Psychiatry Research 267 (2018) 281–288

Contents lists available at ScienceDirect

Psychiatry Research

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

The potential protective effects of cannabinoid receptor agonist WIN55,212-2 on cognitive dysfunction is associated with the suppression of autophagy and inflammation in an experimental model of vascular dementia

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ARTICLE INFO

Keywords:
Autophagy
Cannabinoid receptor
Cognition
Inflammation
Vascular dementia

ABSTRACT

Vascular dementia (VaD) is characteristic of chronic brain ischemia and progressive memory decline, which has a high incidence in the elderly. However, there are no effective treatments for VaD, and the underlying mechanism of its pathogenesis remains unclear. This study investigated the effects of a synthetic cannabinoid receptor agonist WIN55,212-2 (WIN) on VaD, and molecular mechanisms of the effects. VaD model was induced by 2-vessel occlusion (2VO). Spatial reference learning was evaluated by the Morris water maze, and recognition memory was assessed using the novel object recognition test. Autophagy-related proteins [microtubule-associated protein 1 light chain 3 (LC-3) and Beclin-1] were examined by immunohistochemistry and Western blot. Caspase-3 was detected by Western blot. Inflammatory factors, tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β), were estimated by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot. VaD increased the levels of LC-3, Beclin-1, and inflammatory factors, which were reversed by chronic treatment with WIN. WIN decreased the expression of Capase-3, and improved the learning and memory impairment of VaD rats. These data indicate that WIN exerts a neuroprotective effect on the cognitive deficits of VaD rats, which may be associated with the suppression of excessive autophagy and inflammation.

1. Introduction

Vascular dementia (VaD), a common dementing illness in aged people, is characterized by a progressive memory and cognitive decline (Smith, 2017). Increasing evidence suggests that vascular risk factors, including cerebral hypoperfusion (i.e., atherosclerosis, carotid artery stenosis), cerebral angiopathy (i.e., cerebral amyloid angiopathy, cerebral small vessel disease), and cardiovascular disease (i.e., hypertension, diabetes, and hyperlipidaemia), contribute to human VaD (Iadecola, 2013). As a main vascular risk factor of VaD, chronic cerebral hypoperfusion (CCH) usually induces neurodegeneration, neuroinflammation, and white matter injury in patients and animal models (Kitamura et al., 2017). Currently, there are several types of vascular animal models (of which CCH is a common hemodynamic characteristic) are used to study human VaD, such as 2-vessel occlusion (bilateral common carotid artery occlusion, 2VO) model, cerebral small vessel disease model, and hypertensive model (Gooch and Wilcock, 2016). For example, obvious neuronal loss in the cortex and hippocampus of rats with 2VO was proved by the immunochemical staining (Choi et al., 2016). However, the exact pathogenesis and the effective treatment of VaD remain elusive.

The endocannabinoid system (ECS) involves cannabinoid receptor 1 and 2 (CB1 and CB2), two ligands-anandamide and 2-arachidonylglycerol, and the degradative enzymes and monoglyceride lipase (Patel et al., 2017). WIN55,212-2 (WIN), a potent CB agonist, has a key role in the control of the cannabinoid signaling, which modulates the biological activity of ECS (Basavarajappa et al., 2017). WIN exerts protective effects in various neurological disorders, including Alzheimer's disease (AD), VaD, anxiety, and aging, opposing neuroinflammation, neuropathic pain, and cognitive decline (Escamilla-Ramírez et al., 2017). Recently, Navarro-Dorado suggests that WIN and the CB2 selective agonist JWH-133 ameliorate the vascular dysfunction and brain damage in one AD model (Navarro-Dorado et al., 2016). In a 2VO model, administration of a selective agonist of CB2, 1-phenylisatin,

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https://doi.org/10.1016/j.psychres.2018.06.012
Received 8 August 2017; Received in revised form 7 June 2018; Accepted 7 June 2018
Available online 14 June 2018
0165-1781/

reduces brain edema and memory impairment (Jayant and Sharma, 2016). In focal cerebral ischemia, treatment with WIN at 1 mg/kg for 22 h promotes neuronal cells proliferation and enhances synaptic plasticity, which contributes to its neuroprotective effect (Sun et al., 2013). Inversely, acute administration of WIN at a low dose (0.3 mg/kg) disrupts non-associative learning, recognition, and retention of spatial memory in rats (Galanopoulos et al., 2014). Furthermore, studies have shown that WIN involves in the regulation of the autophagic flux in astrocytes and tumor cells (Hiebel et al., 2014; Pellerito et al., 2014). However, the roles of WIN and its influence on autophagy in VaD model remain poorly understood. Therefore, the present study investigated the effects of WIN on VaD, and the molecular mechanism of the effects.

2. Materials and methods

2.1. Animals and VaD model

A total of 60 male Sprague-Dawley rats, weighing 250 ± 10 g, and 1 month old were purchased from the Chinese Academy of Sciences (Shanghai, China). All rats were housed under conventional conditions with constant temperature (24 ± 1 °C) and humidity (60%), and 12/12 h light/dark cycle, being allowed free access to food and water for 1 month acclimatization. All experiments were under the Guide for the Care and Use of Laboratory Animals of National Institute of Health.

Animal model of VaD was induced by 2VO. Rats were anesthetized with 0.15% pentobarbital sodium (40 mg/kg). The bilateral common carotid arteries were exposed through a midline cervical incision, occluded then by 4–0 monofilament nylon suture. Control rats were subjected to the same procedure without ligation of arteries.

2.2. Treatment groups and drug administration

The animals were randomly divided into three experimental groups (n = 20 each group): (1) the Sham group; (2) the 2VO group; (3) the 2VO and WIN group (2VO + WIN). Rats were chronic intraperitoneal injected with WIN (1 mg/kg/day) (Sigma, St. Louis, MO, USA) for 4 weeks (Fig. 1) (Su et al., 2015). The first injection was given at 30 min following 2VO surgery (Sun et al., 2013). Rats were sacrificed 2 h after the end injection. Rats in the Sham and 2VO groups were also administrated with an equal volume of saline. Eight rats with visual impairment were excluded for later experiments.

2.3. Behavior tests

The behavioral tests were performed on the animal at 3 months old, and after 10 min following the injection. The spatial reference learning and memory was assessed by the Morris water maze (MWM) test (n = 10 per group), and the novel object recognition (NOR) (n = 10 per group) task was operated to test recognition memory.

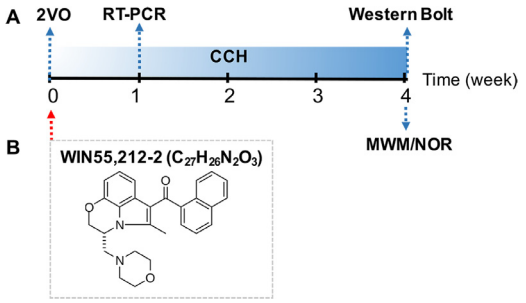

Fig. 1. Experimental protocols. (A) Schematic of experiment design: the CCH phase is 4 weeks; WIN55,212-2 is intraperitoneal injected for 4 weeks; inflammatory factors are estimated by RT-PCR at 1 week of CCH; the MWM/NOR task and Western Blot are used to test the cognition and proteins' level at 4 weeks. (B) The molecular structure of WIN55,212-2. 2VO: 2-vessel occlusion; RT-PCR: reverse transcription-polymerase chain reaction; CCH: chronic cerebral hypoperfusion; MWM: Morris water maze; NOR: novel object recognition.

2.3.1. MWM task

A circular black tank (180 cm diameter, 60 cm high) with water (temperature 25 ± 1 °C, depth 35 cm) was used. The pool was divided into four quadrants with different extra-maze cues. In one of the four quadrants, a black escape platform with a diameter and height of 10 and 24 cm was placed. Spatial training trials were performed for 4 consecutive days. Rats were trained 4 times per day starting in one of the four quadrants. In each training trials, the rats were allowed to swim for 60 s to find the hidden platform (latency to escape). Then spatial probe test was done on day 5 without the platform, and rats were placed in the pool for 60 s. The swimming tracks, the latency to escape, the number of crossing platform, and the time in the target quadrant were recorded by an automatic video tracking system (Ethovision XT, Noldus, Netherlands).

2.3.2. NOR task

The experiment was performed according to previously reported methods (Liu et al., 2015a). It was conducted in an open field box (50 cm × 40 cm × 40 cm). All animals were habituated in the box to decrease their novelty stress to the apparatus during the training phase. In habituation, the rats were allowed to explore the apparatus for 5 min in the absence of objects (Liu et al., 2015a). In training phase, the rats were handled for 5 min in the presence of two identical objects. After inter-trial-interval of 1 h (home-cage), the rats were placed in the box for testing. During the testing phase, one familiar object was replaced by a new object in the same location, the role (familiar or novel object) as well as the relative position of the two objects were counterbalanced and arbitrarily permuted. Each animal was allowed to explore these two objects for 5 min. All objects were cleaned with 70% ethanol for tests. Two sets of objects were used and they were consistent in material, height, and volume, but different in shape and color. The time spent on exploring both objects and each object were recorded. The exploratory time and the discrimination index were estimated.

2.4. Immunohistochemistry

Paraffin-embedded brains (n = 6 per group) were coronally sectioned at a thickness of 4 μm. Sections were briefly deparaffinized, rehydrated, and immersed in phosphate buffer solution and 0.2% Triton X-100 (PBS-T), with 10% normal goat serum for 1 h and then incubated with primary antibody against microtubule-associated protein 1 light chain (LC) 3B (1:100, 2775S) (CST, MA, USA) at 4 °C for 24 h. The secondary antibody and diaminobenzidine were from the ABC kit (Vector Labs, USA). LC3 positive cells and histopathological injury of neurons were counted at 400× magnification from three visual fields by an examiner blinded to groups. For each rat, three consecutive slices were observed.

2.5. Western blot analysis

Forty-five μg of total protein from each sample (n = 6 per group) were separated in 8%, 10%, or 12% SDS-polyacrylamide gel electrophoresis and blotted onto polyvinylidene difluoride membranes. The membranes were probed with the primary antibodies as follows: LC-3B (1:1000), Beclin-1(1:1000), cysteinyl aspartate specific proteinase-3 (Caspase-3) (1:500), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1:5000) (CST, MA, USA); tumor necrosis factor alpha (TNF-α) (1:500), interleukin 1 beta (IL-1β) (1:500) (R&D Systems). After incubating with appropriate secondary antibodies, protein signals were detected by enhanced chemiluminescence system (Millipore, MA, USA), then quantified by ImageJ software (NIH, Bethesda, USA).