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An easy-to-use imaging tool and radiopharmaceutical agent derived from CCK_4 for internal radiotherapy: Synthesis and assessment of an original biovector

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Abstract

S-EOE-MAG_3-β-alanine-CCK_4 vector (S-EOE-MAG_3-β-alanine: S-(1-ethoxyethyl) mercaptoacetyltriglycyl -β-alanine and CCK_4: Trp-Met-Asp-Phe-NH_2) was obtained by successive condensations of activated amino acids on resin with high chemical purity. This biovector was complexed at high dilution scale with ^{99m}Tc as well as at ponderable level with $^{185/187}$Re and ^{99}Tc. In particular, this biovector was efficiently labelled with ^{99m}Tc (yield $>95\%$) in one step under pH 7–8 and 45 °C conditions and then injected, In vivo, for biodistribution and targeting of pancreatic adenocarcinoma tumoral grafts (AR4-J). ^{99m}Tc-MAG_3-β-alanine-CCK_4 exhibits remarkable biological properties related to its low background noise resulting from the fast blood clearance (tissular distribution half-life $\approx$10 min, elimination half-life $\approx$ 20 min), low hepatic uptake (0.03% injected dose/g of tissue 2 h after injection) and good affinity for the CCKB receptors ($IC_{50} = 8 \times 10^{-8}$ M) expressed by tumoral cells.

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Keywords: Technetium; Rhenium; Tetragastrin; CCK4; Biovector

1. Introduction

Cancer constitutes one of the first causes of mortality worldwide. This proliferative disease is characterized by two evolutionary components consisting of regional extension and of metastasis diffusion. The success of cancer therapeutic approaches depends upon early diagnosis, extension assessment and initial treatment of the primitive tumor and its metastatic dissemination. However, a central issue remains drug delivery to tumors because cancer proliferation and metastasis relies on complex interactions between tumor cells and the host and is often resistant to all therapeutic modalities. Investigating new tumor cell targets and increasing understanding of the pathways that control tumor growth and progression represent the basis for developing new treatments. In particular, the development of targeted therapies is providing a new paradigm in the management of cancer, better efficacy while minimizing toxicity being an achievable goal. The work presented here is part of the French National Cancer Fighting Plan as illustrated in South West France with the founding of the ''Canceropole''. In tumoral targeting, biomolecular vectors complexed with two different isotopes (photon and particle emitters) seem to be a suitable strategy. Indeed, technetium-99 m (γ: 140 keV, $T_{\frac{1}{2}} = 6$ h) can be used for diagnosis and rhenium-186/188 for radiotherapy (^{186}Re β: 1.07 MeV, $T_{\frac{1}{2}} = 90.64$ h and ^{188}Re β: 2.11 MeV, $T_{\frac{1}{2}} = 16.97$ h) and cold rhenium-185/187 for chemotherapy [1,2]. The tetragastrin (CCK_4: Trp-Met-Asp-Phe-NH_2), an effector related to gastrin, exhibits specific affinity for CCKB receptors which are preferentially expressed by some tumoral cells in digestive tract [3]. The development of an easy-to-use imaging tool requires radiometalic complexation method

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with high purity under mild pH and temperature conditions in order to avoid biovector peptide denaturation. The aims of this work are (i) synthesis of a β-Ala-CCK$_4$ biovector coupled to SN_3 ligand and (ii) development of technetium and rhenium complexation method using physiological pH and temperature conditions and (iii) pharmacological in vitro and in vivo studies of these labelled vectors.

2. Experimental section

2.1. Chemistry

S-EOE-MAG$_3$-β-alanine-CCK$_4$ vector has been synthesised according to Belhadj-Tahar method [3] consisting in successive condensations of activated amino acids on resin followed by the coupling of S-(1-ethoxyethyl) mercaptoacetyltriglycyl acid (S-EOE-MAG$_3$-β-alanine-CCK$_4$) (Fig. 1).

Synthesis of S-(1-ethoxyethyl) mercaptoacetyltriglycyl acid (S-EOE-MAG$_3$) was described by Brandau and Belhadj-Tahar teams [4,5].

Synthesis of S-EOE-MAG$_3$-β-alanine-CCK$_4$: 0.23 mM of resin were solubilized with 0.55 ml of piperidine and 2.2 ml of *N*,*N*-dimethylformamide (DMF), evaporated in vacuum and then washed by DMF and *N*-methylpyrrolidone (NMP). The activated resin was incubated with a mixture composed of 1.15 mM of protected amino acid (AA-Fmoc) in 2 ml of NMP, 0.2 ml of *N*,*N*-diisopropylcarbodiimide (DIPC) and 1.04 mM of *N*-hydroxybenzotriazole (HOBT). After 9 h agitation, the excess of unreacted amino acid was eliminated by several washings with NMP. The procedure was repeated five times successively with each new amino acid (Asp; Met; Trp; Ala, S-EOE-MAG$_3$). Final peptide was obtained after cleaving resin by a mixture of TFA (94%)/H_2O (2.5%)/ethanedithiol (2.5%)/triisopropylsilane (1%) then filtered and washed with TFA and finally lyophilised.

^{1}H RMN (S-EOE-MAG$_3$-β-alanine-CCK$_4$ in $CDCl_3$); 3.4 (t, CH_3–CH_2-), 3.7 (d, CH_3–CH–), 4.2–4.4 (m, –CH_2), 4.8–5 (m, –CH–).

2.2. Radiochemistry

Labelled biovectors were characterised and compared by means of HPLC on reverse phase C_{18} column LiChrospher 60 RP-select B (125-4; 5 μm) eluted with methanol/H_2O/trifluoroacetic acid (TFA) (30/70/0.01) at a flow rate of 1 ml/min, detection used sodium iodide radiodetector and UV detector.

2.2.1. Synthesis of technetium complex

This method has already been described by our team [6] as follows: typically, 25 μl of $SnCl_2$ at 10^{-2} M were added to 400 μl buffered solution (pH 8) of biovector at 0.1mM (≈0.1 mg/ml) and pertechnetate ($^{99m}TcO_4Na$ 74 Mbq or 0.1 mM de $^{99}TcO_4K$). The reaction mixture was incubated during 20 min at 40 °C.

Radiochemical purity control of complex was determined by TLC chromatography on reversed phase of C_{18} plates eluted with a mixture of methanol (60%)/H_2O (40%)/TFA(0.1%).

The stability control of labelled biovector was carried out as follows: the purified ^{99m}Tc-complex was incubated during 4 h at 37 °C with (i) serum (Calbiochem), (ii) or physiological solution only (iii) or with cysteine, HCl (6.10^{-3} M). The TLC analysis allows us to determine the constant of stability that is expressed in terms of activity ratio related to the complex peak versus the total activity, corresponding to the sum of complexed and hydrolized forms.

2.3. Synthesis of rhenium complex

The labelled biovector at ponderable scale was obtained through ligand exchange reaction [2]:

(a) trichlorobis(triphenylphosphine)rhenium (V) ($ReOCl_3$)(PPh_3)$_2$ was synthetised according to Parshall protocol [7]: 30 mM of potassium perrhenate was dissolved in 250 ml of ethanol and 50 ml of hydrochloric acid at 12 N. The solution was heated under reflux and 170 mM of triphenylphosphine were added. The yellow precipitate was filtered and redissolved in 100 ml of ethanol; then, the complex was extracted with 250 ml of boiling toluene.
(b) the free biovector (0.2 g, 0.2 mM) was incubated with ($ReOCl_3$)(PPh_3)$_2$ (0.18 g, 0.2 mM) in ethanolic solution of $KOC(CH_3)_3$ (0.05 g, 0.4 mM). The mixture was heated overnight, then the volatile solvent was evaporated in vacuum and the residue washed with boiling ether.

^{1}H ($CDCl_3$): 2.9 (t, –CH_2–CH_2–CO–); 3.2 (m, –NH–CH_2–CH_2–); 3.35 (s, –N–CH_2–CO–); 8 (t, –CO–NH–CH_2–).

2.4. Pharmacology

In vitro: labelled biovectors were incubated with CCKB cell receptors and their affinity was tested by tetragastrin competition according to protocol described elsewhere [8].

In vivo*:* (for pharmacological study) ^{99m}Tc-MAG$_3$-β-alanine-CCK$_4$ was injected into Wistar rats and nude mice with pancreatic adenocarcinoma tumoral grafts (AR4-J) were used for tumoral targeting.

3. Results and discussion

The S-EOE-MAG$_3$-β-alanine-CCK$_4$ vector was obtained with high chemical purity and was efficiently labelled by $^{99/99m}$Tc (yield > 95%) in one step under pH 7–8 and 40 °C conditions. Re complexes were obtained with high yield,

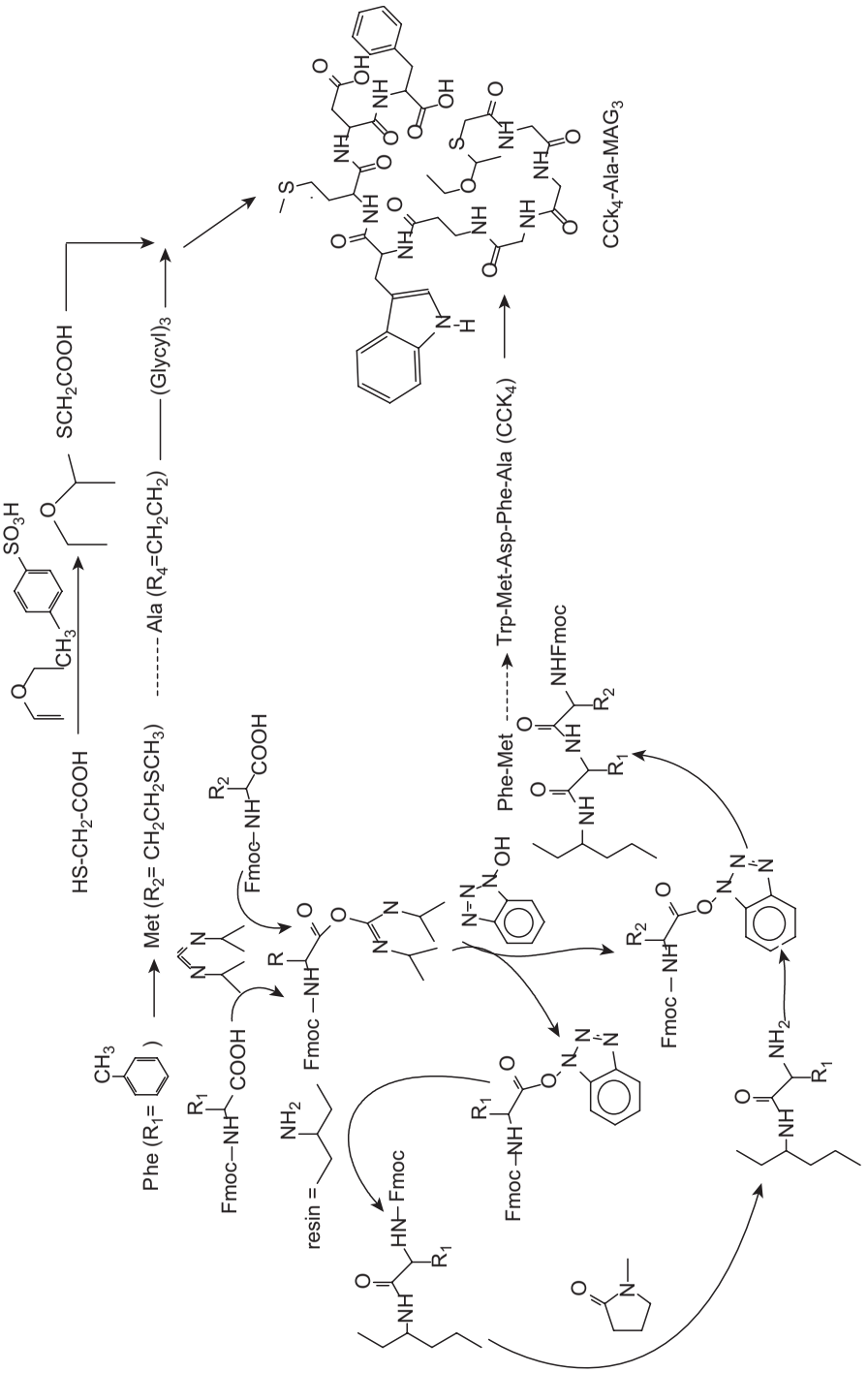

Fig. 1. Synthesis of MAG_3-β-ala-CCK_4.

through exchange ligand reaction, based on an intermediary complex ($(ReOCl_3)(PPh_3)_2$) and exhibits the same physiochemical properties as ^{99m}Tc-complex, as demonstrated by HPLC comparative studies (Fig. 2). These labelled compounds are stable at high dilution rates in the presence of oxygen in plasma or cystein-added medium since the stability constants were above 90%.

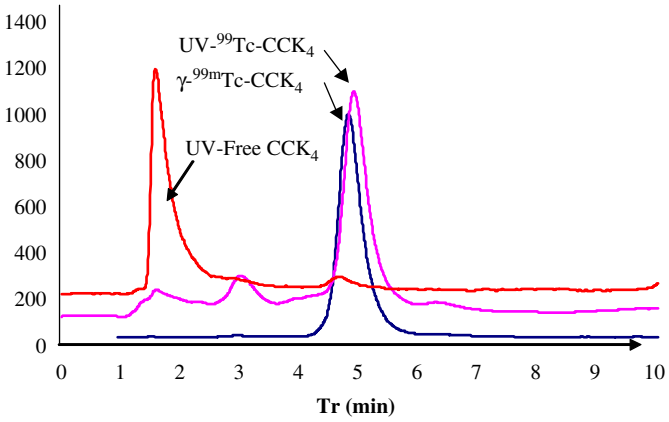

Fig. 2. HPLC patterns of the [$^{99/99m}$Tc]-MAG$_3$-β-ala-CCK$_4$.

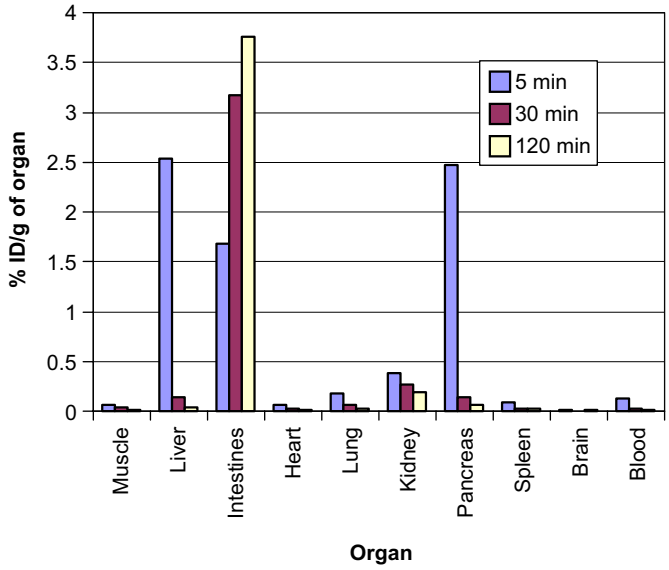

Fig. 3. Biodistribution of ^{99m}Tc-MAG$_3$-ala-CCK$_4$ in the rat.

These studies carried out in vitro with ^{99}Tc-MAG$_3$-β-Ala-CCK$_4$ revealed good affinity for the CCKB receptors ($IC_{50} = 8 \times 10^{-8}$ M) in connection with its in vivo persistent tumoral sequestration of ^{99m}Tc-biovector. The biodistribution shows significant contrast between the subcutaneous tumoral graft and background noise of border tissues as well as, at early vascular time (at 5 min: stroma vascular blood 0.13 ± 0.03 vs. muscle 0.06 ± 0.01 ID/g of tissue) as after diffusion time (at 2 h. tumor: 0.05 ± 0.02 vs. muscle: 0.01 ± 0.01 ID/g of tissue).

Besides, these biovectors present remarkable pharmacokinetic properties related to low background noise resulting from the lack of hepatic uptake (Fig. 3). Indeed, on the one hand the blood clearance of ^{99m}Tc-vector is fast (tissular distribution half-life $\approx$10 min, elimination half-life $\approx$20 min) and on the other hand hepatic and muscular uptakes decrease quickly to reach, respectively, 0.03% and 0.01% ID/g, 2 h after injection. However, the rate of intestinal uptake is significant and increases with time (1.68% ID/g at 5 min and 3.76% ID/g at 120 min) related to tropism of alanine-CCK$_4$ toward the physiological receptors (Table 1).

4. Conclusion

This result allows us to conclude that linking the SN_3 chelating agent to CCK$_4$ does not alter peptide conformation (IC_{50} pentagastin $= 10^{-9}$ M versus IC_{50} ^{99}Tc-MAG$_3$-β-ala-CCK$_4$ $= 8.10^{-8}$ M). In vivo, this biovector exhibits interesting pharmacokinetic properties because of the low background noise due to the lack of hepatic uptake and fast blood clearance. Thus, metallic-MAG$_3$-β-Ala-CCK$_4$ could be used as ^{99m}Tc photon emitter probe for in vivo density receptor quantification as well as ^{99}Tc/$^{185/187}$Re in vitro pharmacological tool in CCKB binding studies.

Table 1
Biological distribution of ^{99m}Tc-MAG$_3$-ala-CCK$_4$ in the rat

	% injected dose/organ			% injected dose/g of organ		
	5 min	30 min	120 min	5 min	30 min	120 min
Muscle	18.30±2.16	9.49±2.76	2.33±20.9	0.06±0.01	0.03±0.01	0.01±0.01
Liver	31.82±0.88	1.83±0.05	0.55±0.11	2.54±0.02	0.14±0.02	0.03±0.01
Intestines	42.46±1.76	85.16±0.10	91.00±1.92	1.68±0.10	3.17±0.08	3.76±0.68
Heart	0.08±0.01	0.02±0.00	0.01±0.00	0.07±0.01	0.02±0.00	0.01±0.00
Lung	0.31±0.08	0.11±0.01	0.05±0.05	0.18±0.04	0.07±0.01	0.03±0.02
Kidney	1.12±0.18	0.63±0.05	0.46±0.19	0.38±0.13	0.27±0.01	0.19±0.07
Pancreas	2.73±0.61	0.15±0.02	0.06±0.01	2.47±0.81	0.14±0.02	0.06±0.02
Spleen	0.07±0.02	0.03±0.02	0.03±0.02	0.09±0.03	0.03±0.03	0.03±0.02
Brain	0.02±0.01	0.01±0.00	0.01±0.01	0.01±0.01	0.01±0.00	0.01±0.00
Blood	3.02±0.73	0.52±0.04	0.09±0.09	0.13±0.03	0.02±0.00	0.01±0.00

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