99mTc-Aprotinin in Diagnosis of Cardiac Amyloidosis

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Aprotinin is a serine protease inhibitor. Several studies investigated the use of ^{99m}Tc-labelled Aprotinin as an amyloid seeker. In vitro tests showed high binding affinity for several types of amyloid fibrils accompanied by an excellent specificity. Initial human studies demonstrated good accuracy in detecting cardiac involvement. Scintigraphy results were confirmed in a group of 28 endomyocardial biopsies. Unfortunately, clinical studies were halted because of a temporary suspension of the vector protein (Trasylol) and public health concerns over prion contamination of the bovine origin compound. To obviate these limitations, efforts have been made to label a recombinant Aprotinin with 99mTc, which exhibits the same affinity for h-insulin fibrils.

Keywords: cardiac amyloidosis ; technetium aprotinin ; recombinant aprotinin ; 30-51 SS cyclic peptide: synthetic insulin fibrils ; radioactive Gallium ; PET

1. Introduction

The advent of effective therapies for the most common types of amyloid cardiomyopathy in recent years has boosted interest in these rare diseases, with the development of new imaging tools ranging from MRI through bone scintigraphy tracers, to PET imaging with radiotracers used for Alzheimer amyloidosis. Cardiac amyloidosis is often misdiagnosed, or its recognition is delayed as a result of both physician- and/or disease-related circumstances. Advances in multi-parametric cardiac imaging, including cardiac echography, have led to a deeper understanding of the disease process and to the tracking of treatment responses. However, no single diagnostic approach is so far able to perform a differential diagnosis, an early diagnosis and—in line with rapidly expanding treatment regimens—to monitor responses and assist with the adjustment of treatment strategies.

When we started our investigation on labeled aprotinin in 1995, the tracer available for systemic amyloidosis was ^{123}I-SAP, which cannot, however, detect cardiac amyloidosis [1]. ^{99m}Tc-labeled aprotinin (TcA) was introduced into the nuclear medicine armamentarium during the eighties due to its high renal tubule uptake reflecting kidney function [2]. Several years later, the same compound was studied for the imaging of cardiopulmonary amyloidosis with promising results [3], although this was scarcely available outside the UK [1] because cardiac involvement was poorly visualized with ^{123}I-SAP.

Aprotinin, a serine protease inhibitor, was commonly employed (as Trasylol) in cardiac surgery to prevent blood loss until the Blood Conservation Using Antifibrinolytics in a Randomized Trial (BART) study initiated in response to concerns about increased mortality associated with this agent induced the EMA to suspend marketing authorization in 2008 [4]. However, the Agency's Committee for Medicinal Products for Human Use (CHMP) found that there were a number of problems with the way the BART study was conducted, and the EMA recommended lifting the suspension of aprotinin in 2012 following the publication of the final BART study and other clinical studies showing that the benefits of aprotinin outweigh its risks in restricted indications [5][6].

2. Binding Mechanism and Specificity

Proteolytic remodeling of the amyloid precursor involving serine-proteases is a critical step in the formation of AL and ATTR amyloid fibrils [7][8].

However, in an elegant in vitro study, Cardoso et al. [9] were able to demonstrate that: (1) there is specific binding of ^{125}I-aprotinin to different types of fibril such as h-insulin (Ka 2.9 × 0.37 μM^{-1}}, TTR V30M, λ-BJP and Aβ (1-42) without interaction with amorphous precipitates and/or soluble fibril precursors; (2) thioflavin and Congo Red do not compete for binding, indicating a different interaction; and (3) aprotinin binding has two major components, namely its interaction with the β-structure elements of both fibrils and ligands and an electrostatic effect.

There are other reports regarding the specificity of Tc-labeled aprotinin, the lack of significant binding to EGFr tumours in a mouse model, and the low non-specific uptake in an experimental rat model of sterile or Staphylococcus-induced inflammation [10][11].

3. Radiopharmacokinetics

Two papers have investigated the kinetics of the radiopharmaceutical after i.v. administration in humans [12][13]. Blood clearance is rapid with a biexponential function, with only 15% of the i.d. still circulating in the blood of a subject with normal kidney function 30 min p.i., and a significant correlation was found between this parameter and creatinine clearance. Cumulative urinary excretion amounted to 8% during the first 6 h in one report and to 3.5% at 4 h in the other.Liver uptake was not negligible, being higher for instance in comparison to the commonly employed renal agent ^{99m}Tc-DMSA. Maximum uptake was observed 90 min p.i., after which, no significant changes ascribable to catabolism were noted.

Sojan et al. determined 84.0 ± 0.8% of urinary radioactivity to be ^{99m}Tc pertechnetate and 10.0 ± 7.0% to be unchanged ^{99m}Tc-Aprotinin. However, some differences are remarkable between this study and the Bellitto et al. report [14] that found that, as with other low m.w. proteins, TcA is taken up by the tubular cells, metabolized, and in part excreted as labeled degradation products with a molecular weight of approximately 1 kDa. There is no immediate explanation for this discrepancy; even if the kits employed are slightly different (direct Sn Cl_2 in one kit and stannous pyrophosphate in the other), the same amounts of aprotinin and reducing agent were employed [15].

4. Endomyocardial Biopsy

Myocardial biopsy represents the gold standard for validating the results of an imaging test—even if this involves the risk of sampling error—and a sufficient number of biopsies is therefore mandatory to reduce the risk of a false negative result; additionally the Congo Red staining is not without problems [16].

Validation of the TcA cardiac scan by myocardial biopsy has been reported by four groups working separately [3][17][18][19][20] and the results are summarized in **Table 1**. The data from the study by Awaya et al. [20] refers only to planar scans—not only to ensure uniformity with the other data, but also due to the discrepancies between the planar and SPECT-CT results that they reported. In fact, five planar scans produced true positives in the five subjects with positive biopsies, while SPECT-CT produced false positives in three out of five patients with negative biopsies [20].

Table 1. Comparison between Tc-A scans and endomyocardial biopsy results in 28 subjects. Data recorded from refs. [3][17][18][19][20].

		Endomyocardial Biopsy	
		+	−
Tc-A scan	+	16	0
	−	1	11

Table also includes necroscopy results. Note that the Awaya et al. results [20] refer only to planar scans.

The negative biopsy group in the table refers not only to amyloidosis patients without cardiac involvement but also to different cardiac diseases such as idiopathic cardiomyopathy (n.2) and infiltrative desmin cardiomyopathy (n.2) [3][18].

5. Safety

No adverse events were reported in either patients or control subjects following administration of 99mTc-Aprotinin. Despite the allergenic potential of aprotinin, it is interesting to note that the amount usually employed is largely inferior to the 3500 KIU usually contained in a multidose vial, while the amount suggested for testing the risk to allergic/anaphylactic reactions is equivalent to 10,000 KIU [21].

6. Dosimetry

Following the i.v. administration of 500–700 MBq, the estimated dose equivalent in healthy subjects has been estimated to be <10 mSv [17], which is in the same range as reported by Sojan et al. [13] of 1.4–2.0 mSv for a dose of 250 MBq and by Han et al. [18] of 1.6 mSv for a dose of 200 MBq.

7. Labeled Aprotinin in the Present Amyloid Cardiac Imaging Landscape

There is a need for a diagnostic radiopharmaceutical for CA which can fulfill the following requirements: (a) readily available, non-invasive, able to quantify the amyloid burden, and easily reproducible; (b) sufficiently sensitive to reveal subtle amounts of fibrils providing an early diagnosis, and (c) adequate for assessing progression or responses to therapy. This last characteristic aroused great interest following the introduction of new drugs able to modify cardiac involvement [22][23]. More than 30 proteins are present in the fibril-associated amyloid, with AL and ATTR being the most common. Therefore, another question arises—namely, whether a radiopharmaceutical with approximately the same sensitivity for all types of amyloid or one which is more selective for a specific type is preferable for assisting in the diagnostic workup.

In the present cardiac amyloid imaging landscape, technetium-labeled phosphonates, especially -DPD, and -PPi in US, are the most commonly employed tracers. They preferentially detect ATTR-associated cardiac amyloid rather than AL, but lack the ability to quantify disease burden over time and to correlate with serum biomarkers [24]. Despite the fact that the vast majority of patients studied with TcA were affected by AL amyloidosis, positive cardiac scans were obtained also in ATTR and AA—thus confirming the in vitro experience with different amyloid fibrils [9].

However, the binding mechanism of labeled aprotinin is highly specific for fibrils and very different from that observed with ^{99m}Tc-DPD and –PPi, which bind amorphous precipitates with the same kinetics of fibrils [25]. More recently, Thelander et al. demonstrated that the binding of bone tracers to amyloid-containing hearts depends on an irregular presence of clouds of very tiny calcifications, which seem not to be directly associated with amyloid fibrils [26].

The introduction of PET radiopharmaceuticals such as ^{11}C-PIB, ^{18}F-florbetapir and ^{18}F-florbetaben has changed the molecular imaging approach. They are potentially able to detect early amyloid deposition and to correlate with disease progression. They preferentially bind to AL fibrils in comparison to ATTR [23]. These tracers demonstrate a preferential affinity for AL rather than ATTR, but the binding site is different from that of aprotinin—which is not displaced by thioflavin in vitro and is stably retained in vivo in amyloid deposits, while PET tracers undergo a washout process [9][23]. This may be a further advantage of aprotinin being either Tc or Ga-labeled, allowing a wider imaging-window time with a unique limit of radioactive decay.

There is only one tandem study by the Japanese group comparing the performance of ^{11}C-PIB PET and TcA SPECT/CT in nine AL patients [27]. Disagreement was reported in five of them, with four apparently false positive TcA scans. From a so-limited number of cases, and for the reasons mentioned above relating to possible technical artifacts with TcA SPECT, it is difficult to draw reliable conclusions.

^{124}I-peptide p5 + 14 is a new emerging PET pan-amyloid radiopharmaceutical, binding via electrostatic interactions to electronegative glycosaminoglycans (GAGs) and protein fibrils—two ubiquitous components of amyloid deposits. Pathological heart activity, either AL or ATTR, increases up to 6 h and remains almost stable for up to 24 h. Uptake correlates with serum NT-proBNP in AL but not in ATTR patients [28][29]. Potential limits relate to the 124 I label (half-life 4.2 days)—with a restricted availability—in vivo dehalogenation with accumulation of free iodine in the stomach, thyroid, and salivary glands, and the elapsed time after injection required for imaging (6 h).

The peptidic approach suggests once again the use of a 30-51SS cyclic peptide. Due to its peptidic structure, it is suitable for Ga labeling via a bifunctional chelator with or without a spacer and, theoretically, could be a PET tracer with a higher affinity and a faster clearance than the parent molecule.

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