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SPECT imaging of peripheral amyloid in mice by targeting hyper-sulfated heparan sulfate proteoglycans with specific scFv antibodies

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Abstract

Introduction: Amyloid deposits are associated with a broad spectrum of disorders including monoclonal gammopathies, chronic inflammation, and Alzheimer's disease. In all cases, the amyloid pathology contains, in addition to protein fibrils, a plethora of associated molecules, including high concentrations of heparan sulfate proteoglycans (HSPGs).
Methods: We have evaluated radioiodinated scFvs that bind HS for their ability to image amyloid deposits in vivo. scFv's with different binding characteristics were isolated by phage display using HS extracted from bovine kidney or mouse and human skeletal muscle glycosaminoglycans (GAGs). Following purification and radioiodination, the biodistribution of ^{125}I-scFv's was assessed in mice with inflammation-associated AA amyloidosis or in amyloid-free mice by using SPECT imaging, biodistribution measurements and tissue autoradiography.
Results: Four different scFv's all showed binding in vivo to amyloid in the spleen, liver and kidney of diseased mice; however, three of the scFv's also bound to sites within these organs in disease free mice. One scFv specific for hypersulfated HSPGs preferentially bound amyloid and did not accumulate in healthy tissues.
Conclusions: These data indicate that HS expressed in amyloid deposits has unique qualities that can be distinguished from HS in normal tissues. A scFv specific for rare hypersulfated HS was used to selectively image AA amyloid in mice with minimal retention in normal tissue.

Keywords: AA Amyloid; Mice; scFv; SPECT imaging; Heparan sulfate

1. Introduction

Amyloid diseases are characterized by organ deposition of fibrillar forms of several different proteins [1,2]. The peripheral amyloids, most cases of which are associated with the deposition of immunoglobulin light chains, serum amyloid protein A (sAA) or transthyretin, can involve a single or many different organs depending on the patient. The ability to image the distribution and extent of disease will contribute not only to diagnostic and prognostic staging but also to planning and evaluation of treatment efficacy. Very early during the course of the disease progression, amyloid deposits acquire accessory molecules including SAP [3] and heparan sulfate proteoglycans (HSPG) [4–6]. HSPGs are a heterogeneous group of glycoproteins in which core proteins are decorated with heparan sulfate-containing oligosaccharides (HS). These HS molecules are a group of related linear polysaccharides that vary in molecular weight and have a high degree of molecular variability that is largely due to differences in epimerization and the extent and pattern of sulfation of hydroxyl groups within the sugars comprising the linear backbone [7]. There is some evidence

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that hypersulfated HS units are selectively found in amyloid deposits [8]. While it is clear that different forms of HS are expressed at different anatomical sites in the body, determining the exact chemical structures has been challenging [9,10]. Thus, understanding the slight differences in structure has been largely empirical. We have identified a number of scFv's that bind to HS from various sources with different avidities [11–14]. In an effort to develop imaging agents that are diagnostic for amyloid deposits, we have tested four of these scFv's in mice for their biodistribution as radioiodinated tracers. The fact that HSPGs are ubiquitously distributed in the plasma and basement membranes of normal tissue complicates the use of many anti-HS reagents for the specific detection of amyloid deposits [15]. We hypothesized that at least three factors would allow us to distinguish HS associated with amyloid from that expressed in normal tissues: 1) very high concentrations of HS are present in the amyloid, 2) unique bio- or electrochemical features of the amyloid-associated HS might be preferentially recognized by an scFv with appropriate specificity, 3) enhanced exposure of the amyloid to the circulation promotes first or early pass association of the targeting molecule to the in vivo injected tracer.

An experimental murine model of systemic AA amyloidosis served as our test system. The transgenic mouse strain, H2-L^d-huIL-6 Tg Balb/c (C) (H2/huIL-6), expresses the human interleukin 6 (hIL-6) pro-inflammatory cytokine constitutively under the control of the murine major histocompatibility complex H2-L^d promoter [16–18]. Consequently, these animals express high levels of acute phase proteins, notably sAA, the precursor of AA amyloid. This results in the natural onset of disease by ~4-5 months of age. Amyloid appears initially in the spleen; however, fueled by circulating sAA levels of >1 mg/mL, it progresses rapidly to involve the liver, pancreas, adrenal, heart, vasculature and kidneys. By ~8 months of age the mice become moribund due to failure of involved organs. The onset of AA in IL-6 expressing transgenic mice can be induced or accelerated by injection of amyloid enhancing factor (AEF), a splenic extract containing pre-formed fibrils isolated from mice with AA [16]. This model of inducible AA has been used repeatedly as a robust, predictable system for in vivo evaluation of the efficacy of amyloid-binding reagents using small animal imaging [17,18].

In this report, we demonstrate that all four anti-HS scFv's localize specifically at the sites of amyloid deposits in mice with heavy amyloid burden and that patterns of deposition can be clearly differentiated from those in normal mice. NS4F5, an scFv recently shown to bind hypersulfated HS moieties [8], proved to be unique in that it did not bind to HS in normal mice, enhancing its value as an imaging agent for diseased tissue. This scFv, or improved molecular forms, may prove useful for imaging peripheral amyloidosis.

2. Materials and methods

2.1. ScFv purification and radiolabeling

Heparan sulfate (HS) reactive scFv's HS4C3 [13], NS4F5 [14] and HS4E4 [19], as well as the chondroitin sulfate (CS)-binding scFv, GD3G7 [20], were derived by panning the phagemid synthetic scFv display Library No. 1 (from Dr. G. Winters, Cambridge University, Cambridge, UK) on HS from bovine kidney (Sigma) or human lung, as well as rat embryo-derived CS, using established techniques [11,21] (Table 1). Antibodies were isolated from periplasmic extracts of phagemid infected HB2151 E. *coli* using nickel affinity chromatography as described previously [11]. Purified scFv's were analyzed on SDS-PAGE and judged to be >95% pure by observation of the amount of CBB stain located in the gel region between 27 and 30 kDa (data not shown). Some aggregation was noted in samples stored for over 1 month or those that were frozen and thawed frequently; therefore, purifications were conducted within about 1 week of radiolabeling. ScFv's (20-100μg) were radiolabeled with 0.5 –2.5 mCi ^{125}I (Perkin Elmer) using 5μg of chloramine T from a freshly prepared water stock solution of 1 mg/mL. The reactions were performed in a final volume of <500μL of 0.1 M sodium phosphate buffer, pH 7.6 for 1 min at RT. The reaction was quenched by addition

Table 1
Binding characteristics of scFv's reactive with GAGs

scFv Antibody	Immunogen	V_H CDR3 Sequence	GAG reactivity	Preferred chemical group	Ref
GD3G7	Rat embryo: glycosaminoglycan extract	GRWTQMT	CS-E	GalNAc4S6S	[20]
HS4E4	Bovine Kidney: HS	HAPLRNTRTNT	HS	*N*-acetylation *N*-sulfation C5-epimerization	[19]
HS4C3	Bovine Kidney: HS	GRRLKD	HS	IdoA2S-GlcNS3S6S *N*-sulfation 2-*O*-sulfation 6-*O*-sufation 3-*O*-sulfation	[13]
NS4F5	Human lung: HS extract	SGRKGRMR	HS	*N*-sulfation C5-epimerization 2-*O*-sulfation (high) 6-*O*-sulfation (high)	[14]