Pathology - Research and Practice xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Intracerebroventricular injection of $A\beta_{1\text{-}42}$ combined with two-vessel occlusion accelerate Alzheimer's disease development in rats

Shi Jie Dai[a,1], Jie Ying Zhang[a,1], Yu Ting Bao[a], Xiao Jie Zhou[a], Lu Ning Lin[a], Yun Bo Fu[a], Yu Jia Zhang[a], Chang Yu Li[a], Yuan Xiao Yang[b,*]

[a] Zhejiang Chinese Medical University, Hangzhou, Zhejiang, People's Republic of China
[b] School of Basic Medical Sciences and Forensic Medicine, Hangzhou Medical College, Hangzhou, Zhejiang, People's Republic of China

ARTICLE INFO

Keywords:
Alzheimer's disease
Aβ**1-42**
Two-vessel occlusion
Cerebral ischemia

ABSTRACT

Numerous experimental studies and clinical observations suggest that cerebral ischemia may contribute to the pathogenesis of Alzheimer's disease (AD). Two-vessel occlusion caused cerebral ischemia model is often used in the study of vascular dementia (VaD). But how cerebral ischemia works on AD rat model which induced by intracerebroventricular injection of $A\beta_{1\text{-}42}$ remains unclear. In the following study, we investigated the characteristics of rat model caused by intracerebroventricular injection of $A\beta_{1\text{-}42}$ or two-vessel occlusion (2-VO) only and by both of the two operations. The animal cognitive functions were accessed by the Morris water maze. Regional cerebral blood flow was detected by Laser Doppler Blood Flowmeter. HE&Nissl staining, Congo red staining and immunohistochemistry were used to observe the status of neuronal loss, Aβ deposition and the phosphorylated tau expression in hippocampus, respectively. We also measured the contents of AchE and ChAT in serum and hippocampus by Enzyme Linked Immunosorbent Assay. The MWM results showed that rats of $A\beta_{1\text{-}42}$ + 2-VO group had a disorder in cognitive functions, at an early stage of one week after modeling, comparing with rats of sham group. The regional cerebral blood flow (rCBF) was significantly reduced in $A\beta_{1\text{-}42}$ + 2-VO and 2-VO group one week after modeling, and still maintained low perfusion levels four weeks after modeling. HE and Nissl staining showed that $A\beta_{1\text{-}42}$ + 2-VO rats' hippocampal CA1 neurons were in disorder, degeneration and necrosis, severe neuronal loss from the first week to the fourth week, while this phenomenon only appeared in the fourth week after modeling in rats of $A\beta_{1\text{-}42}$ group and 2-VO group. Congo red staining showed that $A\beta_{1\text{-}42}$ + 2-VO group rats' hippocampus CA1 had amyloid deposits from the first week to the fourth week, $A\beta_{1\text{-}42}$ group were not find amyloid deposition significantly until four weeks after modeling, however, 2-VO group had no significant amyloid deposition all the time. Notably, IHC showed that, two weeks after modeling, the p-tau positive total area and integrated optical density of hippocampal CA1 region were significantly increased in $A\beta_{1\text{-}42}$ + 2-VO group rats, while 2-VO group and $A\beta_{1\text{-}42}$ group rats had no significantly changes all the time. We also found that the content of AchE was increased both in serum and hippocampus of $A\beta_{1\text{-}42}$ + 2-VO group rats, and ChAT was decreased. However, there was no significantly change in cortex of content of AchE: acetylcholinesterase (AchE) and choline acetylase (ChAT) all three groups. Together, our study suggest that intracerebroventricular injection of $A\beta_{1\text{-}42}$ combined with two-vessel occlusion may accelerate Alzheimer's disease development in rats. Also, this may serve as a less-time consuming new model to study the Alzheimer's disease and especially AD accompanied by cerebral ischemia.

1. Introduction

Alzheimer's disease (AD) is the main cause of dementia and one of the great health-care challenges of the 21 st century [1]. In 2016, it was estimated that approximately 44 million people worldwide suffer from Alzheimer's and related dementia [2]. AD is characterized by progressive memory loss and cognitive dysfunction. Accumulation of extracellular plaques made of beta-amyloid (Aβ) and intracellular neurofibrillary tangles (NFT) made of tau protein, are considered the main pathological features of AD [3–5].

* Corresponding author.
E-mail addresses: 597383375@qq.com (S.J. Dai), cecezone@163.com (J.Y. Zhang), 674552915@qq.com (Y.T. Bao), 1053144865@qq.com (X.J. Zhou), 603297331@qq.com (L.N. Lin), 782531142@qq.com (Y.B. Fu), 393635423@qq.com (Y.J. Zhang), lcyzcmu@sina.com (C.Y. Li), yyx104475@163.com (Y.X. Yang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.prp.2018.07.020
Received 17 May 2018; Received in revised form 2 July 2018; Accepted 22 July 2018

Table 1
Neurological score sheet.

Experiment	Neurological scoring standard			
	0	1	2	3
① Autonomous movement	No autonomous movement	Move infrequently	Move and touch at least one side of the cage wall	Move and touch at least the three side of the cage wall
② Symmetry of limbs movement	No movement of the affected side	Slight movement of the affected side	Slow movement of the affected side	Bilateral symmetry
③ Extension movement of fore limb	Left forelimbs without extension	Slight extension of the left forelimbs	Left forelimbs with extension, but not to the right	Both sides extend symmetry
④ Climbing ability	No reaction	Can't climb up	Left side is inferior to the right	Climb normally
⑤ Both sides of the body tactile reflex	No reaction	No reaction on the left	Left side reaction was weaker than right	Reaction equally
⑥ Bilateral whisker tactile reflex	No reaction	No reaction on the left	Left side reaction was weaker than right	Reaction equally

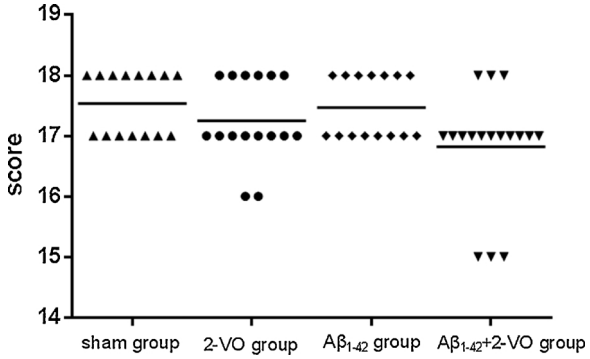

Fig. 1. Effects of 2-VO operation on the neurological function of rats.

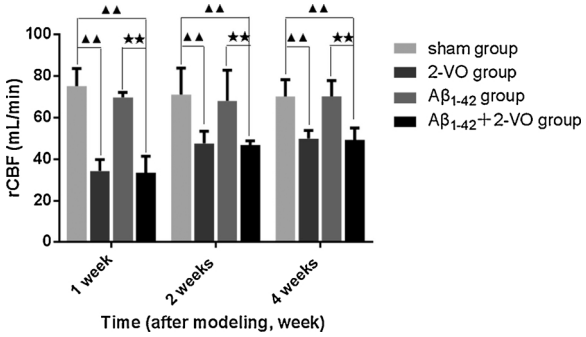

Fig. 2. Effects of different modeling methods on regional cerebral blood flow (rCBF) in rats. (mean ± SD, n = 3). (▲: $P < 0.05$, ▲▲: $P < 0.01$; ★: $P < 0.05$, ★★: $P < 0.01$).

There is growing evidence that cerebral ischemia may increase the risk of AD. One study reported that elderly people with cardiovascular risk factor had hazard risk (HR) of 3.7 for developing dementia during the observational period [6]. In addition, the recent development of neuroimaging techniques has revealed that many brain regions susceptible to AD pathology, display a significant decline in cerebral blood flow at the mild cognitive impairment phase, which consequently leads to AD diagnosis. Furthermore, stroke has been shown to be closely associated with Alzheimer's disease [7–10]. Also, several reports have indicated that cerebrovascular dysfunction is an early event in AD pathogenesis [11–15], which would also imply that local hypoxia/ischemia is an important enhancer of AD pathogenesis and progression, rather than a consequence of the pathological changes observed in AD.

Intracerebroventricular injection of Aβ in the rodent brain can mimic some aspects of AD. Over the last 15 years, substantial evidence has suggested that soluble Aβ oligomers (AβOs) play a pivotal role in the synaptic dysfunction, neuroinflammation, and cognitive deficits present in AD [16,17]. Aβ peptide comprises the main component of amyloid plaques. $Aβ_{1-42}$'s accumulation in the brain plays a crucial role in AD pathogenesis and has been proposed as a trigger for AD onset and progression [18,19].

Bilateral common carotid artery occlusion (two-vessel occlusion (2-VO)) was often used in vascular dementia rats [20–22]. Studies [23,24] have reported that two-vessel occlusion will result in a 22%–30% reduction of hippocampal blood flow that will stabilize after several weeks without further reduction. Other studies also reported that 2-VO rats which can mimic human mild cognitive impairment [25,26]. Liu et al. [27] proved that cerebrovascular hypoperfusion induced by bilateral carotid occlusion surgery in adult rats induces spatial memory impairment, synaptic changes, pyramidal cell death in the CA1 hippocampal subfield, and amyloid-β oligomerization in rats.

The present study is based on the β amyloid cascade hypothesis, which argues that the neurodegeneration in AD is caused by abnormal accumulation of Aβ plaques in various areas of the brain, and the relationship between AD and cerebral ischemia. The experiment animals were divided into 4 groups, including sham operation, single injury (ICV injection of $Aβ_{1-42}$ or 2-VO), and combined injury ($Aβ_{1-42}$ + 2-VO) groups. The rat models were evaluated by detecting the learning and memory ability, regional cerebral blood flow, pathological events in the hippocampal tissue, deposition of amyloid protein, phosphorylated tau protein and cholinergic system level. We were supposed to find out the effects of two-vessel occlusion on pathological changes in AD rats modeling by intracerebroventricular injection of $Aβ_{1-42}$.

2. Materials and methods

2.1. Animal

The animal study was approved by the Ethics of Committee of Zhejiang Chinese Medical University. Male SD rats in a SPF grade (age, 9 weeks; weight 250 ± 30 g) were purchased from Chinese Academy of Sciences of Shanghai Branch Sippr-BK Laboratory Animal Center (Animal production license No.: SCXK (Shanghai) 2013-0016). Animals were housed at a constant room temperature (20 ± 2℃) and supplied with sterilized food and water (Laboratory rearing room Permit No. SYXK (Zhejiang) 2013-0184). All animals were subjected to acclimatization for a week before the experiment started.

All SD rats were carried out Morris water maze (Smart-Mass 0800916 s, Panlab, Spain) to filter out SD rats with no significant difference in the ability of learning and memory according to the references [28,29]. After two-vessel occlusion operation (2-VO), neurological scoring was performed on rats, those with serious hemiplegia, mobility impairments, akinesia and the ones that scored below 17 points were excluded from the study. Remaining 56 rats were randomly divided into 4 groups (14 rats/group): 2-VO group (rats subjected to