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Direct labeling of chemotactic peptide ForNIeLFNIeYK with radioiodine -in vivo- stability evaluation.

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Abstract

Some peptides are naturally occuring inflammatory mediators which specifically bind to receptors abundantly present in the area of inflammation, and owing to their small size, they rapidly clear from all non-target tissues. ForNIeLFNIeYK is a synthetic chemotactic peptide with high affinity to receptors on the white blood cell membranes. This hexapeptide contains a tyrosine residue susceptible to iodination by oxidative eletrophilic substitution - direct labeling. The aim of this study was the radioiodination of ForNIeLFNIeYK using the direct method (chloramine T) and its in vivo stability evaluation. The labeled compound was obtained in a short reaction time with high radiochemical purity (96.8 ± 0.84%) and remained stable over 48 hours when stored at low temperature. Biological distribution studies showed an uptake in inflammated tight significantly greater than the normal tight ($p < 0.05$, Student t test), and some *in vivo* dehalogenation of the compound.

Keywords: Radioiodination; chemotactic peptide; direct labeling; peptide ForNIeLFNIeYK

Introduction

Various conventional radiopharmaceuticals are currently available for scintigraphic imaging of infection and inflammation. Although a wide variety

of infectious and inflammation focus can be detected with these agents, several disadvantages limit their application. These limitations have stimulated the search for new radiopharmaceuticals. Nature has designed peptides to stimulate, inhibit or regulate many body functions and the development of radiolabeled peptide-based radiopharmaceuticals for imaging a variety of tumors, infection/inflammation and thrombus has introduced a new era in nuclear medicine (1,2,3).

Some peptides are naturally occuring inflammatory mediators which specifically bind to receptors abundantly present in the area of inflammation. In addition, owing to their small size, they rapidly clear from all non-target tissues. For-MLF is a bacterial product that iniciates leukocyte chemotaxis by binding to high affinity receptors on the white blood cell membranes. These receptors are present on both polymorphonuclear leukocytes (PMNs) and mononuclear phagocytes. There are many synthetic analogs with equal or greater affinity compared to the native peptide. ForNIeLFNIeYK is one of these synthetic analogs and this hexapeptide contains one tyrosine residue susceptible to iodination by oxidative electrophilic substitution (1,4,5,6,7,8).

Radioiodination of peptides and proteins is well established. The most common procedure is the *in situ* production of electrophilic radioiodine species with functional groups on a native protein, referred to as "direct" labeling. These reactions require the production of the electrophilic radioiodine through oxidation of radioiodide ions by suitable oxidants (chloramine-T, iodogen or enzymatically by lactoperoxidase) and involves the substitution of the iodine ortho to a hydroxyl group on an aromatic ring (activated phenolic ring of tyrosine residue of the protein) (9,10).

One of the major problems in the use of radioiodinated proteins in vivo is their deiodination that may occurs by the action of specific enzymes, probably because of the structural similarity between these iodophenyl groups and thyroid hormones (9,10,11).

The aim of this work was to study the radioiodination of the peptide ForNIeLFNIeYK using direct method (chloramine T) and verify the in vivo stability of this labeled compound and its specifity by inflammation focus.

Materials and Methods

Synthetic ForNIeLFNIeYK, dimethylformamide (DMF), chloramine T, sodium methabisulfite, trifluoracetic acid (TFA) and acetonitrile were purchased from Sigma-Aldrich; [^{131}I] NaI solution was obtained from Nordion in pH 11 sodium hydroxide and processed at IPEN-CNEN/SP. *Swiss* mice were obtained from IPEN-CNEN/SP biotery.

Peptide radioiodination

The synthetic ForNIeLFNIeYK (25 µg dissolved in 5 µL of DMF) was placed in a conical vial followed by addition of 5 µL of chloramine T (1,0 mg/mL DMF) and 10 µL of [^{131}I] NaI solution (3.7 - 7.4 MBq). The reaction was conducted at room temperature and gentle stirring for 10 minutes. The reaction was terminated by the addition of 5µL of sodium methabisulfite solution (2,0 mg/mL distilled water). Radiochemical purity of labeled peptide was determined by horizontal zone electrophoresis (Whatman 1 paper, 0.05M barbital buffer, pH 8.6, 295V for 40 minutes) and by HPLC using a C18 column 4.6 x 150 mm (Dionex) eluted with acetonitrile: TFA 0,1% (35:65), 220nm, and 1mL/min flux.

Biological distribution studies

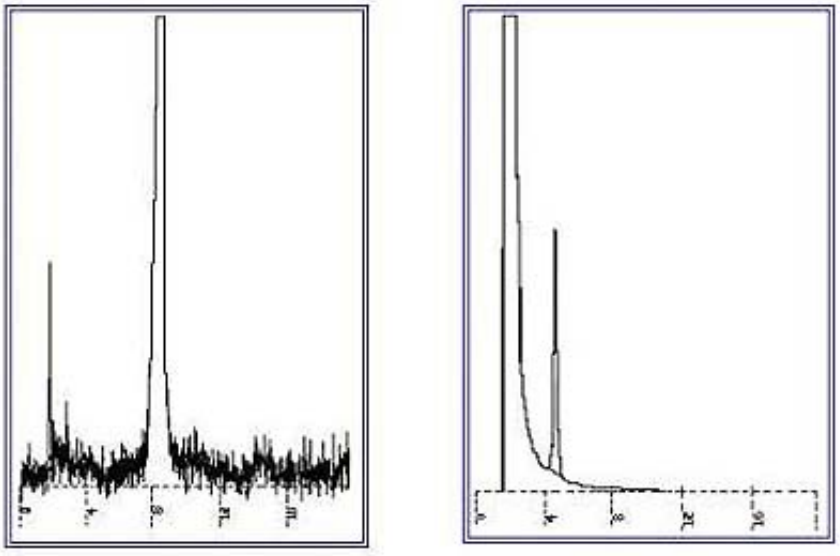
The biodistribution studies were developed in a group of normal Swiss mice

(control group) and a group of Swiss mice with turpentine promoted inflammation focus developed on the right thigh. Eighteen animals of each group received 0.48 - 0.55 MBq/100µL of the labeled FNIeLFNIeYK both intravenously via tail vein. Blood samples were collected by an orbital bleed 1, 4 and 24 hours after the dose administration and the animals were sacrificed, the tissues of interest removed, washed, weighed and counted for ^{131}I activity using a gamma counter. The percent injected dose for each tissue was calculated by comparing the activity in each tissue to injection standards of suitable count rate.

Results

Radiochemical purity of FNIeLFNIeYK determined by electrophoresis was 96.8 ± 0.84 % (N=6). Figure 1 shows the HPLC profile of the labeled mixture.

Figure 1.
HPLC profiles of the labeled mixture - left: radioactive chromatogram - Rt (free iodine) = 1.7 minutes; Rt (radiolabeled peptide) = 8.3 minutes; righ: UV chromatogram - Rt (peptide) = 4.7 minutes.



Tables 1 and 2 and figures 2 and 3 show the results obtained in biological distribution studies.

Table 1.
Percent dose/organ of radioiodinated FNIeLPNIeYK in normal *Swiss mice*.

ORGANS	% Administered dose /organ		
	1 hour	4 hours	24 hours
Brain	0.195 ± 0.044	0.196 ± 0.056	0.064 ± 0.053
Thyroid	4.954 ± 0.733	13.965 ± 3.275	20.156 ± 3.788
Lung	2.719 ± 1.048	1.690 ± 0.344	0.232 ± 0.147
Heart	0.317 ± 0.058	0.332 ± 0.094	0.043 ±0.019
Spleen	1.427 ± 0.288	0.766 ± 0.453	0.103 ±0.043
Liver	12.525 ± 1.431	5.082 ± 0.903	0.635 ± 0.253
Stomach	8.997 ± 2.758	9.620 ± 2.970	0.998 ±0.940

Total muscle	18.303 ± 2.505	14.815 ± 1.604	2.254 ± 1.380
Kidney	1.513 ± 0.340	1.706 ± 0.541	0.168 ±0.101
Small Intestine	9.059 ± 1.937	10.708 ± 2.021	1.313 ± 0.556
Large Intestine	5.147 ± 1.096	8.027 ± 2.155	1.994 ± 0.875
Total Blood	11.560 ± 1.178	11.296 ± 1.011	1.332 ± 0.759

Figure 2.
Percent dose/organ in normal Swiss mice

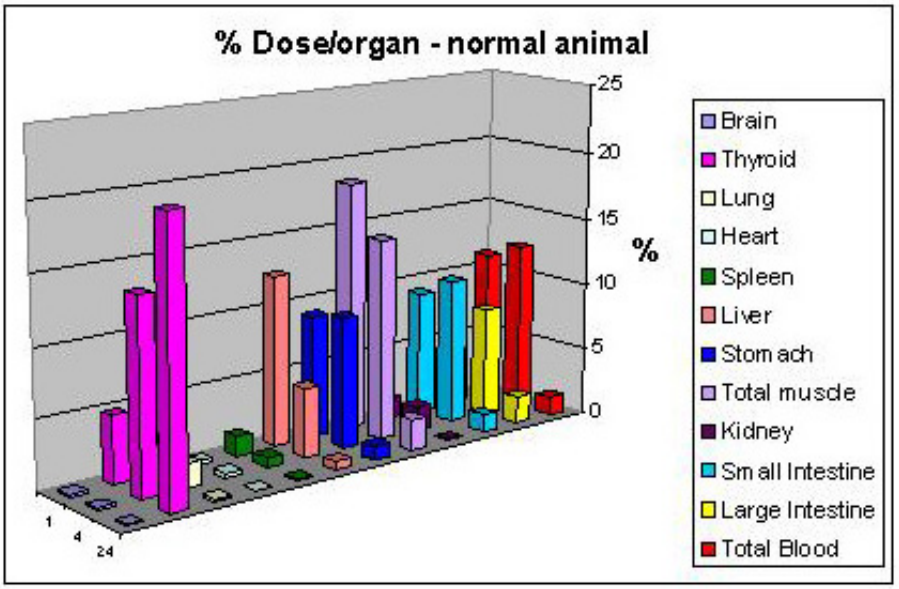
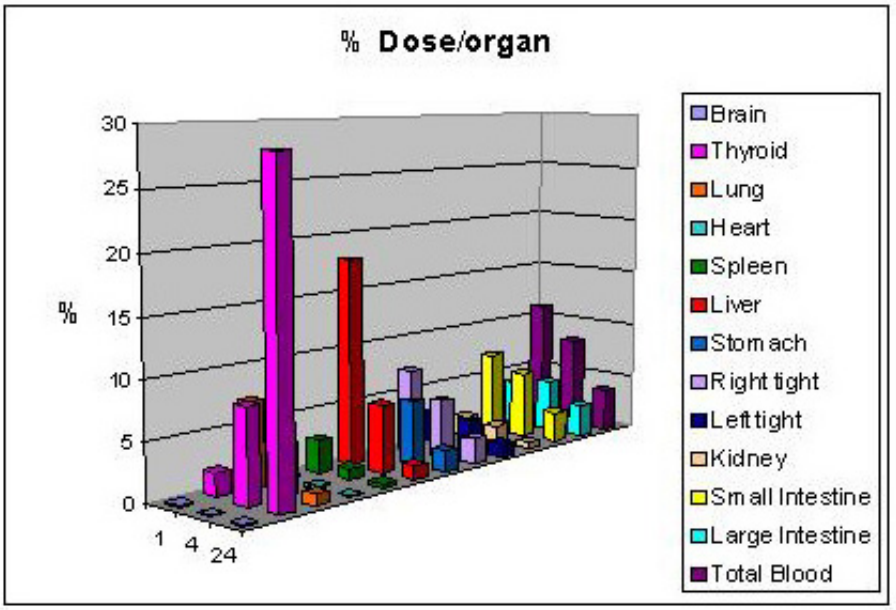


Table 2.
Percent dose/organ of radioiodinated FN1eLPN1eYK in Swiss mice whit turpentine promoted inflammation focus.

ORGANS	% Administered dose /organ		
	1 hour	4 hours	24 hours
Brain	0.182 ± 0.046	0.172 ± 0.042	0.106 ± 0.080
Thyroid	2.144 ± 0.387	8.059 ± 1.903	28.229 ± 5.272
Lung	7.293 ± 1.139	4.272 ± 2.301	1.070 ± 0.596
Heart	0.291 ± 0.056	0.210 ± 0.047	0.097 ± 0.034
Spleen	2.846 ± 0.804	1.031 ± 0.310	0.334 ± 0.050
Liver	17.986 ± 2.596	5.797 ± 0.831	1.217 ± 0.105
Stomach	3.953 ± 1.082	5.475 ± 1.304	1.877 ± 0.534
Right tight	7.251 ± 0.912	5.083 ± 0.836	2.265 ± 0.679
Left tight	2.888 ± 0.560	2.551 ± 0.991	1.036 ± 0.201
Kidney	1.873 ± 0.325	1.494 ± 0.292	0.607 ± 0.191
Small Intestine	7.243 ± 1.260	5.875 ± 0.572	2.757 ± 0.250
Large Intestine	3.910 ± 0.489	4.579 ± 0.714	2.797 ± 0.779
Total Blood	10.983 ± 1.808	8.093 ± 1.396	3.639 ± 0.497

Figure 3.
Percent dose/organ in Swiss mice with turpentine promoted inflammation focus.



Discussion and Conclusion

The peptide FNIeLFNIeYK was radioiodinated with high radiochemical purity using chloramine T as oxidative agent in a direct labeling approach.

The *in vitr*o stability was evaluated and the product remained stable over 48 hours when stored at low temperature (5-10ºC).

The *in vivo* stability of the labeled peptide was evaluated in normal Swiss mice and the thyroid uptake increased in time from 4.9 ± 0.7% at 1 hour after the dose administration to 20.15 ± 3.78% at 24 hours that indicates the in vivo dehalogenation of the compound.

When administrated to normal Swiss mice, the peptide cleared fast from the blood and from all non-target organs. When administrated to Swiss mice with turpentine promoted inflammatory focus, the uptake in the right tight (inflammated tight) was significantly greater than the left tight (normal tight) ($P < 0.05$, Student t test) for 1, 4 and 24 hours after the dose administration. The right tight/blood relations were 0.66, 0.63 and 0.62 respectively for the data colected at 1, 4 and 24 hours. Despite the greatest uptake in the right tight was observed 1 hour after the dose administration, the right tight/blood rate remained almost inalterated for 1, 4 and 24 hours. This result suggests that the uptake in inflammated area is proportional to the blood flow.

Further studies will be concentrated on labeling the FNIeLFNIeYK peptide with 123-iodine in order to investigate the scintigraphic potencial of the radiopharmaceutical in the diagnostic of inflammation focus.

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