

Comparative Biodistribution Profile of [¹³¹I]VIP and [¹³¹I]VIP10⁻²⁸

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ABSTRACT

Various tumor cells express significantly higher amounts of VIP receptors (VIPR) that provided the basis for the clinical use of radiolabeled VIP for the in vivo localization of tumors. This work studied the labeling of VIP and VIP10-28 with iodine-131 to compare the biological distribution of the labeled compounds in Nuce mice and the affinity for tumor cells. Both VIP and VIP10-28 peptides contain two tyrosine residues, in positions 10 and 22, that are theoretically equally susceptible to radioiodination employing oxidative electrophilic substitution using oxidizing agents like Chloramine T. Radiochemical purity of the reaction mixture was determined by electrophoresis and HPLC. The VIP peptide and the fragment were labeled with radioiodine with good radiochemical yield (above 96%). Suitable, but important differences can be observed in biological distribution studies. Comparatively, blood clearance was faster for labeled VIP and perhaps because of this, the uptake in tumor was lower, especially during the first hour. These differences observed in the biological distribution of the compounds can be related to the lipophilicity of the labeled compounds.

Key words: Radiopharmaceuticals, protein radioiodination, VIP, VIP10⁻²⁸, adenocarcinomas

INTRODUCTION

Vasoactive Intestinal Peptide (VIP) is a 28-amino acid peptide. Various tumor cells express significantly higher amounts of VIP receptors that provide the basis for the clinical use of radiolabeled VIP for the *in vivo* localization of adenocarcinomas, breast cancer, melanomas, neuroblastomas and pancreatic carcinomas (Hessenius, C., et al., 2000, Reubi, J.C., et al., 1999, Virgolini, I., et al., 1994).

VIP10-28 is a fragment of the VIP peptide that preserves the amino acid sequence responsible for the interaction with the specific receptors. Both VIP and VIP10-28 peptides contain two tyrosine residues, in positions 10 and 22, that are

theoretically equally susceptible to radioiodination employing oxidative electrophilic substitution using oxidizing agents like Chloramine T.

This work studied the labeling of VIP and VIP10-28 with iodine-131 to compare the biological distribution of the labeled compounds in Nuce mice and the affinity for tumor cells.

MATERIAL AND METHODS

Labeling of VIP and VIP10-28 with 131-iodine

To the peptide VIP or VIP10-28 (Sigma), in a reaction vial (25 µg/20 µL 0.2 M phosphate buffer pH 7.5) were added 10 µL [¹³¹I]Na (Nordion) (555-666 MBq) and 5 µL of chloramine T solution

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(1 mg/mL). The reaction occurred during one minute at room temperature with gentle stirring and was terminated by the addition of 5 μ L of sodium metabisulfite (2 mg/mL).

Radiochemical purity analysis

Radiochemical purity of the reaction mixture was determined by electrophoresis (Amersham-Pharmacia) on Whatman No. 1 paper with 0.05 M sodium barbital buffer, pH 8.6, using 300V for 40 minutes. The electrophoresis profile of the [131 I]Na solution was also determined under the same conditions.

The identification of the radiochemical species in the reaction mixture was performed on a high-performance liquid chromatography system (HPLC), column RP-C18 (5 μ m, 4.6x 250 mm, Waters) eluted isocratically with acetonitrile/0.1% aqueous trifluoroacetic acid solution (TFA) with a flow rate of 0.5 mL/minute.

Biological distribution

Biological distribution studies were developed in Nude mice. All animals presented a tumor mass in the dorsal region, developed by the inoculation of 5×10^5 cells of human colon adenocarcinoma, recuperated from the culture medium in 0.1 mL of PBS. The animals were used in the experiments 7-10 days after the cell inoculation, when the tumor mass was approximately 5 mm in diameter.

The animals received the radiopharmaceutical, [131 I]VIP ou [131 I]VIP10-28 (0.50MBq/0.1mL) by intravenous administration and, after 1, 4 and 24 hours, each group (5 animals/group) was sacrificed and the tumor mass and organs of interest were removed, washed, weighed and counted by radioactivity determination in a gamma counter (Packard). Blood samples were collected (0.1 mL) using a capilar from the coroid plexus

RESULTS

The radiochemical purity of labeled mixtures employed in biological studies was superior to 96%. The electrophoresis profile of the VIP and VIP10-28 labeling mixtures and radioiodine is presented in Fig. 1. A good separation of labeled peptide species from free radioiodine was obtained.

The radioactive HPLC profile for the VIP labeling mixture is presented in Fig. 2. The peak with Rt 5.3 minutes corresponds to the free radioiodine. Four peaks correspond to the VIP radioiodinated species. The results of biological distribution of [131 I]VIP and [131 I]VIP10-28 are presented on Tables 1 and 2, respectively. Table 3 considered the tumor/blood ratio (% dose/g tumor/% total blood) for the compounds.

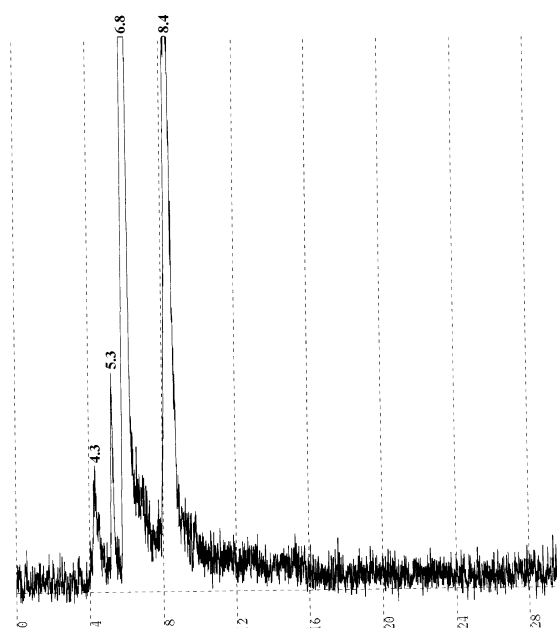


Figure 1 - Electrophoresis profile of [131 I]VIP, [131 I]VIP10-28 and [131 I]Na

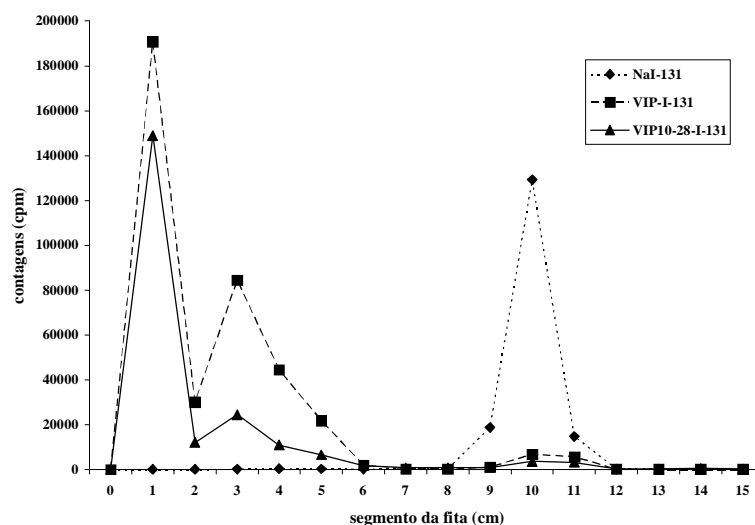


Figure 2 - HPLC profile of [¹³¹I]VIP

Table 1 - Biodistribution of [¹³¹I]VIP in *Nude* mice with tumor

Organs	%dose/organ			%dose/gram		
	Time (hours)					
	01	04	24	01	04	24
Brain	0.13 ± 0.02	0.03 ± 0.01	0.04 ± 0.01	0.25 ± 0.02	0.09 ± 0.02	0.09 ± 0.02
Thyroid	3.37 ± 0.35	5.63 ± 0.72	7.04 ± 0.84	-	-	-
Lung	0.56 ± 0.08	0.21 ± 0.07	0.08 ± 0.01	4.11 ± 0.69	0.76 ± 0.07	0.27 ± 0.01
Heart	0.21 ± 0.05	0.05 ± 0.01	0.02 ± 0.01	1.53 ± 0.32	0.53 ± 0.03	0.07 ± 0.01
Splen	0.29 ± 0.03	0.08 ± 0.01	0.03 ± 0.01	2.04 ± 0.27	0.59 ± 0.02	0.34 ± 0.01
Liver	2.32 ± 0.15	0.08 ± 0.01	0.14 ± 0.02	1.54 ± 0.01	0.67 ± 0.01	0.10 ± 0.02
Stomach	0.94 ± 0.03	0.77 ± 0.13	0.03 ± 0.01	6.92 ± 1.29	1.53 ± 0.06	0.16 ± 0.04
Muscle(1g)	-	-	-	0.41 ± 0.04	0.22 ± 0.01	0.10 ± 0.02
Total Muscle	4.14 ± 0.36	1.78 ± 0.02	1.34 ± 0.24	-	-	-
Kidneys	2.25 ± 0.51	0.72 ± 0.09	0.13 ± 0.03	5.95 ± 0.78	2.52 ± 0.14	0.37 ± 0.03
Fine intestine	6.39 ± 0.85	0.72 ± 0.09	0.20 ± 0.04	2.96 ± 0.32	1.51 ± 0.24	0.13 ± 0.02
Large intestine	2.72 ± 0.39	2.35 ± 0.37	0.13 ± 0.02	2.70 ± 0.41	1.81 ± 0.12	0.09 ± 0.01
Total blood	6.22 ± 0.98	1.77 ± 0.06	0.33 ± 0.05	-	-	-
Tumor	2.34 ± 0.05	1.51 ± 0.09	0.04 ± 0.01	2.71 ± 0.27	0.63 ± 0.04	0.11 ± 0.02

N=5

Table 2 - Biodistribution of [¹³¹I]VIP10-28 in *Nude* mice with tumor

Organs	%dose/organ			%dose/gram		
	Time (hours)					
	01	04	24	01	04	24
Brain	0.31 ± 0.04	0.21 ± 0.05	0.01 ± 0.00	0.72 ± 0.05	0.41 ± 0.08	0.03 ± 0.01
Thyroid	3.53 ± 0.62	6.53 ± 0.71	6.41 ± 0.54	-	-	-
Lung	1.42 ± 0.30	1.56 ± 0.45	0.02 ± 0.00	10.91 ± 0.06	5.78 ± 0.39	0.16 ± 0.03
Heart	0.33 ± 0.02	0.22 ± 0.02	0.01 ± 0.00	4.54 ± 0.94	3.03 ± 0.41	0.14 ± 0.01
Splen	0.37 ± 0.07	0.24 ± 0.03	0.01 ± 0.00	8.67 ± 0.33	6.05 ± 0.98	0.24 ± 0.01
Liver	4.50 ± 0.20	3.40 ± 0.33	0.09 ± 0.01	4.71 ± 0.30	3.90 ± 0.55	0.07 ± 0.00
Stomach	4.90 ± 0.01	2.37 ± 0.01	0.03 ± 0.01	36.53 ± 1.34	19.84 ± 0.18	0.17 ± 0.01
Muscle(1g)	-	-	-	3.41 ± 0.76	2.15 ± 0.03	0.08 ± 0.01
Total Muscle	18.13 ± 0.94	14.41 ± 0.52	0.47 ± 0.03	-	-	-
Kidneys	1.66 ± 0.29	1.55 ± 0.06	0.03 ± 0.01	7.16 ± 0.58	6.08 ± 0.57	0.22 ± 0.01
Fine intestine	7.86 ± 0.03	8.24 ± 0.40	0.13 ± 0.01	7.81 ± 0.82	8.62 ± 0.21	0.15 ± 0.01
Large intestine	3.68 ± 0.14	2.95 ± 0.76	0.06 ± 0.01	5.36 ± 0.28	4.75 ± 0.01	0.09 ± 0.01
Total blood	15.69 ± 0.25	9.05 ± 0.78	0.19 ± 0.01	-	-	-
Tumor	4.04 ± 0.73	2.11 ± 0.01	0.01 ± 0.00	11.37 ± 0.76	4.51 ± 0.25	0.05 ± 0.01

N=5

Table 3 - Tumor/blood ratio (%dose/g tumor/%total blood) at different time after dose administration

Time (hours)	%dose/g tumor/%total blood	
	[¹³¹ I]VIP	[¹³¹ I]VIP10-28
1	0.44	0.72
4	0.36	0.49
24	0.33	0.26

DISCUSSION

The VIP peptide and the fragment were labeled with radioiodine with good radiochemical yield. In the electrophoresis profile, two peaks that correspond to the labeled peptide, probably the di-iodinated form (the first peak) and the mono-iodinated form (the second peak), can be seen. The multiple species of radioiodinated VIP identified by HPLC are also probably related to the mono and di-iodinated peptide. Furthermore, the methionine residue in position 17 of VIP molecule is susceptible to oxidation to methionine sulfoxide by the oxidizing agent employed and can form two corresponding species (Marie, J.-C. et al., 1985, Martin, J.-L. et al., 1986). The HPLC profile of the fragment is similar that of the VIP.

Suitable, but important differences can be observed in biological distribution studies. The uptakes in thyroids were relatively low, thus showing the stability of the compounds towards the *in vivo* dehalogenation. Part of the thyroid uptake could be related to the free radioiodine present in the preparation (about 4%) because the labeled compounds were not previously purified. The uptakes by the stomach and intestines were high, particularly for [¹³¹I]VIP10-28.

Comparatively, blood clearance was faster for labeled VIP, and, perhaps because of this, the uptake by the tumor was lower, especially in the first hour. These differences observed in the biological distribution of the compounds can be related to the lipophilicity of the labeled compounds. The introduction of one iodine atom in the peptide molecule increases the lipophilicity.

The di-iodinated form of the peptide is already more lipophilic and presents a slower blood clearance. One can suppose that the fragment of VIP is more susceptible to the formation of the di-iodinated form of the peptide (as evidenced in electrophoresis profile). Considering the tumor/blood ratio (Table 3), the better target for back-ground ratios was obtained one hour after the administration of the dose to the labeled VIP and VIP10-28, but the retention of the peptide in the tumor was greater in the case of VIP, as observed at 4 and 24 hours after the administration of the dose.

Although the literature considers that the interactions of the VIP10-28 with VIP receptors are preserved (Blok, D. et al., 1999), the introduction of an iodine atom, especially with the production of the di-iodinated form of the peptide, interfered in the kinetic and tumor uptake of this radiopharmaceutical compared to the labeled VIP.

RESUMO

Várias células tumorais expressam significativamente uma alta quantidade de receptores VIP (VIPR) que determinam a base para o uso clínico de VIP radiomarcado para localização de tumores *in vivo*. Foi estudado neste trabalho a marcação do VIP e do fragmento VIP10-28 com iodo-131 comparando a distribuição biológica dos compostos marcados em camundongos *Nude* e sua afinidade pelas células tumorais. Ambos os peptídeos, VIP e VIP10-28, contém dois resíduos de tirosina nas posições 10 e 22, que teoricamente são igualmente susceptíveis pela substituição eletrofílica oxidativa do radioiodo utilizando Cloramina T como agente oxidante. A pureza radioquímica da mistura de reação foi determinada por eletroforese e cromatografia líquida de alta eficiência (CLAE). O VIP e fragmento foram marcados com radioiodo com bom rendimento radioquímico (superior a 96%). Importantes diferenças foram observadas nos estudos de distribuição biológica. Comparativamente, o clareamento sanguíneo foi mais rápido para VIP marcado e por esta razão, a captação no tumor foi inferior, especialmente na primeira hora. Estas diferenças observadas na distribuição biológica dos compostos podem estar relacionadas com a lipofilicidade dos compostos marcados.

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