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Bombesin derivative radiolabeled with technetium-99m as agent for tumor identification

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ABSTRACT

A bombesin derivative was successfully radiolabeled in high labeling yield. Biodistribution studies and scintigraphic images in Ehrlich tumor-bearing mice were performed. This compound showed high accumulation in tumor tissue with high tumor-to-muscle and tumor-to-blood ratios. Thus, ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ could be used as an agent for tumor diagnosis.

A multitude of pathologically up regulated physiological processes allow to differentiate a tumor from a normal tissue.[1] Cancer imaging techniques using radiotracers targeted to specific receptors have yielded successful results demonstrating the utility of such approaches for developing specific radiopharmaceutics.[2] Small receptors-binding peptides are currently the agents of choice for receptor imaging and tumor targeting. Several malignant tumors overexpress cell surface receptors, which can be targeted with radio labeled receptor-specific peptides for imaging and therapy.[3] In vivo functional imaging technique can help to diagnose and stage tumors, optimize drug scheduling, and predict response to a therapeutic modality, which would be advantageous to both patient and oncologist.[4,5] In this manner, this method allows for the viewing of physiopathological process in the initial stages, which runs contrary to the images conventional methods based on anatomical alterations.[6,7]

Bombesin (BBN) is tetradecapeptide initially isolated from skin of the frog *Bombina bombina*. The mammalian counterpart is the gastrin releasing peptide (GRP), which has almost an identical C-terminal sequence as bombesin. A variety of tumors have been found to express receptors for these peptides, such as lung, prostate, breast, pancreas, and colon.[8] Radiolabeled BBN analogs having high affinity for these receptors might therefore be used for scintigraphic imaging of those tumor types.[9–11] Several of these analogs bind selectively and avidly to GRP receptors on cancer cells when the truncated amino acid sequence (BBN$_{(7-14)}$NH$_2$) was used. It has been shown that the C-terminal amino acid sequence is necessary to retaining receptor binding affinity. Thus, the N-terminal region of the peptide can be used for radiolabeled.[3]

Technetium-99m (^{99m}Tc) has been mostly used for labeling radiopharmaceuticals owing to its suitable physical and chemical characteristics and inexpensive isotope cost.[12,13] 2-Hydrazinonicotinamide (HYNIC) is an attractive bifunctional chelating ligand for preparing ^{99m}Tc-labeled peptides[14] because it show a high labeling efficiency and its usage with various co-ligands (e.g., ethylendiaminediacetic acid (EDDA), tricine, and glucoheptonate) allows for easy modification of the hydrophobicity and pharmacokinetics of the ^{99m}Tc-labeled small peptides.[15]

The purpose of this study was to conjugate the HYNIC-BBN analog with technetium-99m and performed the biodistribution studies and scintigraphic images for evaluate the feasibility of the complex ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ as candidate for tumor-diagnosis agent.

The HYNIC-Bombesin$_{(7-14)}$ (Fig. 1) analog was purchased by GL Biochem (Shangai). It was synthesized by automated solid-phase synthesizer employing traditional Fmoc chemistry. The peptide was characterized by ESI-MS analysis (m/z = 1147.05 [M+H]$^+$). In our laboratory RP-HPLC[16] was performed showing high purity for the final product (Fig. 2).

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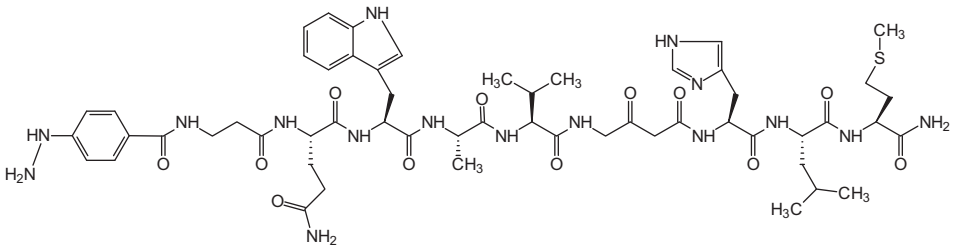

Figure 1. Structure of the HYNIC-Bombesin$_{(7-14)}$ analog.

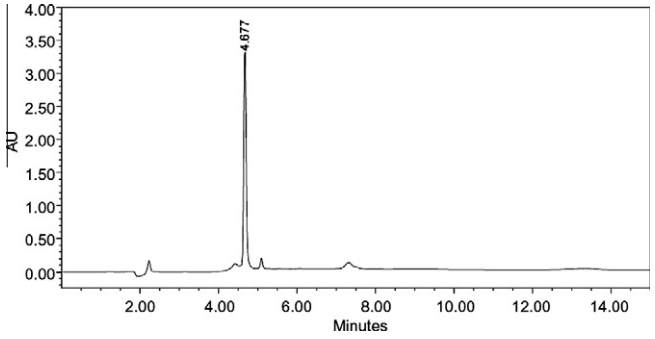

Figure 2. RP-HPLC profile of HYNIC-Bombesin$_{(7-14)}$.

The complex ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ was prepared in presence of tricine and EDDA at pH 7. In these conditions the chelating group can form a stable complex with technetium-99m.[17,18] The radiolabeling yield of ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ was determined by Instant Thin Layer Chromatography (ITLC) on two solvent systems: methylethylketone (MEK) to determine TcO_4^-, and acetonitrile/water (1:1) to determine TcO_2, as published elsewhere.[3] The radiochemical purity was higher than 95%. The in vitro stability was performed in NaCl 0.9% at room temperature and after 1, 2, 4, 6, and 24 h aliquots were analyzed by ITLC. In all times evaluated the complex showed high stability (over 95%).

Biodistribution of this complex was performed in Ehrlich tumor-bearing Swiss mice (25–30 g) at 60, 240, and 480 min post injection. This experiment was performed using five animals to each time investigated. The results are summarized in Table 1. ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ was excreted rapidly through kidneys and presented low heart uptake. It had high tumor-to-muscle (T/M) ratio that increased up to 480 min post injection. The results showed also fast blood clearance with high tumor-to-blood (T/B) ratio. It has been considered in the literature[19,20] that radiotracers that have a target/non-target ratio greater than 1.5 (50% greater capture in the target tissue) may be considered to be potential diagnostic agents.

When the biodistribution studies were performed with an unspecific radiopharmaceutical (^{99m}Tc-DTPA) in the same times intervals, a tumor-to-muscle ratio was about 1.0 (data not shown). Therefore, the data of the present study suggest a tropism of ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ to tumor, due to the presence of the specific receptor to GRP.

To confirm this hypothesis we performed a biodistribution study with receptor (GRP) blocking by coadministration of 10-fold cold HYNIC-Bombesin$_{(7-14)}$ (Table 2). In this specific study we used three mice to each investigated group. As shown, ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ uptake in the tumor decreased considerably as compared to biodistribution data shown in Table 1, indicating competition for the receptor between cold HYNIC-Bombesin$_{(7-14)}$ and ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$. This result suggests that ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ binds selectively to GRP in tumor cells (Fig. 3).

Scintigraphic images showed a greater uptake of radioactivity by the tumor (right thigh) when compared with the left thigh, used as control (Fig. 4). Quantitative analysis of scintigraphic images obtained from Ehrlich tumor-bearing mice with ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ presented tumor-to-muscle ratios similar with the obtained in biodistribution studies (data not shown). These results showed the tropism of the ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ to the tumor during the whole experiment.

In summary, the bombesin derivative was radiolabeled in high yield. The complex ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ showed high tumor-to-muscle ratio during all time studied and in both studies, biodistribution and scintigraphic images. These data suggest that ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ could be used as an potential agent

Table 1
Biodistribution of ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ in Ehrlich tumor-bearing mice (% ID/g)[a]

Tissue	60 min	240 min	480 min
Liver	0.38 ± 0.05	0.24 ± 0.06	0.21 ± 0.01
Spleen	0.32 ± 0.05	0.12 ± 0.03	0.09 ± 0.01
Kidney	4.72 ± 0.85	2.73 ± 0.67	1.90 ± 0.21
Stomach	0.73 ± 0.08	0.50 ± 0.18	0.78 ± 0.08
Heart	0.28 ± 0.03	0.10 ± 0.01	0.07 ± 0.01
Lung	0.51 ± 0.08	0.19 ± 0.03	0.13 ± 0.01
Blood	0.44 ± 0.08	0.11 ± 0.01	0.07 ± 0.01
Bladder	79.90 ± 8.46	22.76 ± 5.75	14.76 ± 3.08
Pancreas	1.66 ± 0.32	0.41 ± 0.10	0.22 ± 0.03
Thyroid	0.59 ± 0.11	0.30 ± 0.09	0.26 ± 0.07
Tumor	0.87 ± 0.19	0.39 ± 0.03	0.30 ± 0.02
Muscle	0.30 ± 0.02	0.08 ± 0.01	0.05 ± 0.01
T/M	2..90 ± 0.67	4.78 ± 0.81	6.15 ± 0.54
T/B	1.97 ± 0.53	3.53 ± 0.53	4.51 ± 0.54

[a] All data are the mean percentage (n = 5) of the injected dose of ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ per gram of wet tissue ± the standard deviation of the mean.

Table 2
Biodistribution of ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ in Ehrlich tumor-bearing mice with blocked receptor (% ID/g)[a]

Tissue	60 min	240 min	480 min
Liver	0.82 ± 0.18	0.22 ± 0.04	0.23 ± 0.04
Spleen	0.33 ± 0.05	0.09 ± 0.02	0.11 ± 0.03
Kidney	4.85 ± 0.87	2.34 ± 0.32	0.64 ± 0.12
Stomach	0.40 ± 0.17	0.26 ± 0.06	0.49 ± 0.08
Heart	0.51 ± 0.18	0.07 ± 0.01	0.09 ± 0.02
Lung	0.32 ± 0.07	0.10 ± 0.01	0.10 ± 0.01
Blood	0.41 ± 0.05	0.09 ± 0.01	0.07 ± 0.01
Bladder	78.07 ± 1.18	26.71 ± 4.70	7.68 ± 1.03
Pancreas	0.85 ± 0.02	0.14 ± 0.02	0.14 ± 0.05
Thyroid	0.18 ± 0.01	0.06 ± 0.01	0.15 ± 0.02
Tumor	0.43 ± 0.07	0.17 ± 0.03	0.10 ± 0.02
Muscle	0.22 ± 0.04	0.09 ± 0.02	0.12 ± 0.08
T/M	1.95 ± 0.12	1.89 ± 0.13	0.83 ± 0.14
T/B	1.08 ± 0.30	1.97 ± 0.39	1.46 ± 0.38

[a] All data are the mean percentage (n = 3) of the injected dose of ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ per gram of wet tissue ± the standard deviation of the mean.

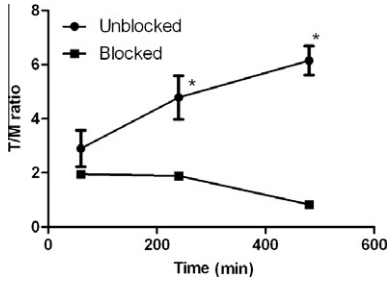

Figure 3. Tumor-to-muscle ratio after intravenous administration of ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ (unblocked) and ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ + cold HYNIC-Bombesin$_{(7-14)}$ (blocked) in Ehrlich tumor-bearing mice. Results are expressed as means ± standard error. The asterisks indicate a statistically significant difference between unblocked and blocked (p <0.05).

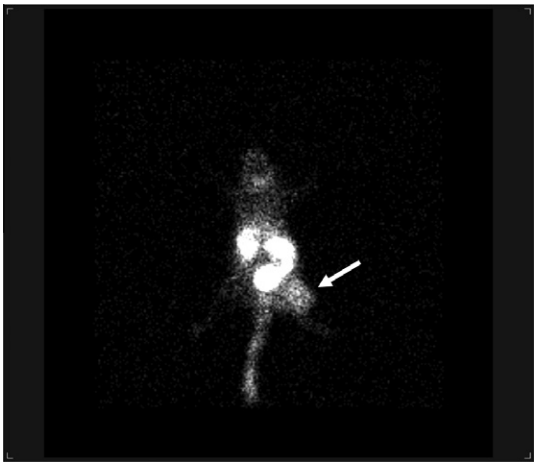

Figure 4. Scintigraphic image 480 min after intravenous administration of ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ in Ehrlich tumor-bearing mice. While under ketamine/xylazine anesthesia, 3.7 MBq of ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ was inject into the tail vein (posterior view).

for tumor identification. Further studies will be carried out to evaluate the real potential of to ^{99m}Tc-HYNIC-Bombesin$_{(7-14)}$ for identification of the human tumors such as prostate, breast and lung and will be reported in due course.

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