two time points the tumour receptor expression was determined using *in vitro* autoradiography: first, when the CA20948 tumours were declining in size and second, when regrowth occurred (as indicated in Fig. 3b,c). With autoradiography, clear receptor expression was found in both control tumours and ¹¹¹In-treated tumours; when an excess of octreotide (1 μ M) was co-incubated, a clear decrease in radioactivity was found (Fig. 4a). Quantification showed a higher receptor density, taking into account only the “vital” parts of the tumour (Fig. 4a,c), when the tumours regrew ($p < 0.001$) (Fig. 4b). No significant difference was found when the tumours were in regression.

Discussion

Our results show impressive radiotherapeutic effects of ¹¹¹In-labelled somatostatin analogues in this rat tumour model. Complete responses (up to 50%) were observed only in the animals bearing small but palpable tumours after at least 3×111 MBq/370 MBq ¹¹¹In-DTPA-octreotide.

The success of the therapeutic strategy depends on the amount of radioligand that can be concentrated within tumour cells, which is in turn determined by (among other factors) the rates of internalisation of both ligand and

Fig. 3. Tumour growth of groups of rats bearing CA20948 tumours treated with 185 MBq $^{111}\text{In-DOTA-Tyr}^3\text{-octreotate}$.

a Tumour growth in the control group. **b** Tumour growth in rats treated with 185 MBq $^{111}\text{In-DOTA-Tyr}^3\text{-octreotate}$, euthanised when the tumour was declining in size. **c** Tumour growth of rats treated with 185 MBq $^{111}\text{In-DOTA-Tyr}^3\text{-octreotate}$, euthanised after regrowth of the tumour following initial tumour shrinkage

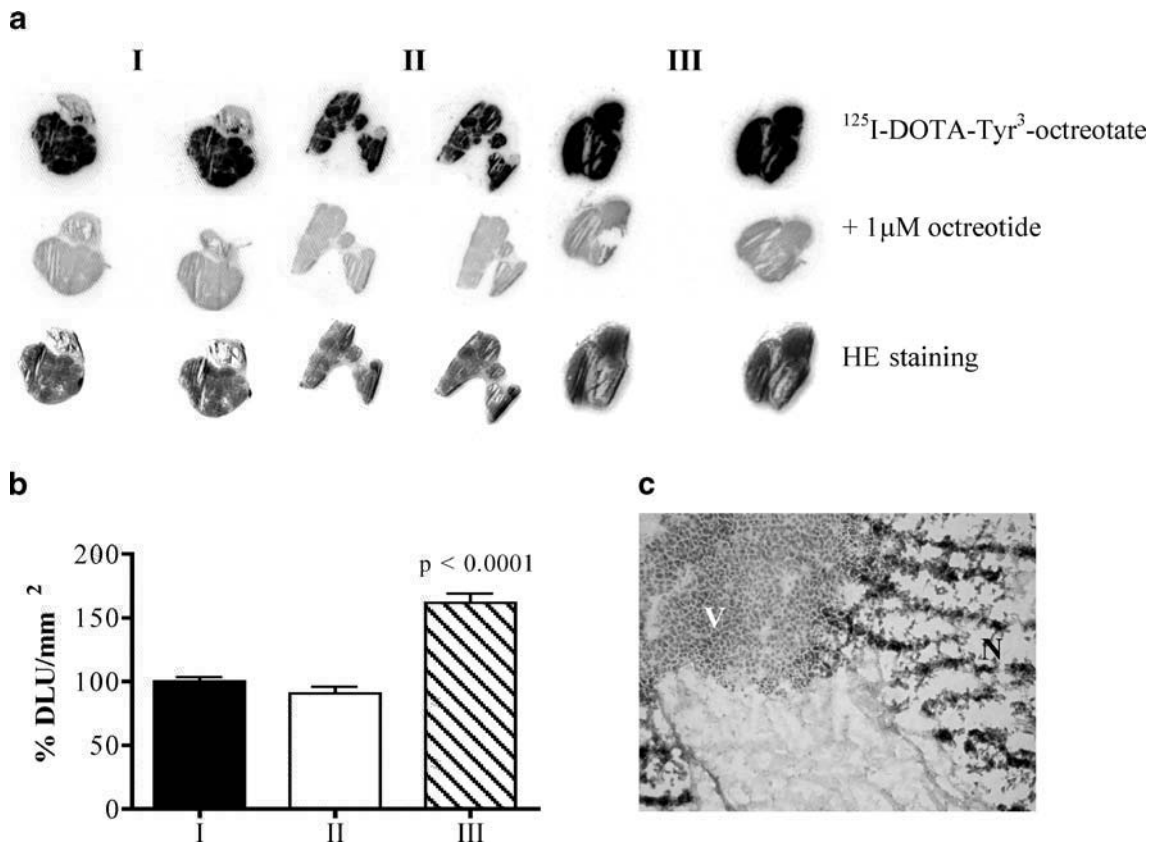
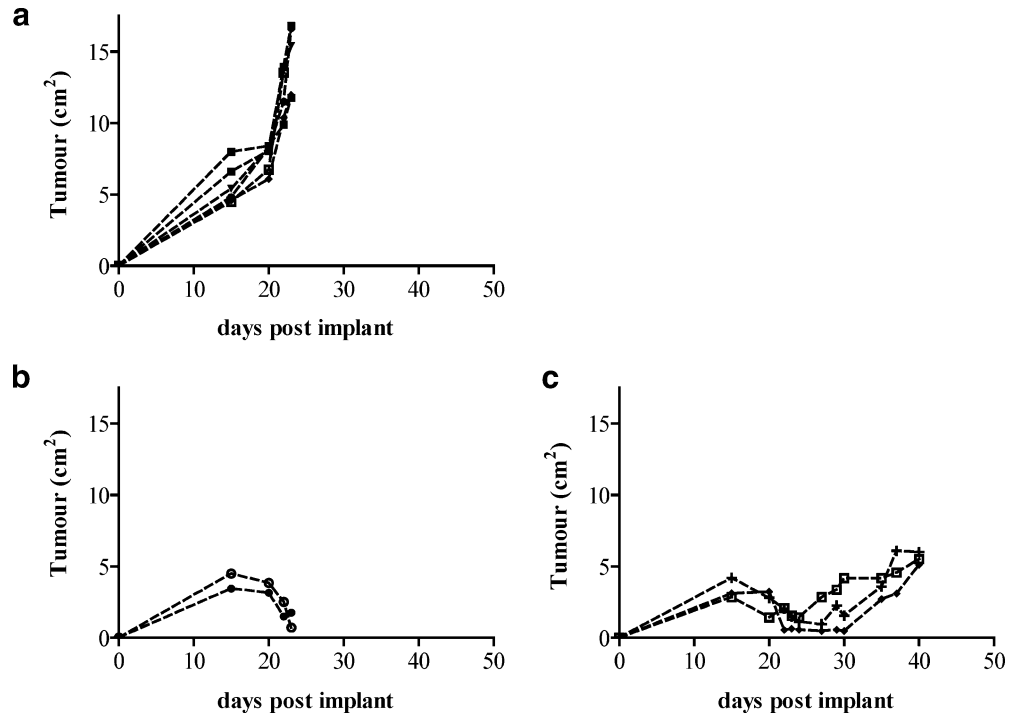


Fig. 4. a Autoradiography with $10^{-10}\text{M DOTA-}^{125}\text{I-Tyr}^3\text{-octreotate}$ of CA20948 tumours treated with saline (control) (I) or 185 MBq $^{111}\text{In-DOTA-Tyr}^3\text{-octreotate}$ (II and III) to determine the density of the somatostatin receptors when the tumour was declining in size (II) or when regrowth had occurred after the initial tumour shrinkage (III). For determination of receptor specificity of the radiopharmaceutical, adjacent slides were co-incubated with $10^{-6}\text{M octreotide}$

(middle row). Histology was determined using HE staining (third row). **b** Quantification of the somatostatin receptor density using autoradiography of treated and control tumours, performed when the tumour was declining in size (in regression) or at regrowth after an initial reduction in size. The receptor density is expressed in % density light units (DLU) per mm² relative to control. **c** HE-stained tumour section (200 \times) presenting vital (V) and necrotic (N) tissue

receptor. We studied the internalisation of radiolabelled DTPA-octreotide in tumour cells and observed that, in accordance with the findings of Andersson et al. [20], this process appeared to be receptor specific and temperature dependent [21]. Receptor-mediated internalisation of ^{111}In -DTPA-octreotide will most probably result in degradation to ^{111}In -DTPA-D-Phe. This metabolite is not capable of passing the lysosomal membrane [22], thereby contributing to the long residence time of radioactivity in the target cells (see below). Furthermore, the amount of radioligand taken up in the tumour may depend on the specific activity of the radiolabelled peptide used, as we have shown that tumour uptake of radiolabelled peptides is dependent on the amount of unlabelled peptide that is co-injected with the radiolabel [23].

The rat pancreatic CA20948 flank tumour has been shown to be a good model for therapy using radiolabelled somatostatin analogues. This is in agreement with the antiproliferative effects of ^{111}In -DTPA-octreotide found in a rat liver metastasis model [15]. Administration of 370 MBq ^{111}In -DTPA-octreotide on day 1 and/or day 8 after intraportal CA20948 tumour cell inoculation induced a significant decrease in the number of hepatic metastases at day 21. These findings showed that after radionuclide therapy, reduction in the tumour volume can be obtained because of the radiotherapeutic effect of ^{111}In -labelled octreotide. Recently we reported an *in vitro* colony-forming assay in which we studied the therapeutic effects of ^{111}In -DTPA-octreotide using the rat pancreatic tumour cell line CA20948 [11]. In this *in vitro* system we showed that ^{111}In -DTPA-octreotide can control tumour growth, the effects being dependent on incubation time, radiation dose and specific activity. Similar concentrations of ^{111}In -DTPA, which, unlike ^{111}In -DTPA-octreotide, is not internalised into sst-positive tumour cells, did not influence tumour survival. This demonstrates that the therapeutic effect of ^{111}In is dependent on internalisation, enabling the Auger electrons with their very short particle range to reach the nucleus. These findings hold promise for the application of therapy with ^{111}In -labelled octreotide in an adjuvant, micrometastatic setting and are consistent with the findings of the present study.

After therapy with diverse doses of ^{111}In -labelled octreotide we found various responses. CRs were only observed in the small tumours. Cure (up to 50%) was found in the animals bearing small ($\leq 1 \text{ cm}^2$) tumours after at least three injections of 111 MBq or a single injection of 370 MBq ^{111}In -DTPA-octreotide, leading to a dose of 6.3–7.8 mGy/MBq (1–10 g tumour). In the rats bearing the larger ($\geq 8 \text{ cm}^2$) tumours, the effects were much less pronounced and only partial responses were achieved in these groups.

Preclinical PRRT studies *in vivo* with the β -emitting radionuclides ^{177}Lu and ^{90}Y showed very promising results [24, 25]. The cure rate of the rats was, however, again dependent on the tumour size. Based on a mathematical model, the tumour curability was examined in relation to the tumour size [26]. It was calculated that the optimal tumour diameter was 34.0 mm for ^{90}Y and 2.0 mm

for ^{177}Lu . Tumours smaller than the optimal size will not absorb all the energy of the β -emitting radionuclide. In larger tumours, more clonogenic, presumably hypoxic, cells will be present, thereby limiting radiocurability. We did not investigate the presence/absence of areas of hypoxic cells in the tumour in relation to tumour size, and we cannot exclude the possibility that this factor might play a role especially in the therapeutic response of larger tumours. Furthermore, it could be that larger tumours have more areas with poor vascularisation and enhanced interstitial pressure [27], which might lead to less accessible areas for octreotide targeting. Another possible explanation for relapse in larger tumours is that they probably display greater tumour heterogeneity owing to tumour differentiation. A potential consequence would be loss of expression of the somatostatin receptor by certain tumour cells, leading to a heterogeneous receptor distribution in larger tumours. At present we do not have a clear indication of sst receptor-negative cells in tumours causing a heterogeneous distribution of the radioligand in the tumour. This will be investigated further in micro-autoradiography studies (the autoradiography studies presented here did not have the spatial resolution required to investigate this). As already mentioned, a heterogeneous receptor distribution in larger tumours may result in a reduction in the anti-tumour effect of PRRT owing to the lack of crossfire from ^{111}In . It is therefore important to know the receptor expression of the tumour at various stages of tumour development. Since 50% of the tumours regrew after $3 \times 370 \text{ MBq } ^{111}\text{In}$ -DTPA-octreotide, we investigated the sst₂ expression in tumours after ^{111}In therapy. The sst₂ expression was determined when tumours were either declining in size or when regrowth had occurred after an initial decline in size. The receptor expression was compared with that in control tumours, which did not receive PRRT.

We therefore investigated whether the somatostatin receptors were still present after therapy with 185 MBq ^{111}In -labelled somatostatin analogue. It was expected that after this relatively low tumour dose of 3.3–4.1 Gy, there would be an initial partial tumour response (decline in size, regression) followed by regrowth. We showed that a single dose of 185 MBq ^{111}In -DOTA-Tyr³-octreotate resulted in a clear decline in tumour size but that all tumours regrew. In this study we observed sst₂ receptor expression in the control tumours as well as in the tumours treated with ^{111}In . A significantly ($p < 0.001$) higher receptor density was found after regrowth in comparison with the control tumours; however, the tumour histology should also be taken into account, to investigate differences between the two tumour types (control vs regrowth) in terms of cell size, shape or cellularity. To further investigate the effects of PRRT on receptor expression, studies including these items are ongoing.

Recently, B  h   et al. [28, 29] also found an increase in receptor density after irradiation. AR42J cells were irradiated with a dose of 4, 8 or 16 Gy (external beam irradiation). Subsequent binding assays showed a time-dependent upregulation of somatostatin and gastrin re-

ceptors for all doses. The tumoural uptake in vivo was also increased, indicating that fractionation of radiolabelled peptides is feasible.

The results reported here with regard to the radiotherapeutic effects in small and large tumours may be of high clinical significance. They hold promise for application of radionuclide therapy with ^{111}In -labelled octreotide (as well as ^{177}Lu) in an adjuvant, micrometastatic setting, e.g. after surgery to eradicate occult metastases. This is in accordance with our earlier findings that high radioactive doses of ^{111}In -DTPA-octreotide inhibited the growth of somatostatin receptor-positive micrometastases in a rat liver tumour model [15]. Furthermore, the data reported here point to the importance of early initiation of radionuclide therapy during tumour development, whereas the phase I studies performed in our hospital using ^{111}In -DTPA-octreotide included only end-stage patients who often had a large tumour load.

Finally, we showed that PRRT leads to an increase in somatostatin receptor density. This upregulation of the somatostatin receptor may result in higher uptake of radiolabelled peptides in therapeutic applications, probably making repeated injections of radiolabelled peptides more effective. The observations in this study also stress the importance of careful timing of repeated administrations if the best results are to be obtained. Based on this study it appears that the effect of repeated injections could be enhanced when the dose is given relatively late, when the tumour is already declining in size.

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