

The Effect of Amyloid on Infarct Size in a Rat Model

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Introduction: Alzheimer's disease affects over 26 million people worldwide and is predicted to affect 1 in 85 people globally by 2050 (1). For years, neuroscientists have known that the risk of Alzheimer's disease is nearly doubled among people who have had a stroke. After a stroke there is an increase in the production of the toxic amyloid beta (A β) peptides that are implicated in the Alzheimer disease cascade (1,2). The current work investigates the effect of A β peptide on infarct size in the rat brain. The hypothesis is that infarcts induced in the presence of A β will be larger than infarcts that occur in the absence of this peptide.

Methods: Four groups of animals were studied. Group 1 (N=2) consisted of animals that received a ventricular A β injection; Group 2 (N=4) consisted of a group of animals that received A β injection and had sub-cortical stroke induced; Group 3 (N=3) consisted of A β sham animals (saline injection); and Group 4 (N=3) consisted of stroke sham animals (saline injection). For groups 1-3 A β or saline was injected separately into both ventricles by stereotaxic injection. For groups 2 and 4, stroke was induced by stereotaxic injection of 60 pM endothelin into the right striatum. The animals were anaesthetized with 1.5% isoflurane for imaging on a 9.4 Tesla Varian (Palo Alto, CA) small animal MRI scanner. T2 weighted 2D FSE and TrueFISP imaging (TR/TE = 4000/29 ms, image matrix size 256 x 256 x 64, FOV 38.4 x 38.4 x 19.2 mm³, thickness: 150 x 150 x 300 μ m³) was performed on each animal on days 9, 19 and 29 post surgery. Images were acquired on days 9, 19 and 29 after surgery and then animals were sacrificed for histology. OX-6 and Fluoro-Jade® B staining was performed. The stroke volumes were manually calculated using ImageJ plugins for volume calculation. Similarly, the ventricle sizes were manually segmented from 3 consecutive slices in the brain, also using ImageJ software for data analysis.

Results: The combined average stroke volume in Groups 2 (stroke+A β) and 3 (stroke only) was 2.35 ± 1.06 mm³ and there was no difference in stroke volume between groups. No significant differences were found between ipsilateral and contralateral ventricle volume in all animals. Figure 1 shows a control animal (Group 3) and an animal from Group 2 on day 29 demonstrating the significant difference in ventricle appearance. However, the average ventricle volume in Groups 1 and 2 combined (8.7 ± 7.0 mm³, A β injection) compared to Group 3 (2.1 ± 0.4 mm³, A β sham) were not significantly different. In combined A β /endothelin-treated rats, there was a greater intensity of staining of both OX-6-positive microglia, compared with endothelin or A β -treated rats (Figure 3). Brown regions in Fig. 3 signify the presence of inflammation in the brain. Fluoro-Jade® B is a polyanionic fluorescent derivative which sensitively and specifically binds to degenerating neurons. Green regions in Figure 3 signify the presence of dead cells in the magnified areas of OX6 stained cells, as shown within the rectangles.

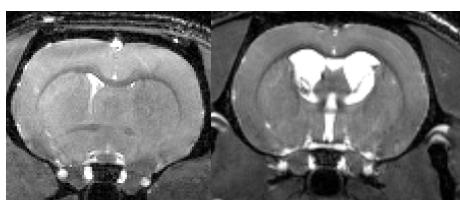


Fig.1 demonstrates the presence of enlarged ventricles in a representative animal from the control group (left) and the combined ET1/ A β group at day 29 (right)

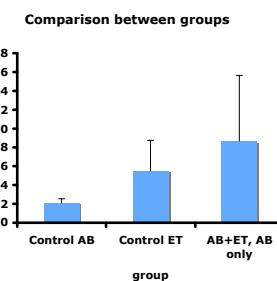


Fig.2 Ventricle size comparison

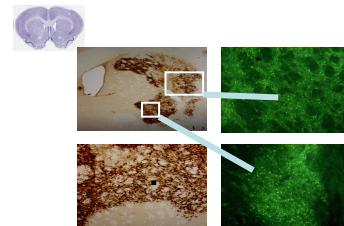


Fig.3 Histology image of a representative animal, showing two types of stain- OX6 section in brown and Fluoro-Jade staining in green.

Discussion: These preliminary data show that ventricle enlargement was present in many animals that received A β injection and that tissue damage was visible in the striatum in animals receiving endothelin injection. Alzheimer's disease is characterized by a loss of neurons and synapses in the cerebral cortex and some subcortical regions. Therefore, the observed ventricle enlargement could be the result of atrophy of adjacent brain tissue following A β injection. Endothelin-induced ischemia resulted in an extensive increase in immunostaining in the right striatum in the region of the infarct. OX-6-positive stained microglia covered a large area of the striatum, signifying the presence of inflammation as a result of the injection.

References: (1) Alz.org Alzheimer's association education and awareness. (2) Alzheimer's society of canada (website).