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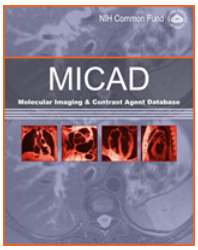

^{125}I-Labeled heparin-binding peptides that target heparan sulfate proteoglycans for the *in vivo* imaging of peripheral amyloidosis

[^{125}I]-Peptides

Arvind Chopra, PhD[1]

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Chemical name:	^{125}I-Labeled heparin-binding peptides that target heparan sulfate proteoglycans for the *in vivo* imaging of peripheral amyloidosis	
Abbreviated name:	[^{125}I]-Peptides	
Synonym:		
Agent Category:	Peptides	
Target:	Heparan sulfate proteoglycans	
Target Category:	Proteins (proteoglycans)	
Method of detection:	Single-photon emission computed tomography (SPECT); gamma planer imaging	
Source of signal / contrast:	^{125}I	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available in PubChem.

Background

[PubMed]

Peripheral amyloidosis is the extracellular deposition of insoluble protein fibrils in various organs of animals, including humans, and these deposits are considered to be biomarkers for diseases such as Alzheimer's disease (AD), light chain amyloidosis (AL), etc (1). The fibrils are made up of disease-specific aggregated proteins or peptides (e.g., Aβ peptides for AD, light chains for AL or multiple myeloma, and reactive amyloidosis (AA)) that incorporate heparin sulfate proteoglycans (HSPG; heparin belongs to the heparan sulfate family of proteoglycans that contain the $(GlcNS6S\text{-}IdoA2S)_3$ motif) and the serum amyloid P component (SAP) within their structure during disease progression. A characteristic feature of the protein fibrils is that the constituent proteins form a secondary cross-β pleated sheet structure that is resistant to proteolytic digestion (for structural details, see Goldsbury et al. (2)). The HSPG contain diverse types of oligosaccharides that are sulfated on the

Author Affiliation: 1 National Center for Biotechnology Information, NLM, Bethesda, MD 20894; Email: micad@ncbi.nlm.nih.gov.

hydroxyl moieties to varying degrees, and these hypersulfated structures are distinct, are found specifically in the amyloid deposits, and differ from one another depending on the organ where they are located (3). In addition, clinical symptoms in patients are influenced by the degree to which an organ is involved in the disease (4). Because the HSPG are hypersulfated compared to proteoglycans found in normal tissues, HSPG are considered to be relevant biomarkers for use with noninvasive imaging to detect, diagnose, and monitor amyloidosis progression and to determine the prognosis for a patient with amyloidosis (1).

Whole-body scintigraphy with radioiodinated SAP is commonly used in Europe for the detection of amyloidosis in the various parts of the body, but this technique is not approved for clinical use in the United States by the U.S. Food and Drug Administration because the SAP in the tracer is isolated from human sources (1). In an attempt to develop an amyloid imaging agent that does not require the use of materials of human origin, Wall et al. studied the biodistribution of seven ^{125}I-labeled synthetic heparin-binding peptides with small-animal single-photon emission computed tomography (SPECT) to evaluate their use in the detection of amyloid deposits in mice with severe systemic AA amyloidosis (1).

Related Resource Links

Related chapters in MICAD

Clinical trials related to amyloidosis

Amyloidosis in Online Mendelian Inheritance in Man Database (OMIM)

Protein sequence of human amyloid

Crystal structures of human amyloid proteins

Symptoms, diagnosis, and treatment of amyloidosis (at Cedars-Sinai Medical Center, LA)

Synthesis

[PubMed]

The ^{125}I-labeled heparin-binding peptides (designated p1 thru p7; see table below for amino acid sequences) were obtained in a semi-pure form from commercial sources and purified with reversed-phase liquid chromatography using a C_3 reversed-phase column as described by Wall et al. (1). The mass of each purified peptide was confirmed with mass spectroscopy. The amino acid sequences of the various peptides used in the study are given below:

Peptide	Amino acid sequence	Net charge
p1	CGGYS SSRPV RRRRR PRVSR RRRRG GRRRR	+16
p2	CGGYG DAKKK KDGKK AEPKN PRENK LKQPG	+6
p3	CGGYP KKGSK KAVTK AQKKD GKKR	+9
p4	CGGYS RPRAR ARARD QTR	+5
p5	CGGYS KAQKA QAKQA KQAQK AQKAQ AKQAK Q	+8
p6	CGGYP RRRRS SSRPI RRRRP RRASRR	+13
p7	CGGYF AKLNC RLYRK ANKSS K	+6

Each peptide was labeled with ^{125}I using the chloramine-T method, and the purity of the labeled product was determined with SDS/PAGE followed by analysis with a phosphor imager. The radiochemical yield and purity of

the ^{125}I-labeled peptides were not reported. The specific activity of the labeled peptides was calculated to be ~0.75 MBq/μg (~20 μCi/μg).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The heparin-binding characteristics of the various purified peptides were investigated with high-salt elution of the peptide from a heparin column (1). The heparin-binding activity of the peptides was reported to be p1 > p6 > p4 > p5 = p3 > p7 > p2 and was dependent on the net charge (see table in Synthesis).

Using histochemical techniques (exposure of tissue sections to biotinylated p% peptide followed by detection with streptavidin-conjugated horse radish peroxidase), the p5 peptide was shown to bind the Aβ amyloid deposits in the brain sections of patients with AD (1).

Animal Studies

Rodents

[PubMed]

Wall et al. investigated the biodistribution of the various ^{125}I-labeled peptides in H2-L^{d}-huIL-6 Tg BALB/c transgenic mice (AA mice; constitutively express the human IL-6 transgene) 3–6 weeks after the induction of AA amyloidosis in the animals (1). The animals (n = 3 mice/radioiodinated peptide/time point) were injected with 3.70–7.40 MBq (100–200 μCi; 5–10 μg protein) of each ^{125}I-peptide through the tail vein. For use as controls, the labeled peptides were administered to the same number of wild-type (WT) mice as described above. The mice were subsequently euthanized at 1 h and 4 h postinjection (p.i.) to determine the amount of radioactivity accumulated in the major organs. Data obtained from this study were presented as percent of injected dose per gram tissue (% ID/g). At 1 h p.i., the amount of radioactivity accumulated in the liver with peptides p1, p2, p3, p4, p5, p6 and p7 was 11.6 ± 3.4% ID/g, 3.1 ± 1.0% ID/g, 6.0 ± 1.4% ID/g, 5.8 ± 0.7% ID/g, 12.5 ± 2.5% ID/g, 6.6 ± 1.5% ID/g, and 4.1 ± 0.1% ID/g, respectively; in the pancreas, accumulation was 7.1± 2.1% ID/g, 3.5 ± 1.4% ID/g, 5.6 ± 2.3% ID/g, 6.3 ± 1.9% ID/g, 10.0 ± 4.5% ID/g, 4.8 ± 0.3% ID/g, and 3.7 ± 0.5% ID/g, respectively; in the spleen, accumulation was 11.5 ± 2.2% ID/g, 2.4 ± 0.4% ID/g, 3.0 ± 1.7% ID/g, 5.6 ± 0.4% ID/g%, 15.9 ± 5.2% ID/g, 6.3 ± 0.4% ID/g, and 2.8 ± 0.6% ID/g, respectively. Presence of AA amyloid in the various tissues was confirmed with Congo Red staining (this stain is selective for amyloid deposits). From the biodistribution study it was evident that the amount of radioactivity in the organs of mice with AA amyloidosis was at least two- to seven-fold higher than radioactivity in organs of the WT animals. Among all the peptides, the uptake of radioactivity in the liver, spleen, and pancreas was highest with ^{125}I-labeled p5. The AA amyloidosis/WT uptake (AA/WT) ratios for the liver and spleen were ~5 and 8, respectively, which increased to ~10 and >20, respectively, at 4 h p.i. On the basis of these observations, the efficacy of the peptides to bind the amyloid deposits was determined to be p5 > p1 > p6 > p4 ~ p3 > p7 > p2. These observations were confirmed with microradioautography of the liver and spleen tissues.

SPECT/ computed tomography imaging of the AA and WT mice at 1 h p.i. showed that the amyloid deposits could be visualized in the liver, pancreas, spleen, and kidneys of AA animals only with p1, p5, and, to some extent, p6 (1). At 4 h p.i., the liver and spleen were visible only in AA animals injected with the p1 or p5 peptides. In comparison, regardless of the radioiodinated peptide injected, SPECT images of the WT mice acquired at 1 h p.i. showed that the label was present only in the thyroid and the stomach of the animals.

In another study, the amyloid binding of radioiodinated p1 and p5 (because with these peptides the different organs showed the highest uptake compared to the other peptides) was studied in mice (n = 3 animals/time point) up to 24 h p.i (1). Except for the liver, pancreas, and spleen, all amyloid-free organs in the mice showed

rapid loss of radioactivity during this period. In addition, the retention of radiolabeled p5 was higher than that from labeled p1 in all organs with amyloid deposits. The whole-body clearance $T_{1/2eff}$ values of ^{125}I-labeled p1 and p5 for the WT mice were calculated to be 1.2 ± 0.01 h and 1.1 ± 0.1 h, respectively. The $T_{1/2eff}$ values of the radioiodinated peptides in the AA mice were 3.3 ± 0.08 h and 2.3 ± 0.3 h, respectively. SPECT images of the mice acquired at 24 h p.i. showed that the liver and spleen were visible only in animals injected with p5. These organs were not visible after 8 h p.i. in the animals injected with the labeled p1 peptide. The precise location of amyloid deposits observed in the liver and spleen of AA animals injected with the labeled p5 peptide was confirmed with autoradiography and by the co-localization Congo Red stain in the tissue sections of these organs.

From these studies, the investigators concluded that the p5 peptide is a potential noninvasive imaging agent for the detection and monitoring of amyloidosis in patients (1).

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.

Supplemental Information

[Disclaimers]

No information is currently available.

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