I-123 LABELED 1.3.8-TRIHYDROXY-6-METHYL-ANTHRAQUINONE AS AN IMAGING AGENT FOR TARGETING *HER-2/neu* RECEPTOR

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In this study, we conducted the synthesis, purification and biological evaluation for ^{123}I labeled emodin as SPECT imaging agent for HER-2/neu receptor overexpressed breast cancer. [^{123}I]emodin was labeled using modified chloramine-T method with 85% of radiochemical yield and purity was >98%. The cellular uptake increased in a time dependent manner for both breast cancer cell lines SK-BR-3 and MCF-7. Interestingly, HER-2/neu receptors overexpressed SK-BR-3 showed higher uptake than that of MCF-7. In vivo SPECT imaging showed the specific accumulation of [^{123}I]emodin in MCF-7, HER-2/neu receptor expressed breast cancer, bearing mouse model. This preliminary study suggests that [^{123}I]emodin can be used as an imaging reagent for breast cancer.

I. INTRODUCTION

Breast cancer is a malignant cancer that is prevalent in women all over the world. Enhanced expression of *HER-2/neu* is known to be involved in breast cancer. *HER-2/neu* represent 25 ~30 percent of breast cancers[1], and several efforts have been made for the development of the drug to suppress *HER-2/neu* receptors. Anthraquinone derivatives are well-known for being able to target *HER-2/neu*. As a protein tyrosine kinase inhibitor, Emodin (6-methyl-1,3,8-trihydroxyanthraquinone) is an anthraquinone derivative isolated from Rheum Palmatum, has potent antioxidant and anticancer activity[2]. *HER-2/neu* positive SK-BR-3 has a high level of expression (*HER-2/neu* mRNA level of actin: 476) on the other hand, MCF-7 has a low level of expression (*HER-2/neu* mRNA level of actin: 2.38).[3] Emodin has shown to inhibit *HER-2/neu* tyrosine kinase activity and to repress the change of *HER-2/neu* receptor by overexpressing breast cancer cells through repression of *p185/ neu* tyrosine kinase. In this study, we have evaluated ^{123}I labeled emodin for *HER-2/neu* receptor targeted SPECT image.

I.A. Experimental

I.A.1. Synthesis of ^{123}I labeled emodin

Emodin (2 mmol) in 200 μL of DMF was mixed with Na^{123}I (37 MBq) and chloramine-T of 10 mg at pH 12. After the reaction the mixture was stirred for 10 min and radioactivity of free iodine and labeled emodin were measured with a radioisotope dose calibrator. The total reaction time of [^{123}I]emodin was ~10 min.

I.A.2. Preparation of biological study

In vitro cellular uptake of radioactive [^{123}I]emodin was studied using the SKBR-3 and MCF-7 breast cancer cell lines. Cancer cells were seeded into each well of 24-well culture plates in 5% CO_2 and 37 ^{0}C and [^{123}I]emodin (185 kBq/240 μL) was added at various time points (15, 30, 60, and 120 min) following the addition of [^{123}I]emodin. Ex vivo studies were carried out in female nude mice of 6 weeks weighing 20–25 g. Tumor (size ~ 20 mm) bearing mice was injected with 1 × 10^7 cells of SKBR-3 and MCF-7 subcutaneously for biodistribution studies. The [^{123}I]emodin solution was intravenously injected in 12 mice at a dose of 3.7 MBq/ 100 μL/ mouse. Blood samples were collected from the heart of the anesthetized mice. Further, Tissues including blood, muscle, bone, liver, lung, kidney, stomach, intestine, fat, brain, spleen, and tumor were collected and then analyzed by γ-counter. The percent injected dose per gram of tissue (%ID/g) was calculated by comparing the tissue counts to the initial dose measured at the various time point. In vivo SPECT images were obtained

using MCF-7 xenografted mice. SPECT images were achieved after 0.5, 1, and 2 h of intravenous injection having 3.7 MBq/100 μL of [^{123}I]emodin.

I.A.3. Results and Discussions

^{123}I labeled emodin was synthesized in high purity via a one-step reaction that was performed in oxidizer under room-temperature conditions using [^{123}I]. The reaction was monitored by radio-TLC and [^{123}I] labeled emodin was purified on C18 chromatography using an isocratic elution with 0.25 M NH_4OAc/MeOH solution. Radiochemical yield and purity was 85% and 98%, respectively. The cellular uptake values were 1.18% at 15 min, 1.20% at 30 min, 1.27% at 60 min, 1.25% at 120 min for MCF-7 and 1.51% at 15 min, 1.61% at 30 min, 1.71% at 60 min, 1.83% at 120 min for SK-BR-3. The cellular uptakes of [^{123}I]emodin increased in a time dependent manner for breast cancer cell lines SK-BR-3 and MCF7. Biodistribution studies of [^{123}I]emodin at 0.5 and 2 h in the breast tumor mice model exhibited a significant uptake in SKBR-3 tumors, with 1.63 ± 0.18 and 3.17 ± 0.27% ID/g at 0.5 and 2 h respectively, on the other hand MCF-7 bearing mice showed 0.79 ± 0.20% and 1.36 ± 0.39% ID/g at 0.5 and 2h respectively. SPECT image of [123I]emodin was obtained in MCF-7 bearing mice at 1.5 h and 3 h. SPECT image clearly showed significant accumulation at tumor site. After 90 min post injection, uptake in the tumor is pronounced for [^{123}I]emodin. Over the time, tumor to background contrast was gradually reduced at 180 minutes.

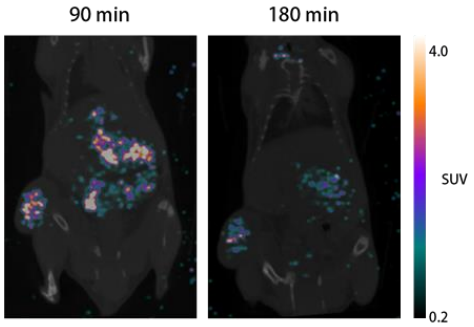

Fig.1. SPECT image of [^{123}I]emodin in MCF-7 bearing mice

II. CONCLUSIONS

We successfully synthesized and evaluated a ^{123}I labeled emodin as a probe for diagnosis of breast cancer. The [^{123}I]emodin was labeled at room temperature within 10 min with high radiochemical yield and purity. [^{123}I]emodin showed significant uptake in *HER2/neu* receptor overexpressed breast cancer. These results suggest that this probe could be used for its potential application in HER-2/neu receptor targeted agent for SPECT imaging.

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