

In vivo Tracking of the Neurodegeneration Induced by Intrahippocampal Injections of β -Amyloid

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Introduction

Alzheimer's disease (AD) is characterized by the development of intracellular neurofibrillary tangles (NFT) and extracellular neuritic plaques (NP) in various areas of the brain [1]. These pathological developments have been linked to the neurotoxicity of β -amyloid (β A), a small protein that ranges from 39 to 42 amino acids in length and forms the core of the NP. Autopsy studies have shown a reduction of neurons and a buildup of NFTs and NPs in regions of the hippocampus that correlate with the severity of AD [1]. This study capitalized on two important features of β A: 1) that β A-mediated cytotoxic actions seem to be localized in the fragment containing amino acids 25-35 of the peptide chain [2] and 2) that *in vivo* animal studies are similar in the neurodegenerative phase of β A mediated toxicity [3]. The reverse fragment of β A, amino acids 35-25 is non-toxic and provides a suitable control to determine the specific action of the 25-35 chain [4]. Here we report our apparent diffusion coefficient (ADC) results testing the hypothesis that β A could induce early degenerative changes near the injection site in the CA1 region of the hippocampal formation (HF).

Methods

Male Sprague Dawley rats (200-250 grams) were anesthetized with ketamine HCl (100 mg/kg) and xylazine HCl (10 mg/kg) (i.p.) and immobilized in a stereotaxic apparatus. A 1.8 mm burr hole was drilled above the CA1 region of the left dorsal HF (Bregma- A/P 3.6 mm, M/L 2.4 mm). The incision was closed after piercing the dura. Following a 7-day recovery period, the rats were imaged under anesthesia for pre-treatment scans prior to β A injections. One group of rats (n=6) received a single 1 mM β A 25-35 injection dissolved in ddH₂O (1 mL volume) into the CA1 region (D/V 2.6 mm). A second group (n=4) received a similar concentration and volume of the reverse peptide, β A 35-25 in the same hippocampal region. All injections were delivered with a 1 mL syringe at an infusion rate of 0.1 mL/min for 10 minutes, minimizing mechanical damage to the HF. The needle was left in place for 6 minutes prior to removal to reduce the dispersion of the injection fluid along the cannula tract. MR scanning was conducted at 0.25, 1, 24 hours and 7 and 14 days post treatment. Imaging was performed using a Siemens 1.5T (63.0 MHz) SP Magnetom scanner. Animals were anesthetized as above and placed on a plastic stereotaxic head holder fitted under a 150 mm diameter small field of view (FOV) Helmholtz coil. Spin echo T1-weighted (T1W) (TR/TE: 480/15 ms) and DW (TR/TE: 2200/111 ms, $b = 1228$ s/mm²) data sets were collected with 2 acquisitions in a 128x128 matrix with a 50 mm FOV. ADC maps were computed from a two point fit to: $ADC = \ln(So/Sn)/b$.

Image/Data Analysis - Cheshire™ (Hayden Image Processing Group) was used to outline and analyse the region of interest (ROI), ~8 pixels each: the dorsal mid-section of the CA1 region of the HF (Bregma-A/P 3.6 mm). Each region was traced bilaterally on T1W images, copied and adjusted on T2 images ($b=0$), then overlaid onto corresponding ADC maps. Mean values were calculated for the ROIs and normalized according to $Observed\ value/Pre-treatment\ value \times 100$ (Observed value = mean ADC at any given time point). Two-tailed Student's t-tests were used to analyse differences between hemispheres and treatment groups.

Results and Discussion

β A 25-35 - Mean ADC values in the ipsilateral ROI did not change significantly during the first 7 days (168 hours) of observation (Fig. 1). At 14 days (336 hours) post β A injection, there was a highly significant (*, $p=0.01$) increase in mean ADC (17%) in the ipsilateral (injected) area of the CA1 region compared to normal. Similarly, no changes in ADC values in the contralateral side were observed during the first week. At 14 days post treatment ADC values were 15% lower than normal (85.2 ± 6.5 ; $p=0.01$) (Fig. 1). **β A 35-25** - From 0.25 hours to 14 days post β A injection, no significant differences in ADC values were observed. Values fluctuated within 15% of normal on the ipsilateral and contralateral sides (Fig. 2).

The ADC increase (Fig. 1) temporally correlates with histological reports of extensive neuronal damage near the injection site,

macrophage invasion within the outer walls of blood vessels and phagocyte deposition [5]. The reduction in mean ADC at 14 days observed in the contralateral CA1 suggests a secondary response in the pyramidal cells of this region, possibly mediated through axonal projections between the CA fields of the HF [6]. Histological studies report an incidence of abnormal swelling with distended neurons distal to the site of injection [4]. The reduction in ADC is consistent with migration of extracellular water, having high intrinsic diffusion, into intracellular compartments [7]. Water diffusion is restricted within the more viscous cytosolic compartment. The fluctuation of ADC values in the β A 35-25 treated animals is more likely due to mechanical damage from insertion of the cannula and support previous reports of the inert nature of this peptide [4]. In conclusion, we observed specific β A-induced neurotoxicity using DWI. This model and our approach hold great promise for understanding the process of β A-mediated neurodegeneration and its role in the development of AD.

Fig. 1- ADC within CA1 after β A 25-35

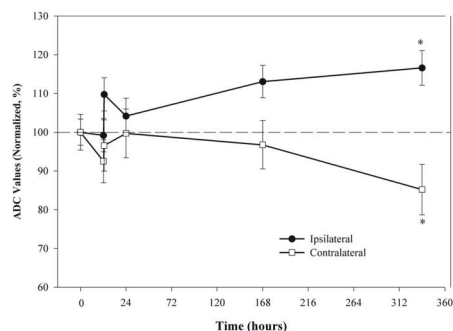
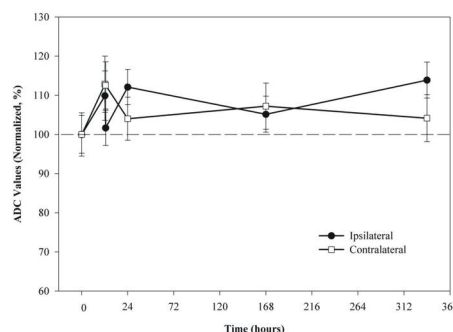


Fig. 2- ADC within CA1 after β A 35-25



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