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Research Report

Caffeine treatment aggravates secondary degeneration after spinal cord injury

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ABSTRACT

Spinal cord injury (SCI) often results in some form of paralysis. Recently, SCI therapy has been focused on preventing secondary injury to reduce both neuroinflammation and lesion size so that functional outcome after an SCI may be improved. Previous studies have shown that adenosine receptors (AR) are a major regulator of inflammation after an SCI. The current study was performed to examine the effect of caffeine, a pan-AR blocker, on spontaneous functional recovery after an SCI. Animals were assigned into 3 groups randomly, including sham, PBS and caffeine groups. The rat SCI was generated by an NYU impactor with a 10 g rod dropped from a 25 mm height at thoracic 9 spinal cord level. Caffeine and PBS were injected daily during the experiment period. Hind limb motor function was evaluated by the Basso, Beattie, Bresnahan (BBB) locomotor rating scale at 1 week and 4 weeks after the SCI. Spinal cord segments were collected after final behavior evaluation for morphological analysis. The tissue sparing was evaluated by luxol fast blue staining. Immunofluorescence stain was employed to assess astrocyte activation and neurofilament positioning, while microglia activation was examined by immunohistochemistry stain.The results showed that spontaneous functional recovery was blocked after the animals were subjected caffeine daily. Moreover, caffeine administration increased the demyelination area, promoted astrocyte and microglia activation and decreased the quantity of neurofilaments. These findings suggest that the neurotoxicity effect of caffeine may be associated with the inhibition of neural repair and the promotion of neuroinflammation.

1. Introduction

Spinal cord injury (SCI) causes central nervous system (CNS) damage, which leads to paralysis, distonia, sensory loss, and even death. Extreme sports, car accidents, war and violent assault are the most common causes of SCI. Unfortunately, spontaneous recovery from injury is rare in adult humans, where tissue necrosis and local hemorrhaging are found at the injury epicenter as the primary injury. During the following days to weeks after an SCI, secondary injury results in

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neuronal loss, axonal destruction, inflammation and demyelination at the injury site (Maegele et al., 2005). This neurological disorder causes life-long muscle denervation, pain, and bowel/bladder dysfunction (Fine et al., 1982). Currently, treatment strategies for SCI are limited, with investigations ongoing around the world (Rabchevsky et al., 2011). Over the past decade, the standard treatment of SCI has targeted decreasing neuroinflammation to improving functional recovery; however, outcomes after therapy remain inadequate

Recently, therapy for SCI has focused on preventing secondary injury to reduce inflammation and preserve more tissue sparing, which might improve functional outcomes (Bao et al., 2003; Cuzzocrea et al., 2006; Genovese et al., 2006; Glaser et al., 2006). A previous study has shown that adenosine receptors are a major regulator of inflammation after an SCI (Song et al., 2009); however, the adenosine receptor has multiple effects in a CNS injury. For instance, the blocking of adenosine receptors has been shown to provide a neuro-protective effect against CNS injury and reduce hyperalgesia (Palacios et al., 2012; Salvemini et al., 2013; Stone et al., 2009). In contrast, promoting adenosine receptors via an agonist has also been demonstrated to act as a potential neuro-protective treatment for CNS injury (Okonkwo et al., 2006; Reece et al., 2004).

Caffeine has been the most widely used psychoactive substance in the world for hundreds of years. Numerous plants can synthesize caffeine, such as coffee, cocoa, yaupon and guarana. The chemical formula for caffeine is 1,3,7-trimethylpurine-2,6-dione, and is an alkaloid chemical that acts as a non-selective adenosine receptors (ARs) blocker. Adenosine binds with ARs, and pertains to the G-protein couple receptors (GPCR) of cell surface proteins. Some evidence, including clinical or preclinical investigations, has suggested that anti-inflammation effects may be attributed to ARs (Fishman et al., 2006; Mabley et al., 2003; Szabo et al., 1998). AR activation decreases TNF-α release by immune cells after a CNS injury (Cassada et al., 2002). ARs are involved not only in inflammatory regulation, but also in neuropathic pain reduction after an SCI (Horiuchi et al., 2010).

However, the role of the adenosine receptors involved in SCI remains unresolved and whether caffeine influences hind limb functional recovery after an SCI has not been well elaborated. In the present study, we used the morphological approach to investigate the effects of caffeine in spontaneous functional recovery after SCIs in rats.

2. Results

2.1. *Caffeine administration blocked spontaneous functional recovery after SCI*

Compared with the functional outcome in sham at post-injury day 7, the PBS and caffeine groups revealed hind limb paralysis and intact fore limb motor ability. For the PBS group, the BBB score increased at day 28 after the SCI compared with 7 days after injury (1.188±0.53 vs. 9.833±3.61), which indicated spontaneous functional recovery had occurred. Interestingly, this spontaneous functional recovery was blocked by caffeine administration. In comparing the PBS and caffeine groups, the PBS group exhibited a significantly higher BBB score than the caffeine group 4 weeks after SCI (BBB score PBS vs. caffeine; 9.833±3.61 vs. 2.00±1.41, see Fig. 1). There were no significant weight difference between caffeine and PBS group during entire experiment period (data no show).

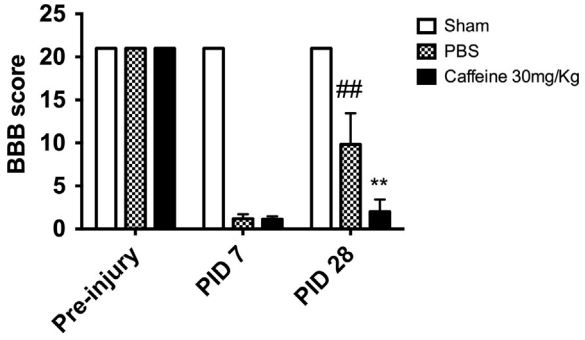

Fig. 1 – Contusion spinal cord injury caused paralysis 7 days after surgery. The hind limb motor function had spontaneous recovery 4 weeks after the SCI in the PBS group (##$p<0.01$). Caffeine injection prevented recovery ($p<0.01$). Data are expressed as mean ±SD with each group, $n=10$.**

2.2. *Caffeine treatment increased demyelination area after SCI*

To examine the effect of caffeine in demyelination, luxol fast blue staining was performed. Fig. 2 shows that the spinal cord demyelination area in the PBS group was smaller than the caffeine group. The demyelination at the epicenter in the caffeine group was markedly larger than that of the PBS group 28 days after SCI (82.83±8.22% vs. 33.73±9.23%, see Fig. 2). The longitudinal section also revealed the same pattern: the caffeine group had larger demyelination areas than did the PBS group (data not shown).

2.3. *Caffeine treatment-induced microglia activation*

To evaluate the effect of caffeine in neuroinflammation after an SCI, we performed IHC to quantify the microglia and observe microglia activation in the spinal cord. At 3 mm rostral to the epicenter, the density of the microglia was significantly higher in the caffeine group than in the PBS group (553.7± 128.1 vs. 395.2±77.86 per mm^2, see Fig. 3C). The caffeine treatment after SCI seemed to affect not only the microglia number but also the activation. Microglia activation was observed more in the caffeine group (marked with arrows in Fig. 3A) than in the PBS group (Fig. 3B). The arrows in Fig. 3D showed neuron necrosis in caffeine group but undetected in the PBS group (Fig. 3E) 28 days after SCI. The neurons in the PBS group revealed a greater number of normal morphological features, such as clear nuclei staining, nissl body and intact cell membrane (double arrows in Fig. 3E). The neuron cells underwent apoptosis were found in PBS group with shrinkage, dense staining (arrowheads in Fig. 3E).

2.4. *Caffeine treatment increased astrocyte activation and decreased neurofilament numbers after SCI*

Glial scar formation after a CNS injury causes failure of nerve conduct regeneration. To evaluate astrocyte activation and neurofilament (NF) number, we performed immunofluorescence