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Aβ(42) induced MRI changes in aged rabbit brain resembles AD brain

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ABSTRACT

Alzheimer's disease is the most common form of dementia and is structurally characterized by brain atrophy and loss of brain volume. Aβ is one of the widely accepted causative factors of AD. Aβ deposition is positively correlated with brain atrophy in AD. In the present study, structural brain imaging techniques such as Magnetic Resonance Imaging (MRI) were used to measure neuroanatomical alterations in Alzheimer's disease brain. MRI is a non-invasive method to study brain structure. The objective of the present study was to elucidate the role of Aβ on brain structure in the aged rabbit brain. Among 20 aged rabbits, one batch ($n = 10$) rabbits was injected chronically with Aβ(1–42) and another batch ($n = 10$) with saline. The MRI was conducted before Aβ(1–42)/saline injection and after 45 days of Aβ(1–42)/saline injection. All the aged rabbits underwent MRI analysis and were euthanized after 45 days. The MRI results showed a significant reduction in thickness of frontal lobe, hippocampus, midbrain, temporal lobe and increases in the lateral ventricle volume. We also conducted an MRI study on AD ($n = 10$) and normal ($n = 10$) cases and analyzed for the thicknesses of frontal lobe, hippocampus, midbrain, temporal lobe and lateral ventricle lobe. We found significant reductions in thickness of the frontal lobe and the hippocampus. However, no significant reduction in the thickness of midbrain, temporal lobe or increase in the lateral ventricle volume was observed compared to normal. Correlations in brain atrophy changes between rabbit brain and human AD brain were found for frontal lobe and hippocampal regions. In contrast, other regions such as midbrain, temporal lobe, and lateral ventricles were not correlated with rabbit brain atrophy changes in the corresponding regions. The relevance of these changes in AD is discussed.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in elderly people (Hardy and Higgins, 1992). AD is structurally characterized by brain atrophy due to loss of neurons (Vemuri et al., 2009). Among many risk factors involved in the cause of AD, Amyloid beta (Aβ) is considered as key player (Selkoe, 2001). The reasons for neuronal cell death in AD is still not clear (Gupta et al., 2006). Further, there are no reliable biomarkers for AD (Georganopoulou et al., 2006). It has been attributed that CSF and plasma Aβ as biomarker and many researchers even try to correlate the Aβ versus brain atrophy. Recent studies clearly indicated that MRI changes in brain may support as good imaging biomarker for early diagnosis of AD (Kantarci, 2005; Rabinovici and Jagust, 2009). But still scientists are debating on this relation (Rombouts et al., 2005). The MRI measures the change in whole brain atrophy and different regional atrophy of the brain (Jack et al., 2005, 2004; Thodberg, 2003). Studies involving AD patients have shown early involvement of medial temporal lobe structures such as entorhinal cortex and hippocampus in the disease initiation (Barnes et al., 2004; Lerch and Evans, 2005).The decrease in the hippocampal volume is associated with severity of the AD (Jack et al., 2005, 2004). The study of Archer et al. (2006) reported that there is a positive correlation between amyloid load and cerebral atrophy in AD. Further studies indicated that the Aβ deposition load is found to have correlation with the degree of neuronal damage and cognitive deficits (Davies et al., 1988; Mann et al., 1985). The microinjection of Aβ into cortex, hippocampus or amygdala has been reported to produce neuron loss and cholinergic degeneration (Chen et al., 1996). Jack et al. (2010) reported that time required to progress from mild cognitive impairment to AD by measuring the brain atrophy and Aβ load. They found that MCI subjects with high amyloid load take less than two years to progress to AD while, MCI subjects with less amyloid load takes more time to progress to AD. Additionally, MCI subjects with hippocampal atrophy coupled with high amyloid load levels still take less time to progress from MCI to

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AD. However there is no animal model to mimic the total AD pathology (Bharathi et al., 2006). Recently, Ramesh et al. (2010) has reported that intracisternal injection of Aβ(1–42) to aged rabbits has significantly altered the DNA conformation in frontal cortex, hippocampus and midbrain. Also, Aβ injection altered DNA stability in brain regions. But these changes do not correlate with the changes in the rabbit brain atrophy. Ramesh et al. (2010) observed that 25 days of Aβ injection is not sufficient to induce the brain atrophy though there are significant DNA instability and DNA conformation changes.

The present study is planned to investigate the effect of intracisternal injection of Aβ(1–42) on aged rabbit brain in 45 days. The brain structural changes in terms of thickness of frontal lobe, hippocampus, temporal lobe, midbrain and volume of lateral ventricle are analyzed. Additionally, the MRI data of rabbits is compared with AD patients MRI. This study will give an insight in understanding the role of Aβ(1–42) in inducing the brain atrophy in AD.

2. Materials and methods

Aβ(1–42) was purchased from BZ Biolab Limited, USA. Saline was purchased from local medical store.

2.1. Animal protocol

All animal procedures were carried out in accordance with Indian National Science Academy (India) animal protocol guidelines and rules framed by JSS Animal Ethical Committee, JSS Medical College, and Mysore, India. All the animals were housed in JSS Animal house in separate stainless steel cages. 10 New Zealand white aged rabbits (4.0 yrs old) received intracisternal injections of 100 μL of normal saline (control) and another batch of 10 aged rabbits received 100 μL of (1 mg/mL) Aβ(1–42) in saline. The injections were carried out under calmpose anesthesia, according to the modified protocol (Savory et al., 1999). The MRI was done before Aβ(1–42)/saline injection and after 45 days of Aβ(1–42)/saline injection. All the aged rabbits were subjected for MRI euthanized after 45 days. Aβ(1–42) injected animals have developed neurological symptoms including forward head tilting, hemiplegic gait, loss of appetite, isolation behavior, splaying of extremities and paralysis. These behavioral changes were the results of observations made on regular basis of every day from date of injection. The control animals did not display any behavioral disturbances.

2.2. MRI protocol for scanning aged rabbit brain

The rabbits' brain were imaged using MRI before injecting the Aβ(1–42). MRI images of aged rabbits injected with Aβ(1–42) were taken after 45 days of injection. Scanning was done on Siemens Aavanto 1.5 T MRI scanner. Animal head was scanned using small flex coil for better positioning of the head in center of the magnetic field so as to obtain better signal noise ratio. Animals were under the influence of diazepam (10 mg) for about 20 min, which was sufficient to complete the MRI protocol. Aged rabbits were scanned in prone position. Images were acquired in coronal plane using T1, T2 and 3D Gradient echo sequences. T2 sequences were obtained by using TR3500ms, TE79ms, slice thickness of 3 mm, and FOV 100 mm. T1 sequences were obtained by following protocol TR-613ms, TE 11ms, 256X256 MATRIX FOV 100 mm and slice thickness of 2 mm. 3D GRE sequences were obtained by following protocol-TR9.5 ms TE-4.76 ms FOV-100 ms and slice thickness of 1 mm. The comparison of control animals with treated animals were done with the aim of demonstrating any structural change in the brain parenchyma which points towards loss brain volume. The thickness of frontal lobe, hippocampus, midbrain, temporal lobe and lateral ventricle was using system-attached software in MRI machine. Measurements were obtained for T2 weighted images.

2.3. MRI protocol for scanning normal and human AD brain

Ten normal and 10 AD patients were selected for the present investigation. The AD patients were diagnosed by NIH protocol by Psychiatry Professor of JSS Medical College, Mysore. MRI was done on all the above persons. Multiplanar, multisequence MRI was done on Siemens Avanto 1.5T system using following protocol. Axial –T1, T2 and FLAIR (Fluid Attenuated inversion recovery). Sagittal –T1, Coronal –T2, 3D -gradient echo. Susceptibility weighted imaging –in axial plane, for T1–TR 550ms and TE of 8.7ms was used for T2–TR of 5000ms and TE of 118ms was used for FLAIR sequence-TR was 9000 and TE was 102ms FOV for all sequence was 230 mm. 5 mm slice thickness was employed for all sequences, other than gradient sequence. 3D Gradient sequence was done in all patients in coronal plane with slice thickness of 1 mm. All patients were co operative and did not need any sedation. Typical total scan time for entire study was about 20 min.

2.4. Statistical analysis

The thickness and volume measurement data was analyzed using student *t* test in origin 6.0.

3. Results

3.1. MRI study of aged rabbits with or without Aβ(1–42) injection

The MRI is done for aged rabbits injected with Aβ(1–42)/saline. The results on the effect of Aβ(1–42) on thickness of different regions of aged rabbit brain frontal lobe, hippocampus, midbrain, temporal lobe and lateral ventricle is given in Tables 1 and 2. Fron-

Table 1
The mean of thickness (mm) of different brain regions (frontal lobe, hippocampus, midbrain, temporal lobe and lateral ventricle) of Aβ(1–42) injected and control rabbits. $n = 10$.

Regions	Mean		Inference
	Saline	Aβ(1–42)	
FL-L	2.72 ± 0.44	2.22 ± 0.26*	Significantly different
FL-R	2.76 ± 0.37	2.36 ± 0.26*	Significantly different
H-L	2.6 ± 0.54	1.93 ± 0.22*	Significantly different
H-R	2.52 ± 0.53	1.97 ± 0.22*	Significantly different
M	3.88 ± 0.84	3.76 ± 0.50	Significantly not different
TL-L	5.78 ± 8.84	4.72 ± 0.71*	Significantly different
TL-R	5.94 ± 0.65	5.07 ± 0.57*	Significantly different
LV-L	1.35 ± 0.34	1.81 ± 0.21*	Significantly different
LV-R	1.47 ± 0.37	1.9 ± 0.29*	Significantly different

* $p < 0.05$.

Table 2
The mean of the thickness (mm) of healthy and AD brain regions (frontal lobe, hippocampus and midbrain, temporal lobe and lateral ventricle) $n = 10$, $p < 0.05$.

Regions	Mean		Inference
	Control	AD	
FL-L	48.05 ± 2.08	42.3 ± 1.17	Significantly different
FL-R	46.2 ± 0.82	41.2 ± 2.03	Significantly different
H-L	19.2 ± 1.6	19.3 ± 2.08	Significantly not different
H-R	17.6 ± 0.82	14.5 ± 3.25	Significantly not different
M	23.75 ± 2.25	12.86 ± 2.71	Significantly different
TL-L	45.76 ± 1.69	38.6 ± 5.71	Significantly not different
TL-R	44.02 ± 2.92	37.42 ± 3.97	Significantly different
LV-L	10.28 ± 2.35	11.16 ± 2.26	Significantly not different
LV-R	9.72 ± 2.09	10.9 ± 1.97	Significantly not different