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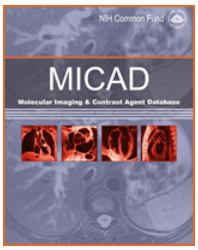

A15 (NIRF agent)

The MICAD Research Team

Created: May 4, 2006; Updated: May 30, 2006.

Chemical name:	A15
Abbreviated name:	A15
Synonym:	
Agent Category:	Peptide
Target:	Activated coagulation factor XIIIa (FXIIIa)
Target Category:	Binding
Method of detection:	Optical imaging (NIRF)
Source of signal:	A15
Activation:	No
Studies:	• *In vitro* • Rodents

Background

[PubMed]

Thrombosis plays a major role in many cardiovascular diseases such as myocardial infarction, pulmonary embolism, deep venous thrombosis or cerebral venous thrombosis (CVT) (1). Unfortunately, the clinical diagnosis of CVT is complex, as most of its symptoms are non-specific and the well-established modalities such as conventional angiography, CT or MR venography, do not offer readily available information on chronicity or activity without serial anatomic testing over time (2).

Thrombosis occurs by an activation process of thrombin, which then converts fibrinogen into fibrin. Thrombin initiates the crosslinking of the polymerized fibrin via the activation of a transaminase called coagulation factor XIII (FXIIIa = activated factor XIII) (3). FXIIIa augments acute thrombus stability by rapidly crosslinking fibrin α- and γ-chains, and by covalently binding to alpha-2 antiplasmin (α2AP) (4) FXIIIa has a catalytic half-life of ~ 20 min, *in vivo* (5). Optical imaging with a FXIIIa probe may be a useful tool in the diagnostic of thrombosis (including CVT) and may provide valuable information currently unavailable through anatomically-based imaging modalities (6, 7).

A15 is a novel near-infrared fluorescence (NIRF) probe recognized by the enzyme FXIIIa and that covalently binds to fibrin by means of the transglutamase activity of FXIIIa. Preliminary research has shown that A15 is capable of testing the activity of FXIIIa and visualizing thrombus *in vivo* (8, 9). A15 may find applications as a molecular diagnostic tool for therapy with fibrinolytic or anti-FXIIIa agents, direct or indirect inhibitors, and other drugs for diseases such as CVT, ischemic stroke or myocardial infarction. Additional and more extensive

research studies are needed to fully assess the potential and possible clinical benefits of the A15-NIRF imaging modality (9).

Synthesis

[PubMed]

A15 can be prepared by solid-state peptide synthesis, as described by Tung et al. (10). Briefly, an acetyl glycine group is added to the N terminus of the α2AP fragment for capping, and a tryptophan residue and a cysteine residue are then added to the C terminus for quantitation and conjugation purposes. A15 is then labeled with a sulfhydryl reactive fluorochrome (Alexa Fluor 680C2 maleimide, AF680) via the C-terminal cysteine side chain (in sodium acetate buffer, at pH 6.5). After purification by HPLC, the final A15 product shows an excitation wavelength of 679 nM and an emission wavelength of 702 nm (9).

In addition to synthesizing A15, Tung et al. (10) and Jaffer et al (8). also prepared a control probe CA15 by replacing the glutamine residue at position 14 with an alanine residue. The fluorescent signal obtained with CA15 was less than 3 fold higher than the background signal, compared with a 11 fold enhancement for A15.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Tung et al. (10) performed *in vitro* assays using both A15 and CA15 probes (1μM) to assess the importance of the glutamine residue at position 14 in the crosslinking reaction with FXIII. The experimental protocol involved using mouse blood clots that were homogenized, washed in phosphate buffered saline and incubated with either A15 or CA15. Subsequent imaging of the clots (imaging system with a 630 + 15 nm excitation and a 700 + 20 nm emission) revealed the critical role of the glutamine residue. An intense fluorescence signal (fluorescence intensity ~ 760 AU, n = 3 clots tested) was obtained for the clot incubated with A15 (11 fold higher than the background of the blood), whereas a much weaker signal (fluorescence intensity ~ 200 AU, n = 3) for the clot incubated with the control probe CA15 (less than 3 fold higher than background).

Using human plasma clots, Jaffer et al. (8) investigated the ability of A15 to be crosslinked by human FXIIIa. The experimental protocol involved creating clots by mixing 90 μl of fresh frozen plasma with 3 μl of A15, CA15 (control probe), AF680 (free NIRF fluorochrome) or normal saline. All imaging agents were used at a concentration of 100 μmol/l, based on AF680. After incubation of the plasma clots with the individual imaging agents (90 min, 37°C), NIRF imaging was performed with each group (11). The collected data showed that in the A15 group, thrombi had a 347-fold NIRF signal increase compared with the saline (1562 ± 193.8 arbitrary units (AU) for A15 and 4.5 ± 0.6 AU for saline, $P<0.001$ A15 vs. each other group) and a 4.8 to 6.3-fold increase over the other control groups (247.5 ± 65.6 AU for CA15 and 328.0 ± 72.9 AU). Pre-incubation of FFP thrombi with IAA, a FXIII inhibitor, substantially reduced the A15 thrombus enhancement by 92.9% ($P<0.001$), supporting the fact that A15 is 'FXIIIa-specific'.

The authors also showed that A15 was crosslinked to fibrin by FXIIIa, by performing a Western blot protein analysis (5) that used denaturated clots subjected to immunoblotting, and a polyclonal antibody that bound to the N-terminal peptide of the α_2AP factor forming the backbone of A15. Clots formed with A15 (mass of A15 < 3kDa) showed a band at ~70 kDa that appeared strongly immunoreactive with the antibody to the N- terminus of α_2AP. (No band was detected with CA15).

Animal Studies

Rodents

[PubMed]

Several studies aimed at assessing the ability of A15 to detect FXIII activity *in vivo* have been reported in the literature (8, 9, 12). One of them (8) was based on an intravascular model of thrombosis using 11 mice injected with A15 following the topical application of ferric chloride ($FeCl_3$) onto femoral vessels exposed by blunt dissection. 6 mice received A15 (n = 3) or CA15 (n = 3), 30 min after application of $FeCl_3$. 2 mice received A15 at either 0 or 120 min after application of $FeCl_3$, and 3 mice received A15 28 hours after application of $FeCl_3$.

Fluorescence signals obtained for all animals having received the A15 showed a NIR enhancement characteristic of acute thrombi, with particular enhancements in the region of the femoral artery and vein within 30 to 45 min after application of $FeCl_3$. In mice that received the imaging agent 30 min after application of $FeCl_3$, the peak contrast -to-noise ratio between thrombus and background was 15.0 ± 6.7% by 90 min. In comparison, the value obtained for the mice having received the control probe CA15 was -1.4 ± 2.5% (P = 0.039). No thrombosis enhancement in the femoral vessels was observed in mice treated with A15, 28 h after application of $FeCl_3$. This last result was found consistent with an expected decline in FXIIIa activity over time (5). Jaffer et al (8). suggested that crosslinking of A15 to fibrin might explain how thrombus contrast increased over time, and that higher contrast might be obtained in the absence of fibrinolysis (or embolization), for time periods occurring after 90 min post-application of $FeCl_3$.

In a study by Kim et al. (9), thrombi were formed in 12 CD-1 mice by application of paper strips (1 x 5 mm^2) soaked in 10% $FeCl_3$ onto the entire superior sagittal sinus (SSS) exposed by craniotomy (5 mm diameter). After an intravenous injection of A15 (5nmol/150 µl), light (exposure time = 1 sec) and serial fluorescence digital images (exposure time = 3 sec) were recorded at 0, 1, 3, 5, 10, 15, 20 and 25 min.

The following observations were made: the mice that had been exposed to $FeCl_3$ only at the posterior 2.5 mm portion of the SSS exhibited a fluorescence signal limited to this same area. On the other hand, the animals whose entire 5 mm region had been exposed displayed a NIR signal in the entire SS and nearby branching vessels. The mean length of the SS showing the A15 signal appeared significantly longer in the full-length thrombosis group (2.8 ± 0.3) than in the localized thrombosis group (1.8 ± 0.5, $P < 0.05$). After intravenous injection, A15 first produced a cerebral angiogram, and was thereafter rapidly washed out in all but thrombosed areas, resulting in an improvement in target/background signal that allowed thrombosed areas to be identified by 5 to 10 min after injection of A15.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available

References

1. Corti R, Farkouh ME, Badimon JJ. The vulnerable plaque and acute coronary syndromes. Am J Med. 2002;113(8):668–680. PubMed PMID: 12505118.
2. Sampson FC, Goodacre SW, Thomas SM, van Beek EJ. The accuracy of MRI in diagnosis of suspected deep vein thrombosis: systematic review and meta-analysis. Eur Radiol. 2006.
3. Bereczky Z, Katona E, Muszbek L. Fibrin stabilization (factor XIII), fibrin structure and thrombosis. Pathophysiol Haemost Thromb. 2003;33(5-6):430–437. PubMed PMID: 15692256.
4. Muszbek L, Yee VC, Hevessy Z. Blood coagulation factor XIII: structure and function. Thromb Res. 1999;94(5):271–305. PubMed PMID: 10379818.
5. Robinson BR, Houng AK, Reed GL. Catalytic life of activated factor XIII in thrombi. Implications for fibrinolytic resistance and thrombus aging. Circulation. 2000;102(10):1151–1157. PubMed PMID: 10973845.
6. Renowden S. Cerebral venous sinus thrombosis. Eur Radiol. 2004;14(2):215–226. PubMed PMID: 14530999.
7. Connor SE, Jarosz JM. Magnetic resonance imaging of cerebral venous sinus thrombosis. Clin Radiol. 2002;57(6):449–461. PubMed PMID: 12069459.
8. Jaffer FA, Tung CH, Wykrzykowska JJ, Ho NH, Houng AK, Reed GL, Weissleder R. Molecular imaging of factor XIIIa activity in thrombosis using a novel, near-infrared fluorescent contrast agent that covalently links to thrombi. Circulation. 2004;110(2):170–176. PubMed PMID: 15210587.
9. Kim DE, Schellingerhout D, Jaffer FA, Weissleder R, Tung CH. Near-infrared fluorescent imaging of cerebral thrombi and blood-brain barrier disruption in a mouse model of cerebral venous sinus thrombosis. J Cereb Blood Flow Metab. 2005;25(2):226–233. PubMed PMID: 15678125.
10. Tung CH, Ho NH, Zeng Q, Tang Y, Jaffer FA, Reed GL, Weissleder R. Novel factor XIII probes for blood coagulation imaging. ChemBioChem. 2003;4(9):897–899. PubMed PMID: 12964167.
11. Mahmood U, Tung CH, Bogdanov A, Weissleder R. Near-infrared optical imaging of protease activity for tumor detection. Radiology. 1999;213(3):866–870. PubMed PMID: 10580968.
12. Jaffer FA, Tung CH, Gerszten RE, Weissleder R. In vivo imaging of thrombin activity in experimental thrombi with thrombin-sensitive near-infrared molecular probe. Arterioscler Thromb Vasc Biol. 2002;22(11):1929–1935. PubMed PMID: 12426227.