In vivo Imaging Using Tissue Specific Near Infrared Fluorescent Peptide Conjugate, c[RGDyK(HiLyte Fluor™ 750)]

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Introduction

Extracellular matrix proteins that contain the Arg-Gly-Asp (RGD) sequence, and integrin receptors which bind this sequence, constitute a major recognition system for cell migration and adhesion processes. Integrins are heterodimeric transmembrane proteins composed of α and β subunits. Integrins can bind a wide variety of ligands, including extracellular matrix proteins, such as fibronectin and vitronectin. In the case of RGD cyclic peptide c(RGDyK), its structure also confers increased affinity and selectivity for integrin αvβ3, both in cell culture and in living subjects. We report here an in vivo testing of this peptide labeled with a proprietary near infrared fluorescent dye, HiLyte Fluor™ 750-labeled RGD peptide, c(RGDyK(HiLyte Fluor™ 750)), with excitation and emission wavelengths at 750 and 780 nm. We found that for our animal model, this conjugate binds specifically to some tissues in organs that are known to be rich in integrin αvβ3.

Results and Discussion

Preparation of c[RGDyK(HiLyte Fluor™ 750)]:

• Cyclic RGD peptide [c(RGDyK)] (cat# 61183, AnaSpec Inc., San Jose, CA) dissolved in NaHCO3 buffer (pH = 8.5) was mixed with HiLyte Fluor™ 750 Acid, SE (cat# 81266, AnaSpec Inc., San Jose, CA) in DMF. The solution was stirred in the dark at room temperature for 1 hour. The conjugate mixture was analyzed and purified by RP-HPLC using 0.1% trifluoroacetic acid in water (solvent A) and 0.1%, trifluoroacetic acid in acetonitrile (solvent B).

In vivo Imaging:

• 20 nmol of the c[RGDyK(HiLyte Fluor™ 750)] was diluted in 200 µL saline solution.
• The saline solution was injected intravenously (IV) into a Sprague-Dawley (Harlan, Indianapolis, Indiana) rat in two separate instances (experiments).
• A control solution of 20nmol of HiLyte Fluor™ 750 Acid (cat# 81265, AnaSpec Inc., San Jose, CA) was injected in the Sprague-Dawley rat in two separate instances (controls).
• In vivo imaging was made using a Xenogen IVIS® Imaging System 200 (Figures 1 and 2); the animal was imaged at 0, 3, 6, and 24 hours post injection using a Indocyanine Green (ICG)Filter set (excitation 710-760nm, emission 810-875 nm). Organs were dissected and imaged 3 hours after injection of conjugate, and imaged (Figure 3).

Conjugate Compared to Dye Only:

The RGD-HiLyte Fluor™ 750 conjugate shows increased fluorescence in the lung, and prostate and seminal vesicles tissues. High fluorescence in the liver and kidney is expected as part of the conjugate clearance process in the animal (Figure 5).

Reference:


Acknowledgments

Financial Support provided by the NIH-NIBIB grant #1R01 EB001640.