

# BIODISTRIBUTION OF AMYLOID-IMAGING PEPTIDE, p31, CORRELATES WITH AMYLOID QUANTITATION BASED ON CONGO RED TISSUE STAINING

Jonathan S. Wall, Tina Richey, Emily B. Martin, Alan Stuckey, Angela Williams, Sallie Macy, and Stephen J. Kennel  
 Departments of Medicine and Radiology, University of Tennessee Graduate School of Medicine, Knoxville, TN 37920

## Introduction

We have recently developed a peptide, designated p31 that binds specifically and with high avidity a unique form of amyloid-associated hypersulfated proteoglycan and that can be used to image amyloid deposits *in vivo* as evidenced using a mouse model of inflammation-associated (AA) amyloidosis (Fig. 1).

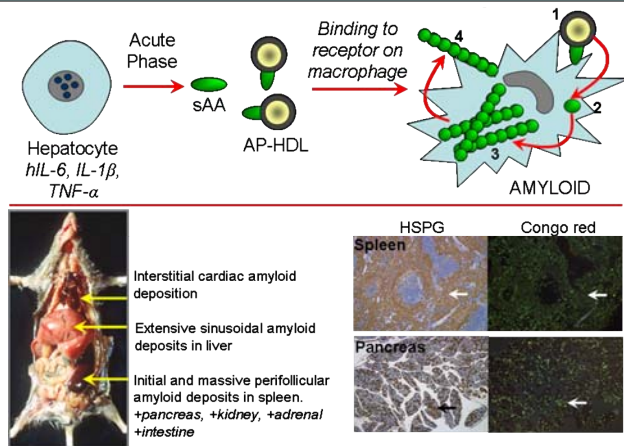


Figure 1. Murine model of AA amyloidosis

## Methods

We have compared the *in vivo* binding of  $^{125}\text{I}$ -p31 in amyloid-laden tissues with the amyloid burden, assessed using Congo red staining. Mice with AA were administered  $\sim 20 \mu\text{Ci}$  of  $^{125}\text{I}$ -p31, sacrificed 2 h pi. Tissues were analyzed for radioactivity and were also formalin-fixed, paraffin-embedded, and sectioned for autoradiography and Congo red staining. The amyloid burden in each tissue was determined by Congo red scoring (0 – 4+), and by measuring the area occupied by Congo red-birefringent material ( $\mu\text{m}^2$ ) in microscope images (Fig. 2).

## Results

Peptide  $^{125}\text{I}$ -p31 was shown by autoradiography to bind specifically to amyloid, as evidenced by the coincidence of silver granules with Congo red-birefringent amyloid seen in consecutive tissue sections (Fig. 3). Comparison of %ID/g  $^{125}\text{I}$ -p31 values for the liver and spleen with Congo red measurements revealed a significant positive correlation ( $>0.84$ ) with  $p$  values from  $10^{-5}$  -  $10^{-13}$  (Fig. 4 and table). Further, we demonstrated that there was a significant positive correlation (0.7) between the amyloid load in the liver and that in the spleen (Fig. 5 and table).

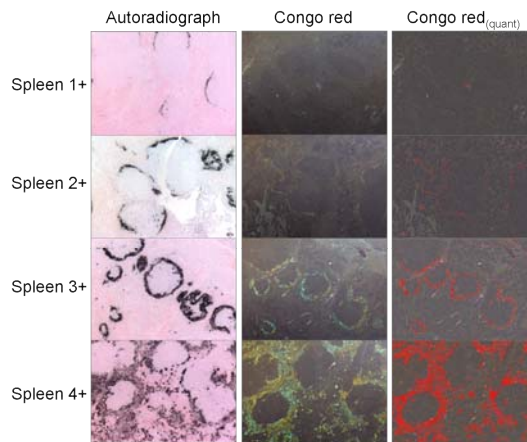


Figure 2. Visual comparison of three methods for quantitatively assessing amyloid load.

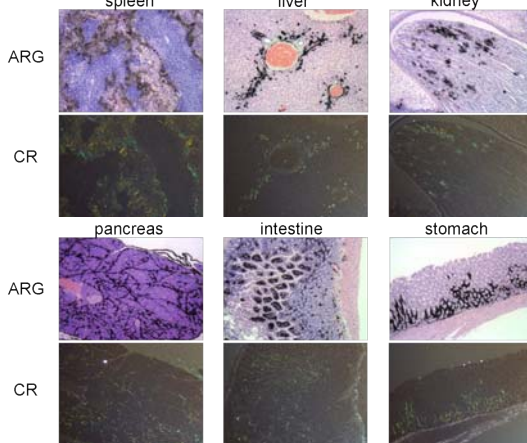


Figure 3.  $^{125}\text{I}$ -p31 binding to amyloid seen in autoradiographs (ARG) correlated with the Congo red (CR) staining.

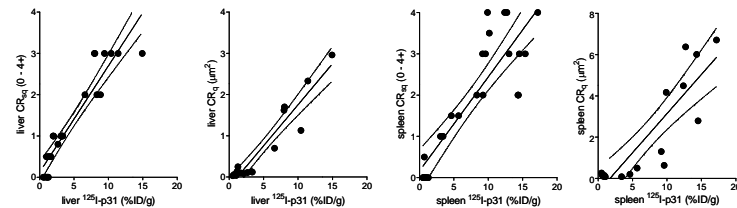


Figure 4. Correlation between  $^{125}\text{I}$ -p31 binding (%ID/g) and amyloid load based on Congo red

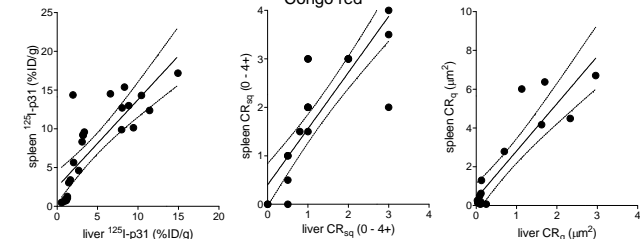


Figure 5. Correlation between amyloid load in the liver and spleen (mean with 95% confidence intervals)

Organ	Correlation	$R^2$ of linear regression	Spearman correlation coefficient	Spearman correlation P value
L	p31 vs L CR <sub>sq</sub>	0.9	0.95	$9 \times 10^{-13}$
L	p31 vs L CR <sub>sq</sub>	0.9	0.89	$5 \times 10^{-6}$
Sp	p31 vs Sp CR <sub>sq</sub>	0.8	0.84	$1 \times 10^{-7}$
Sp	p31 vs Sp CR <sub>q</sub>	0.8	0.85	$3 \times 10^{-5}$
L	CR <sub>sq</sub> vs Sp CR <sub>sq</sub>	0.7	0.69	$2 \times 10^{-9}$
L	CR <sub>sq</sub> vs Sp CR <sub>q</sub>	0.8	0.94	$5 \times 10^{-12}$
L	CR <sub>q</sub> vs Sp CR <sub>q</sub>	0.8	0.72	$2 \times 10^{-3}$

L, liver; Sp, spleen; p31,  $^{125}\text{I}$ -labeled peptide p31 method for estimating amyloid load; CR, Congo red birefringence-based method for estimating amyloid load; <sub>q</sub>, quantitative estimation ( $\mu\text{m}^2$ ); <sub>sq</sub>, semi-quantitative method by scoring (0 – 4+).

## Conclusions

Our analyses demonstrate a significant positive correlation between peptide p31 binding *in vivo* and amyloid load based on Congo red staining and support the hypothesis that radiolabeled p31 imaging (2011 SNM pub. # 83 and 228) can provide an accurate measure of tissue amyloid load as compared to the clinically used histological standard, Congo red with the obvious advantage of non-dependence on tissue biopsy.

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