Alzheimer’s disease Animal Model by Aluminum, Beta-Amyloid and Transforming Growth Factor Beta-1

Fang Fang¹, Ning Yan², Zhanhui Feng¹, Xiangqin Liu¹, Zheng Xiao¹, Ming Wen¹, Hua Huang¹, Yong Yan¹*

Abstract: Alzheimer’s disease (AD) is a chronic, progressing neurological degenerative disease of central nervous system. With the aging of population and prolonging of life expectancy, the incidence of AD is rising. Epidemiologic survey shows that the incidence of AD in China is 3.5% in the elderly (>65y) [1, 2]. So far, the pathogenesis of AD is not very clear, and there is no specific method to cure it. Therefore, in the past few years, more and more scholars have been explored new animal models in order to mimic the clinical manifestations and patho-physiological characteristics of AD. However, all kinds of the models of AD have their merits and drawbacks, and they can’t imitate both the whole clinical praxiological features and the pathological, biochemical characteristics of AD. By analyzing and summarizing the two classic mono-factor animal models which are the one induced by injecting beta-amyloid1-40 (Aβ1-40) into cerebral ventricle [3, 4] and the other induced by aluminium intoxication [5], a new animal model of AD was establish by stereotaxically injecting Aβ1-40 and aluminium into lateral cerebral ventricle, and transforming growth factor β1 into anterodorsal nucleus of thalamus at the same time [6, 7]. The aim was to design a new, multi-factors, ideal animal model of AD that more mimic the complicated etiology, the praxiological features, pathological features and biochemistry changes of AD.

2. Material and method

2.1 Animal and main reagents

4-5 months old male Sprague-Dawley rats, 350-400g in weight, were provided by experimental animal center, Daping hospital of Third Military Medical University. Aβ1-40 is from Anaspec Company. Recombination human TGFβ-1 was purchased from PeproTeCh company, America. ChAT and Aβ1-40 polyclonal antibody is obtained from Bioss Co. Beijing.

2.2 Methods

2.2.1 Animal grouping

The rats were randomly assigned into three groups: sham operation group (normal saline), low dose model group (Aβ1-40 2ug + 1% AlCl3 2ul) and high dose model group (Aβ1-40 4ug + 1% AlCl3 3ul). Each group has 8 rats.

2.2.2 Model preparation

After the rats anesthetized with 3.5% chloral hydrate (10ml/kg, i.p.), two holes were drilled by dental auger at stereotaxically defined points. One point was the lateral cerebral ventricle (behind the bregma 1.2 mm, meta 2mm, deep 4mm), and the other point was the anterodorsal nucleus of thalamus (at the opposite side of the cerebrum, behind the bregma 2.1mm, meta 1.4mm, deep 4.6mm). The catheter was inserted into the lateral cerebral ventricle point [8], fixed at the cranium with dental base acrylic resin powder and 502 glue mixture, and at the same time, the catheter was blocked by the nylon line core which was a little less than the catheter inner diameter in order to prevent from infection and leakage of cerebrospinal fluid.

The sham operation group rats were serially injected normal saline into the lateral cerebral, 4ul per day for 14 days. The low dose model rats were serially injected Aβ1-40 into the lateral cerebral, 2ug per day for 14 days; 1% AlCl3 solution 2ul per day for 5 days. The high dose model rats were serially injected Aβ1-40 into the lateral cerebral, 4ug per day for 14 days; 1% AlCl3 solution 3ul per day for 5 days. Aβ1-40 was infused in the morning and 1%...
AlCl₃ solution in the afternoon. When infusing Aβ₁-₄₀ at the first time, 10 ng TGFβ₁-1 was injected into the cerebral anterodorsal nucleus of thalamus point. In order to guarantee the solution to fully enter into the brain tissue, these materials were slowly injected and kept the needle for 5 minutes.

2.2.3 Ethological test

The ethological test was done a week after the operation for 5 days. Spatial reference and wording memory of the rats was assessed with Morris water maze. In the first 4 days, fixing navigation experiment was done. In this experiment, the latency that rats searched for the platform was recorded. In the fifth day, the spatial exploration test was done. In this experiment, the times that the rats transited the platform in regulated time was recorded [9, 10].

The condition studying memory of the rats was assessed with the shuttle box test. The computer in the shuttle box can automatically record the tip-and-run times and time the rats suffered [11, 12].

2.2.4 Observation of the histopathological changes of the brain

After the ethological experiment, 3 rats of each group selected randomly were perfused with 4% paraformaldehyde. The brains were immersed into the fixation solution containing 4% paraformaldehyde for 24 hours, then embedded in paraffin and slit at 5 mm intervals along the coronal plane. Sections were stained with HE, Congo red and Bielschowsky silver staining.

2.2.5 Observation of the biochemical changes of the brain

The SP method of immunohistochemistry was used in this study. After sections were deparaffinaged, the activity of the endogenous peroxidase was inactivated by 3% hydrogen dioxide, and then leucocyte antigen was repaired by microwave. Normal goat serum was dropped in the paraffin sections and kept for 15 minutes, then anti-rabbit ChAT and Aβ were overnight at 4°C. The sections were incubated with cockheara enzyme labelling strepto-antibiotin fluid for 15 minutes at 37°C, then with biotin labeling goat anti-rabbit IgG for 15 minutes at 37°C, stained by DAB, after stained with hematoxylin and finally fixed and mounted with dimethyl benzene. The anatomical changes of immunoreactivity positive nerve cells in the cortex and hippocampus were studied with the light microscope. 5 sections were randomly selected, and 10 high power fields of vision were selected from each section. In the randomly selected fields of vision, the numbers of the positive nerve cells were counted by the high power lens.

2.3 Statistical analysis

Data were presented as mean ± standard. Group comparison was performed using mono-factor variance analysis. A P-value of 0.05 or less was considered significant.

3. Results

3.1 The learning and memory abilities

The spatial reference and wording memory of rats was assessed with the fixing navigation experiments at the first 4 days. The outcomes were presented in Table 1, the latency period for searching the platform of operation group rats were obviously prolonged as compared with that of the sham operation group rats, both at 1 week and over 3 months after lesion (P<0.01). The latent period for searching the platform of operation group rats at 3 months after lesion were also slightly longer than that at 1 week after lesion, but there was no significant difference (P>0.05). From the second to fourth day, the latent period for searching the platform in the high dose group were manifestly lasting more than that in the low dose group (P<0.05). The spatial exploration experiments at the fifth day were used to examine the spatial memory capability, and the results were presented in Table 2. The times of operation group rats traversing the location of the platform in the defined time (120 sec) at 1 week and 3 months after lesion were obviously decreased as compared with that of the sham operation group rats (P<0.01).

### Table 1 Latency of searching for the platform in Morris water maze test (sec)

<table>
<thead>
<tr>
<th>Day</th>
<th>Control group</th>
<th>Low dose</th>
<th>High dose</th>
<th>Low dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 week</td>
<td>1 week</td>
<td>1 week</td>
<td>3 months</td>
<td>3 months</td>
</tr>
<tr>
<td>1</td>
<td>86.83±23.24</td>
<td>114.89±17.24</td>
<td>119.03±2.94</td>
<td>115.28±8.28</td>
<td>117.08±4.55</td>
</tr>
<tr>
<td>2</td>
<td>44.75±24.88</td>
<td>80.31±36.95</td>
<td>96.52±16.30</td>
<td>87.78±21.67</td>
<td>97.58±14.40</td>
</tr>
<tr>
<td>3</td>
<td>27.28±16.78</td>
<td>45.83±28.44</td>
<td>54.89±13.99</td>
<td>49.13±15.25</td>
<td>58.40±15.86</td>
</tr>
<tr>
<td>4</td>
<td>14.27±7.72</td>
<td>26.23±10.69</td>
<td>31.98±8.24</td>
<td>29.55±11.03</td>
<td>34.25±9.14</td>
</tr>
</tbody>
</table>

3.2 The shuttle box test

The shuttle box test was used to examine the condition learning and retention ability of rats. The results were presented in Table 3. The shocked times of operation group rats were significantly increased compared with the sham operation group rats (P<0.01), and similarly, the shocked time of operation group rats was also obviously more prolonged than the sham operation group rats (P<0.05).

### Table 2 Times of traversing the platform in the Morris water maze test.

<table>
<thead>
<tr>
<th>Times</th>
<th>Control group</th>
<th>Low dose</th>
<th>High dose</th>
<th>Low dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 week</td>
<td>1 week</td>
<td>1 week</td>
<td>3 months</td>
<td>3 months</td>
</tr>
<tr>
<td>1</td>
<td>14±2.78</td>
<td>6.88±1.96</td>
<td>6.13±2.03</td>
<td>7.40±2.30</td>
<td>5.40±1.67</td>
</tr>
</tbody>
</table>

Compared with the control group. *P<0.01

### Table 3 The change of condition learning and retention ability

<table>
<thead>
<tr>
<th>Shocked times</th>
<th>Control group</th>
<th>Low dose</th>
<th>High dose</th>
<th>Low dose</th>
<th>High dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 week</td>
<td>1 week</td>
<td>1 week</td>
<td>3 months</td>
<td>3 months</td>
</tr>
<tr>
<td>Shocked times</td>
<td>8.38±1.69</td>
<td>12±2.39</td>
<td>13.63±3.11</td>
<td>15.4±4.34</td>
<td>17.6±2.51</td>
</tr>
<tr>
<td>Shocked time</td>
<td>21.25±6.04</td>
<td>51.6±25.05</td>
<td>57±32.99</td>
<td>57.6±31.58</td>
<td>79.6±34.20</td>
</tr>
</tbody>
</table>

Compared with the sham operation group. *P<0.01, #P<0.05

3.2 Pathological changes of brain tissues

**HE staining:** The nerve cells of the sham operation group rats were lined up regularly, the structures of neurons were clear with round, big and regular nuclei. On the contrary, the nerve cell nuclei of operation group rats were arranged disorderly, they were obvious pyknosis both at 1 week and 3 months after the operation, and the number of neurons showed decreased relative to the sham operation group.

**Congo red staining:** The amyloid presented red in Congo red staining. The result showed that in the cortex and hippocampus of sham operation group rats, there was no Congo red staining, and the amyloid substances deposited invisibly. However, in the same place of the operation group rats, there was Congo red staining, and the amyloid substances deposited obviously both at 1 week and 3 months after lesion.
Bielschowsky silver staining: The nerve fibers of the sham operation group rats were lined in order and tightly in Bielschowsky silver staining. But at 1 week after the operation, the operation group rats’ nerve fibers became thickened, intensively fused, twisted and the interspace was enlarged, all of the changes progressed over 3 months of observation. SP and NFTs-the marked pathological changes of AD, were scattered in cortex and hippocampus at 3 months after lesion (Figure 1).

Figure 1 Bielschowsky silver staining of nerve fiber. 1: nerve fiber in hippocampus of control, 2: nerve fiber in hippocampus of model group, 3: NFTs of model group after 3 months, 4: SP of model group after 3 months. The scale bar is 25μm.

3.3 Molecular biological changes

Changes of the cholinergic system in the brain: The density of the ChAT-positive substances in the brain tissues of the operation group rats was decreased significantly compared with the controls at both 1 week and 3 months after lesion. The ChAT-positive cells of the controls showed orbicular-ovate, triangle or irregular shape. Both the cytoplasm and membrane of the ChAT-positive cells were colourated, and the ecphyma were deeply stained. However, the positive cells of the sham operation group rats stained lighter. From Table 5, it can be seen that the number of Aβ-positive cells of the operation group both at 1 week and 3 months after the operation were significantly increased when compared to that of the sham operation group (P<0.01).

Table 5 The number of Aβ positive cells in cortex and hippocampus of each group

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Low dose 1 week</th>
<th>High dose 1 week</th>
<th>Low dose 3 months</th>
<th>High dose 3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>12.67±4.32</td>
<td>25.67±7.11</td>
<td>34.5±11.58</td>
<td>26.5±6.06</td>
<td>31.0±10.35</td>
</tr>
</tbody>
</table>

Compared with the sham operation group, *P<0.01

4. Discussion

AD, mainly characterized by deterioration of memory, is a chronic and progressing degenerative disease of central nervous system that was induced by multiple complicated factors [13, 14]. However, most of the classic AD models are mono-factor models [15-17]. Only a few of them are induced by diplo-factors. Tri-factors or more than three factors models are very rare. These models can hardly mimic the whole of the pathological and biochemical features of AD [18, 19]. Therefore, we dropped several factors to accomplish the animal model of AD, which were induced by three factors, for instance Aβ1-40, 1% aluminum chloride solution and TGFβ-1. The water maze, shuttle box test, pathological staining and immunohistochemistry were used to examine the praxiological, pathological and biochemical deterioration of the animal model. It showed that the model was more closed to the features of AD and so could imitate characteristic of AD.

Morris water maze and shuttle box test were used to examine the learning and retention abilities of rats. The results showed that the learning and retention of the model rats were significantly decreased. The cognitive impairment was time-dependent and dose-dependent, and could progress over 3 months. The praxiological manifestations of this model matched well to the cognitive function of AD patients.

We found that the number of nerve cells of the model rats was decreased and they were lined disorderly. The colloid cells were hyperplastic, and the cell nuclei were obvious pyknosis. The cortex and hippocampus had positive Congo red staining. The nerve fibers were thickened and twisted. Overall, SP and NFTs, the marked pathological changes of AD, were appeared at 3 months. All of these pathological changes were well closed to the AD patients.

Immunohistochemistry was used for the immunoreactivity of ChAT and Aβ. The results indicated that expression of ChAT of the model rats was decreased, and cholinergic system was damaged. Moreover, this lesion showed dose-dependent. The expression of Aβ was significantly increased and a lot of amyloid was deposited in the cortex and hippocampus. Furthermore, those changes could progress over 3 months, they perfectly conformed with the amyloid deposition (chiefly refer to Aβ40/42) and stepping down of the ChAT activity in the brain tissues of AD patients [20].

Many data approbated the injected Aβ model which was infused Aβ1-40 or Aβ25-35 into the hippocampus of rats [17, 21,
However, this model is an acute mono-factor model, which is not consistent with the feature that AD is a chronic disease. In the present study, we have adopted the method that serially infused $A\beta_{1-40}$ into the lateral cerebral ventricle of rats for 14 days, which was fully accorded with the fact that the AD slowly onsets. These $A\beta_{1-40}$ models had avoided acutely destroying the brain tissues, the cholinergic system and cognitive function [23, 24]. But the models could not obtain the marked pathological features of AD, such as NFTs. Our results implied that the cholinergic system had been impaired, which induced to cognitive impairment. SP and NFTs had been found at 3 months after injection. Giovannelli et al. [25] suggested that the pathological changes began to fade away 21 days after injected $A\beta_{25-35}$ into basal ganglia, and could exist for 2 months after injected $A\beta_{1-40}$. Our results indicated that praxiological handicap, pathological changes and molecular biological changes could persist more than 3 months after lesion. These results implied that the tri-factors animal model was more durable as compared with the classic injecting $A\beta$ models.

Many researchers have chronically administered rats overload aluminum through digestive duct to induce AD-like cognitive impairment. For example, Miu et al. [26] had injected aluminium solution into the abdominal cavity of rats for 6 months, 3 times per week. Kaneko et al. [27] had continuously fed 6 week old mice with aluminium solution for 120 days. In this study the aluminium solution was lowered after permeating the blood brain barrier so that the damage of brain tissues was lessened. Furthermore, this method was time-consuming and troublesome. Some scholars had infused aluminium solution into the cerebral ventricle for 5-7 days. The results showed that the learning and retention abilities were lesioned, but the marked pathological lesion of AD could not found. All of the mono-factor aluminium overload animal models of AD can describe some parts of the pathological features of AD, however, the SP of age-dependent from $A\beta$ deposited don't emerge. In our research, we had directly injected aluminium solution into the cerebral ventricle of rats for 5 days. Encephalocele injection was very beneficial to aluminium entering the brain tissues to damage the nerve cells. Moreover, our results suggested that not only amyloid deposited and the activity of ChAT decreased, but also SP and NFTs appeared at 3 months after operation. These results indicated that our new model has overcome the shortages of mono-factor aluminium overload models.

The excitotoxin damage model of AD has only imitated cognitive dysfunction induced by the cholinergic nerve system impairment, but the marked pathological changes, SP and NFTs, can't be found in this model. As indicated in this model, the impairment of acetylcholine is reversible [28]. Different from this model, our model had pathological changes of SP and NFTs and a lot of amyloid deposit in brain tissues. Moreover, the cholinergic system damage could last more than 3 months. These indicated that our model could be used for fairly long-term pathogenesis and drug research.

The transgene AD model can more ideally mimic the clinical, pathological and molecular biological manifestations of AD patients, but the transplanted exogenous genes did not consistent with the characteristic of the animal model which is controlled by a group of genes in fact. Furthermore, the manipulation of transgene model is complicated and costly. So, most laboratories can't make such a transgene model. Our model costs reasonably and can be conveniently operated, therefore, it is more be esteemed for common empirical study in general labs.

The existed researches state that aluminium and $A\beta$ can interact and enhance each other, thereby intensify the deterioration of AD. Korchazhkina et al. [29] has suggested that aluminium can inhibit the schizolysis of insoluble $A\beta_{25-30}$ to soluble $A\beta_{29-35}$ to induce the sediment of $A\beta_{25-35}$ accumulation. Kawahara et al. [30] has discovered that aluminium can alter the construction of $A\beta$ to form fibrillate and deposit in the surface of neuron culture. Hedge et al. [31] also has confirmed at genome level that $A\beta$ and aluminium can adjust the spiral and super spiral structure of scDNA, and result in the changes of DNA conformation. But the lasting time is fairly short. The reason lies in that the self-cleaning mechanism of animal is existed in vivo, so that the soluble $A\beta$ could be removed quickly from the hippocampus and cerebral cortex. Frantschy et al. [32] has acquired that little amyloid can be deposited for 30 days after injecting $A\beta_{1-40}$ into cerebral ventricle alone, but a lot of amyloid deposition can be produced after TGFβ-1 is infused simultaneously. The combined $A\beta$ and TGFβ-1-driven pathology recapitulates salient cerebrovascular, neuronal, and cognitive AD landmarks [33]. Lesne et al. [34] deemed that the reason lies in that TGFβ-1 induce the releasing of a great quantity of soluble β-APP in the astrocytes, then $A\beta_{40/42}$ is produced after this disintegration. These researches have elucidated that TGFβ-1 can accelerate the deposition of $A\beta$ in brain, and damage capillary vessel to promote the occurrence of AD [6, 33, 35, 36]. In our research, when the $A\beta$ and aluminium were infused into the lateral cerebral ventricle of rats at the first time, we simultaneously injected 10ng TGFβ-1 into the anterodorsal nucleus of thalamus. The results showed that the TGFβ-1 enhanced the deposition of much amyloid, moreover, it could last a long time more than 3 months. The effect of three materials combined is better than that of one or two material combined, and can more completely mimic the pathological features of human AD, and the duration of pathological change is long.

Therefore, the new animal model may be provided a successful multi-factor and ideal AD model. It is more closed to the process of complicated etiopathogenesis, pathological and biochemical changes of AD than the classic models. It can be used as an ideal experimental model for study of pathogenesis and pharmacological projects of AD and other purposive research.

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References

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