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. In fibronectins and other proteins, the RGD binding sequence is found at the apex of a loop; such conformation has been found to allow for high affinity selectivity to integrin receptors. Cyclic peptides have been shown to be more stable than linear peptides; in the case of RGD cyclic peptide c(RGDyK), its structure also confers increased affinity and selectivity for integrin $\alpha v\beta_3$ 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 $\alpha v\beta_3$.

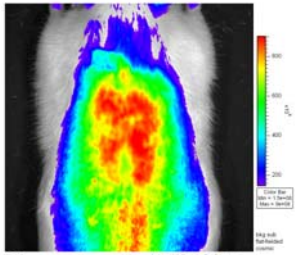

Figure 1. *In vivo* fluorescent imaging of rat injected with 20 nmol c[RGDyK(HiLyte Fluor™ 750)] conjugate 3 hours post injection.

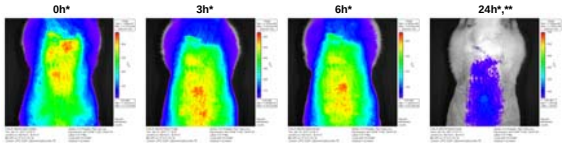

Figure 2. Progression of conjugate clearance over a 24 hour period. *Different scales for Photon Flux (p/s). **48h image not included here, very similar to 24h image and to background.

Results and Discussion

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

• Cyclic RGD peptide [c(RGDyK)] (cat# 61183, AnaSpec Inc., San Jose, CA) dissolved in $NaHCO_3$ 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 r.t. 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%. trifluoracetic acid in acetonitrile (solvent B).
• Pure conjugate was confirmed by MS.

***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) inbred 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).

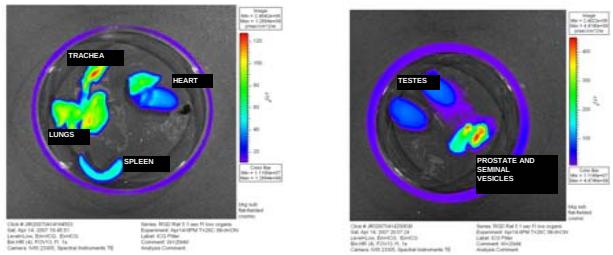

Figure 3. Dissected organs imaged 3 hours post conjugate injection.

Conjugate Compared to Dye Only:

The RGD-HiLyte750 conjugate shows increased maximum fluorescence at 0, 3, and 6 hours when compared to the controls (HiLyte Fluor™ 750 dye only) confirming preferential accumulation of the conjugate in certain organs (Figure 4).

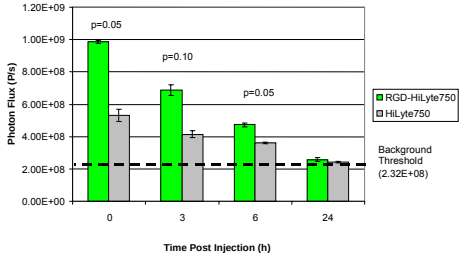

Figure 4. Comparison of fluorescence in the RGD conjugate injections versus controls (HiLyte Fluor™ 750 only).

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).

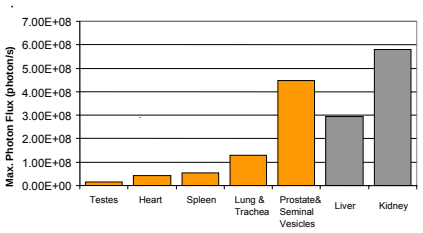

Figure 5. Organ maximum fluorescence.

Reference:

Chen, X., P. Conti, and R. Moats, *Cancer Res.* **64**, 8009 (2004).

Acknowledgments

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