New somatostatin analogues for radiotherapy of somatostatin receptor expressing tumours

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Among various newly synthesized chelator-linked octreotide analogues ^{90}Y-[DOTA-DPhe^1, Thyr^3]-octreotide (^{90}Y-SMT 487) was finally selected for clinical development. In vitro, SMT 487 binds selectively with nanomolar affinity to the somatostatin receptor subtype 2 (IC_{30}=0.39 nM±0.02). In vivo, ^{90}Y-[DOTA-DPhe^1, Thyr^3]-octreotide shows a rapid blood clearance ($T_{1/2\ \alpha}$<5 min) and high accumulation in somatostatin subtype 2 receptor expressing tumours. The in vivo administration of ^{90}Y-[DOTA-DPhe^1, Thyr^3]-octreotide induces a rapid tumour shrinkage in three different somatostatin receptor positive tumour models: CA20948 rat pancreatic tumours grown in normal rats, AR42J rat pancreatic tumours and NCI-H69 human small cell lung cancer both grown in nude mice. The radiotherapeutic efficacy of ^{90}Y-SMT 487 was enhanced in combination with standard anticancer drugs, such as mitomycin C, which resulted in a tumour decrease of 70% of the initial volume. In the CA 20948 syngeneic rat tumour model, a single treatment with 10 µCi/kg ^{90}Y-SMT 487 resulted in the disappearance of 5 out of 7 tumours. Thus the new radiotherapeutic agent showed its curative potential for the selective treatment of SRIF receptor-expression tumours. Clinical Phase I studies with ^{90}Y-SMT 487 were started in September 1997.

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Introduction

A wide variety of human tumours of different origin have a high incidence of somatostatin (SRIF) receptors (e.g. pituitary tumours, endocrine pancreatic tumours, carcinoids, small cell lung cancer, brain tumours and human breast tumours [1,2]). Those tumours most frequently express SRIF receptor subtype 2 (sst_2).

The feasibility of targeting radionuclides to SRIF receptors has already been demonstrated by using ^{111}In-[DTPA-DPhe^1]-octreotide (^{111}In-SDZ 215-811, Octreoscan®111) for the imaging of sst_2 expressing tumours [2,3].

For selective radiotherapy of sst_2 expressing tumours a β-emitter labelled octreotide analogue may be targeted to sst_2 expressing tumours, where a cytotoxic radiation dose will be delivered to the tumour cells. Receptor-mediated radiotherapy of SRIF receptor expressing tumours requires a peptide conjugate that consists of the peptide receptor ligand as the targeting moiety and a chelator that binds the radionuclide.

In order to make such radiotherapeutic treatment of SRIF receptor-expressing tumours possible, we synthesized a series of octreotide analogues that were able to tightly chelate beta emitting rare earth metals (e.g.^{90}Y). Among those compounds were [DTPA-benzyl-acetamide-DPhe^1-Tyr^3]-octreotide [4], [DTPA-benzyl-acetamide-DPhe^1]-octreotide, Tyr^3 [DTPA-succinyl-acetamide-Ala-Pro-Phe-DPhe^1-Tyr^3]-octreotide [5], [DOTA-benzyl-acetamido-DPhe^1]-octreotide, [DOTA-benzyl-acetamide-DPhe^1, Thyr^3]-octreotide. Finally, [DOTA-DPhe^1, Thyr^3]-octreotide (SMT 487) [6] was selected among various DOTA coupled octreotide analogues because of its advantageous biodistribution properties and efficacy in tumour models.

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Somatostatin receptor binding and organ distribution

In vitro, both octreotide and SMT 487 bound with subnanomolar affinity to the human SRIF receptor subtype 2 ($hsst_2$). Receptor binding experiments demonstrated that both octreotide (n-10) and SMT 487 (n-3) inhibited the binding of [^{125}I]Tyr^3-octreotide to CA 20948 rat pancreatic tumour membranes in a monophasic manner, with IC_{50}-values for octreotide of 0.22 nM±0.02 and for SMT 487 of 0.39 nm±0.02.

Pharmacokinetics and tissue distribution

Ideally, a radiotherapeutic drug should be rapidly cleared from the blood with $T_{1/2\ \alpha}$<5 min. Long circulation times would result in a high whole body irradiation which may damage the bone marrow, the liver and other healthy, highly vascularized organs. A disadvantage of labelled mAbs (MW in the 10^4-range) is their long circulation times of 48 to 72 h [7]. In contrast, peptides (MW in the 10^3-range) are cleared much faster from the circulation, thus lowering the whole body radiation exposure. The ^{90}Y-SMT 487 is rapidly cleared from the blood with $T_{39\ alpha}$ of 5 min and $T_{41\ beta}$ of 76 min. As a consequence, already two hours post injection of ^{90}Y-SMT 487, the ratio of activity accumulation of tumour-to-normal tissue was high, with tumour/blood 42:1, tumour/muscle 117:1 and tumour/liver 20:1 in AR42J tumour bearing mice.

The radiotherapeutic dose was excreted mainly via the kidneys. A comparably high accumulation of radioactivity in the kidneys was lowered by 60% of the initial value after co-administration of 2 g/kg of the cationic amino acid L-lysine 15 min prior to the radioligand.

The kidney protective doses of L-lysine did not antagonize the anti-tumour effect of ^{90}Y-SMT 487. On the basis of the pharmacokinetic and tissue distribution studies, dosimetric calculations were made and a radiotherapeutically effective dose that yielded 0.4 Gy/h/24 h to the tumour was calculated to be 500 µCi/mouse.

Therapeutic efficacy

Proof-of-concept studies with the SRIF receptor ligand ^{90}Y-SMT 487 were carried out in three different tumour models: AR42J pancreatic tumour bearing nude mice, NCI-H69 human small cell lung cancer bearing nude mice and CA 20948 rat pancreatic tumour bearing normal rats (syngeneic model). The sst_2 shows high homology (>90%) in all three species (mouse, rat, man) [8]. In AR42J tumour bearing nude mice, a single administration of 500 µCi of ^{90}Y-SMT 487 led to a tumour shrinkage by 60% of the initial volume within 10 days. Similarly, ^{90}Y-SMT 487 exhibited a potent inhibitory effect against the sst_2 receptor-expressing NCI-H69 human small cell lung tumours grown in nude mice. A single dose of 600 µCi/mouse resulted in a tumour shrinkage by 50% of the initial volume within 10 days p.i. (Fig. 1). In contrast, tumours in the control group showed rapid growth and the animals had to be sacrificed after three weeks. The tumour shrinkage in mice treated with 600 µCi ^{90}Y-SMT 487 resulted in a prolonged survival time of about three weeks when compared to controls.

The sst_2 receptor density and the high affinity status of the receptors did not change even after multiple treatment cycles. Since no receptor downregulation occurred during radiotherapy with ^{90}Y-SMT 487, a second dose of the radiotherapeutic agent was administered. Both single and repeated treatments with ^{90}Y-SMT 487 resulted in a significant increase in the survival rate as a consequence of tumour shrinkage (personal unpublished observations).

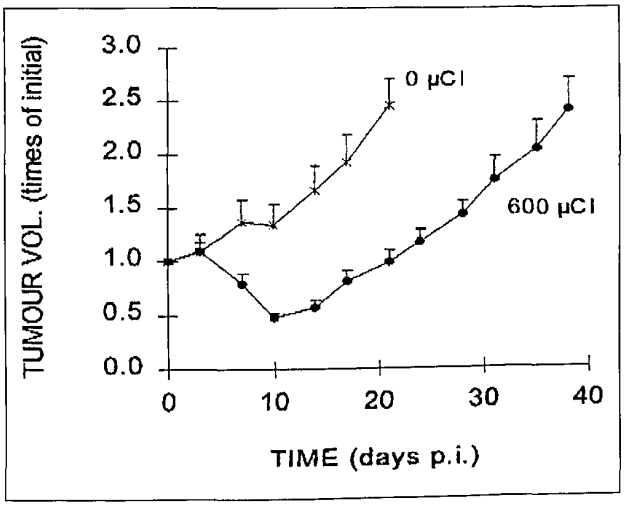

Fig. 1. Radiotherapeutic effect of ^{90}Y-SMT 487 in NCT-H69 human small cell lung cancer xenografts after a single iv injection of 600 µCi ^{90}Y-SMT 487. The initial tumour volume at day 0 was 1203±102 mm^3 (mean±SEM), n=5/group.

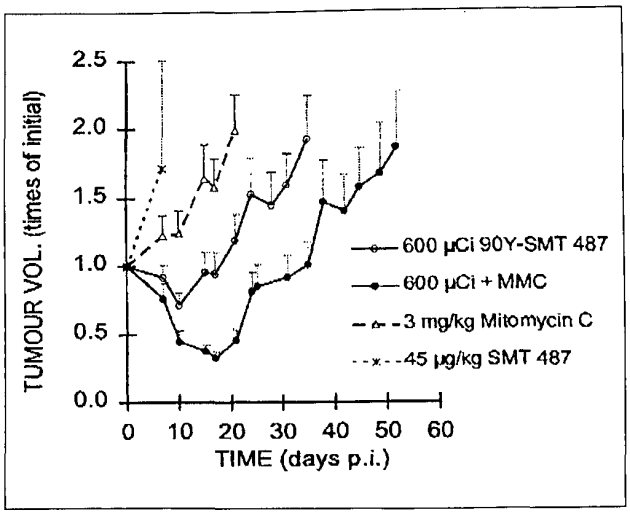

Fig. 2. Radiotherapeutic effect of 600 µCi ^{90}Y-SMT 487 combined with 3 mg/kg mitomycin in C in NCI-H69 human small cell lung cancer xenografts. 45 µg/kg SMT 487 (control), 3 mg/kg mitomycin C and 600 µCi ^{90}Y-SMT 487 were iv injected alone; in one group mitomycin C was injected together with ^{90}Y-SMT 487. The initial tumour volume at day 0 was 1290±81 mm^3 (mean±SEM), n=6/group.

^{90}Y-SMT 487 will most likely be used in combination with existing anticancer drugs which preferably have radiosensitizing properties (e.g. doxorubicin). Therefore, the combination of ^{90}Y-SMT 487 with doxorubicin, 5-fluorouracil and mitomycin-C was tested in the AR42J and the NCI-H69 nude mouse tumour models. The single agent therapy with ^{90}Y-SMT 487 was superior to conventional chemotherapeutic drugs such as doxorubicin, 5-fluorouracil and mitomycin-C. However, the administration of 600 µCi ^{90}Y-SMT 487 in combination with 3 mg/kg mitomycin-C enhanced the extent of tumour shrinkage and resulted in a decrease of 70% of the initial tumour volume in NCI-H69 tumours (Fig. 2) (unpublished data).

The maximum radiotherapeutic affect was achieved in the syngeneic CA20948 rat tumour model[9]. Complete tumour remission was obtained in 5 out of 7 rats, 8 weeks after a single administration of 10 µCi/kg of ^{90}Y-SMT 487. No regrowth occurred during the subsequent observation period (8 months). Upon re-inoculation of tumour material into the cured rats, in 5 out of 7 no tumour growth occurred, whereas in those rats exhibiting tumour growth the tumour disappeared after three to four weeks without any further treatment of the animals. The latter result may indicate a possible immune mediated affect of ^{90}Y-SMT 487. This hypothesis is subject to further research.

Conclusions

The preclinical rationale for the use of ^{90}Y-octreotide analogues for the treatment of sst_2 expressing tumours is based on in vitro and in vivo evidence of a direct antiproliferative effect resulting in tumour regression. The concept of using an ^{90}Y labelled octreotide for the treatment of malignant neoplasms was proven preclinically in sst_2 expressing rodent tumour models. This new radiotherapeutic drug combines several important prerequisites for an effective tumour treatment with targeted radiolabelled peptides. Most importantly, ^{90}Y-SMT 487 has the potency of causing complete tumour remission upon a single treatment as shown, in a syngeneic rat tumour model. Receptor targeted radiotherapy with ^{90}Y-SMT 487 represents a new strategy for the treatment of sst_2 expressing tumours that have previously been diagnosed with OctreoScan111. Together the three compounds, octreotide, the diagnostic octreotide derivative OctreoScan111 and the radiotherapeutic octreotide analogue ^{90}Y-SMT 487, will be complementary in the diagnosis and treatment of sst_2 expressing tumours, especially those of neuroendocrine origin. Clinical Phase I studies with ^{90}Y-SMT 487 are ongoing.

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