Research Report

The synthetic cannabinoid WIN55212-2 ameliorates traumatic spinal cord injury via inhibition of GAPDH/Siah1 in a CB2-receptor dependent manner

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Abstract

The essential role of GAPDH/Siah1 signaling pathway in the pathogenesis of various injurious conditions such as traumatic spinal cord injury (SCI) has been gradually recognized. However, the drugs targeting this signaling pathway are still lacking. The endocannabinoid system, including its receptors (CB1 and CB2), act as neuroprotective and immunomodulatory modulators in SCI. WIN55212-2, an agonist for CB1 and CB2 receptors, has been demonstrated with anti-inflammatory and anti-apoptotic effects in multiple neurological diseases. Therefore, the present study aimed to investigate whether WIN55212-2 could promote functional recovery after traumatic SCI via inhibition of the GAPDH/Siah1 signaling. The traumatic SCI was induced by dropping a 10-g impactor from 25 mm on the dorsal surface of T9 and T10. Our results showed that WIN55212-2 alleviated the activation of GAPDH/Siah1 signaling pathway after SCI, as indicated by the reduction in GAPDH nuclear expression, GAPDH-Siah1 complex formation and iNOS protein expression. Furthermore, WIN55212-2 reduced apoptosis, production of IL-1β and TNF-α and activation of NF-κB signaling in the spinal cord after SCI. The behavioral tests showed that WIN55212-2 improved the functional recovery after traumatic SCI as indicated by sustained increase in the locomotor scores. However, these neuroprotective effects of WIN55212-2 were blocked in the presence of the combined treatment of AM630 (an antagonist of CB2) rather than AM251 (an antagonist of CB1). In conclusion, our study indicates that WIN55212-2 improves the functional recovery after SCI via inhibition of GAPDH/Siah1 cascades in a CB2 receptor dependent manner, indicative of its therapeutic potential for traumatic SCI or other traumatic conditions.

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1. Introduction

Traumatic spinal cord injury (SCI) is a serious disorder that results in a series of neurologic dysfunction. Traumatic SCI has been classified into primary injury and secondary injury. The primary injury is caused by the direct mechanical impact on the injured spinal cord, while the secondary injury is as a result of the following complex pathophysiological alterations including the posttraumatic inflammation and apoptosis (Beattie, 2004; Oyinbo, 2011). Therefore, the prevention of persistent apoptosis and inflammations has been thought to be crucial for functional recovery after SCI.

Accumulating studies have shown that the endocannabinoid system, including its receptors (CB1 and CB2), can act as neuroprotective and immunomodulatory modulators in the lesions of nervous system such as cerebral ischemia-reperfusion injury (Amanetea et al., 2007), traumatic brain injury (Panikashvili et al., 2006) and spinal cord injury (Garcia-Ovejero et al., 2009). WIN55212-2, an agonist for CB1 and CB2 receptors, has been
demonstrated with the potency of inhibiting neutrophil infiltration and pro-inflammatory cytokine production in neurological disorders such as multiple sclerosis (Arévalo-Martín et al., 2008; Di Marzo, 2008). Besides the anti-inflammatory action, WIN55212-2 could also reduce apoptosis and thereafter improve the neural repair in the animal model of neonatal hypoxia-ischemia (Fernández-López et al., 2010). In addition, it is noted that WIN55212-2 can attenuate neuropathic pain after the compression-induced SCI (Hama and Sagen, 2007). However, it is yet unknown whether the treatment of WIN55212-2 would improve functional recovery by directly reduce the apoptosis and inflammation after traumatic SCI.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was originally thought of as merely a housekeeping enzyme. However, studies have shown that GAPDH could be S-nitrosylated by nitric oxide (NO) and then translocated to the nuclei binding with Siah1 (an E3-ubiquitin-ligase), resulting in the activation of apoptotic signaling cascades (Hara et al., 2005; Hara et al., 2006; Hara and Snyder, 2006). Previous studies have suggested that the GAPDH/Siah1 signaling pathway is associated with the progression of various diseases including cerebral ischemia-reperfusion injury (Li et al., 2012) and acute lung injury (Takaoka et al., 2014). Added to these is our recent study, which has shown that the activation of GAPDH/Siah1 cascades is involved in traumatic SCI by inducing apoptosis and inflammation (Huo et al., 2016). Thus, the GAPDH/Siah1 signaling pathway might be a promising target for therapies of apoptosis and inflammation after traumatic SCI. Interestingly, several studies have found that activation of the cannabinoid system is also effective in inhibiting NO signaling (Cabral et al., 2002; González et al., 2011; Molina-Holgado et al., 2002) that has been identified as the trigger of GAPDH/Siah1 cascades. Therefore, the aim of the study is to investigate whether WIN55212-2 could promote functional recovery, and reduce apoptosis and inflammation in the spinal cord after traumatic SCI via inhibition of the GAPDH/Siah1 signaling.

2. Results

2.1. WIN55212-2 promotes functional recovery from traumatic SCI

Firstly, we compared the differences of Basso–Beattie–Bresnahan (BBB) locomotor scores between the WIN55212-2-treated and Control animals within the 4 weeks after traumatic SCI (Fig. 1). The recovery in the Control animals reached a plateau 1 week after SCI, whereas the BBB scores in the WIN55212-2-treated animals were maintained in recovery until 3 weeks, when they scored more than 12. A repeated measures ANOVA analysis showed that the differences between the Control and WIN55212-2-treated groups were significant (Control vs. WIN, $F = 38.49$, $P < 0.0001$, $n = 10$).

Next, we detected the histopathological changes at 24 h postoperatively in the spinal cord after traumatic injury ($n = 3$ per group). As shown in Fig. 1B, the HE staining revealed that hemorrhages and necrotic patches were in the spinal cord of control group. In addition, some cells were found with condensed nucleus and darkly red-stained cytoplasm. However, these histopathological changes were alleviated by the treatment of WIN55212-2, as indicated by markedly less necrotic patches and hemorrhages.

2.2. WIN55212-2 prevents the nuclear expressions of GAPDH and Siah1

Our previous study has indicated that the GAPDH/Siah1 cascades are involved in the traumatic SCI (Huo et al., 2016). Therefore, here we sought to investigate whether the treatment of WIN55212-2 could affect the GAPDH/Siah1 cascades in the spinal cord. Our results showed that the treatment of WIN55212-2 reduced the nuclear protein expression of GAPDH and Siah1 at 48 h after SCI (Fig. 2A; $P = 0.008$ and $P = 0.0236$ for GAPDH and Siah1, respectively, vs. control group; $n = 5$ per group). GAPDH/Siah1 cascade has been implicated as a sensor of NO (Hara et al., 2005). Next, we assessed whether WIN55212-2 could also attenuate the NO signaling after traumatic SCI by determining iNOS protein levels in the spinal cord. We found that the treatment of WIN55212-2 decreased the elevation of iNOS protein expression in the spinal cord at 48 h after SCI (Fig. 2B, $P = 0.037$ vs. WIN group, $n = 5$ per group).

2.3. Treatment of CB2 receptor antagonist reverses the WIN55212-2-induced functional improvement after traumatic SCI

Both CB1 and CB2 receptors can be modulated in response to spinal cord injury (García-Ovejero et al., 2009). However, it is yet unknown which receptor is mainly responsible for the beneficial action of WIN55212-2 for improving functional recovery after traumatic SCI. Thus, AM251 (an antagonist of CB1 receptor) or AM630 (an antagonist of CB2 receptor) was administered 30 min before each dose of WIN55212-2 injection. The behavioral tests of BBB scores showed that the locomotor function improvement induced by WIN55212-2 was abrogated by the combined treatment of AM630, as indicated by the discontinuation of functional recovery from 2 weeks after SCI (WIN vs. WIN+AM630, $F = 23.04$, $P = 0.0001$, $n = 10$), whereas the difference between WIN and WIN + AM251 groups was not significant (WIN vs. WIN + AM251, $F = 1.873$, $P = 0.1879$, $n = 10$).

2.4. Antagonism of CB2 receptor blocks the inhibitory action of WIN55212-2 on GAPDH nuclear expression and GAPDH-Siah1 binding

Either CB1 or CB2 receptors have been reported with inhibitory effects on the NO-mediated neurotoxicity (Molina-Holgado et al., 2002). Given the key role of NO in triggering GAPDH/Siah1 signaling, we next tested which one mediates the WIN55212-2-induced inhibition of GAPDH/Siah1 signaling. Our results showed that the decrease in nuclear GAPDH protein expression and the increase in the cytoplasmic GAPDH protein expression induced by the WIN55212-2 treatment were both blocked by the combined administration of AM630 (Fig. 4A; $P = 0.0022$ and $P = 0.0155$ for nuclear and cytoplasmic GAPDH, respectively, vs. WIN group; $n = 5$ per group) rather than AM251 at 48 h after SCI (Fig. 3A; $P = 0.3942$ and $P = 0.4643$ for nuclear and cytoplasmic GAPDH, respectively, vs. WIN group; $n = 5$ per group), indicating that the WIN55212-2-induced suppression of GAPDH nuclear expression is dependent on its selective activation of CB2 receptor. In addition, we also found that the immunoprecipitated GAPDH by anti-Siah1 antibodies in the extract from the spinal cord after traumatic SCI was reduced by the treatment of WIN55212-2 (Fig. 4B; $P = 0.0008$ vs. control group; $n = 4$ per group). However, the decrease in the immunoprecipitated GAPDH induced by WIN55212-2 treatment was blunted by the antagonism of CB2 receptor with AM630 (Fig. 4B; $P = 0.0139$ vs. WIN group; $n = 4$ per group), suggesting the essential role of CB2 receptor in the inhibition of GAPDH-Siah1 binding by WIN55212-2.

2.5. WIN55212-2 reduces apoptosis in the spinal cord after traumatic SCI in a CB2 receptor-dependent manner

Apoptosis has been demonstrated as an important event in the secondary injury following traumatic SCI (Lu et al., 2000; Springer et al., 1999). Our data showed that the treatment of WIN5512-2 reduced the number of TUNEL-positive cells and the protein level...