Toxicology and Applied Pharmacology 342 (2018) 86–98

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Low, but not high, dose triptolide controls neuroinflammation and improves behavioral deficits in toxic model of multiple sclerosis by dampening of NF-κB activation and acceleration of intrinsic myelin repair

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ARTICLE INFO

Keywords:
Apoptosis
Cuprizone
Demyelination
Multiple sclerosis
Neuroinflammation
Triptolide

ABSTRACT

Cuprizone (Cup) is a copper chelating agent frequently used to study factors that affect oligodendrocytes (OLGs) death and acute demyelination. Triptolide (TP), a nuclear factor-kappaB (NF-κB) blocker, is a major bioactive component of Tripterygium wilfordii Hook f. (TWHf) with various therapeutic activities. In this study, we examined the effects of TP on neuroglia activation, inflammation, apoptosis, demyelination, and behavioral deficits in the Cup-induced toxic model of multiple sclerosis (MS). C57BL/6 J mice were fed with chow containing 0.2% Cup for 6 weeks to induce detectable neuroinflammation and myelin loss. TP was administered intraperitoneally at different doses (125, 250 or 500 μg/kg/day) during the last week of the Cup challenge. Although TP substantially decreased Cup-induced NF-κB extra activation, TNF-α and IL-1 over expression, and gliosis in a dose-dependent manner, only low dose of TP (TP-125) was able to raise the number of OLGs precursor cells ($NG\text{-}2^+/O4^+$), reduce Bax/Bcl-2 ratio and improve behavioral deficits. In addition, TP-125 decreased NF-κB activation on $GFAP^+$ astrocytes more than $MAC\text{-}3^+$ microglial and MOG^+ oligodendrocytes which suggested the possibility of specific dampening of NF-κB signaling in reactive astrocytes. Behavioral assessments by open-field and rota-rod tests showed that only TP-125 notably improved motor function and motor coordination compared to the Cup group. These findings highlight the pivotal role of NF-κB signaling in the oligodendrogenesis and lesion reduction in demyelination diseases such as MS.

1. Introduction

Multiple sclerosis (MS) is a multifocal chronic autoimmune inflammatory disease of the central nervous system (CNS) which is also known as a perivascular demyelinating disease (Romo-González et al., 2012; Trapp and Nave, 2008; Goodin, 2014). It has been hypothesized that auto-reactive lymphocytes pervade the CNS and generate local inflammation together with the resident microglia which leads to further oligodendrocytes (OLGs) damage and demyelination (Sanadgol et al., 2017a). Cuprizone (bis-cyclohexanone-oxalyldihydrazone, Cup) is a copper chelating agent commonly used to study factors that affect OLG death and myelin loss (Abakumova et al., 2015). Cup-associated OLGs apoptosis and myelin loss during early demyelination greatly mimic hallmarks of the pathophysiology of primary progressive MS and

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https://doi.org/10.1016/j.taap.2018.01.023
Received 4 March 2017; Received in revised form 15 January 2018; Accepted 29 January 2018
Available online 31 January 2018
0041-008X/

to a lesser extent progressive relapsing MS (Lucchinetti et al., 2000). Cup influences the normal OLG metabolism similar to type III of MS lesions which more resembles primary OLG dystrophy rather than autoimmunity (Praet et al., 2014). An important advantage of Cup model over other MS animal models such as experimental autoimmune encephalomyelitis (EAE) is the possibility to selectively study molecules and factors that might positively affect remyelination process in the brain either by recruiting or activating oligodendrocyte precursor cells (OPCs) or preventing mature OLGs apoptosis. Since Cup induces mitochondrial dysfunction, it is speculated that its neurotoxic properties are due to a disturbance of oxidative and respiratory related enzymes such as superoxide dismutase (SOD) and cytochrome oxidase and induction of oxidative stress in OLGs. Beside copper chelating nature of Cup, other mechanisms such as gliosis-induced inflammatory responses and inhibition of autonomous repair mechanisms have also been suggested (Kipp et al., 2009).

It has been shown that neuroinflammation can have both harmful and beneficial effects on the neuronal and glial cells function and transcription factor nuclear factor-kappaB (NF-κB) has a determinant role in controlling this process (Camandola and Mattson, 2007). NF-κB is a general term referring to several transcription factors that are engaged in controlling a large number of normal cellular processes including inflammation (Ghosh et al., 1998). Since persistent inflammatory reactions has been shown to be involved in the development of numerous neuronal disorders (Lynch and Mills, 2012), selective anti-NF-κB therapeutic strategies could be beneficial for minimizing damages during acute and chronic inflammation. Genes that can be induced by NF-κB signaling include those that encode important molecules such as tumor necrosis factor alpha (TNF-α), interleukin 1 (IL-1), interleukin 6 (IL-6), SOD, B-cell lymphoma 2 (Bcl-2), and inhibitors of apoptosis proteins (IAPs) (Pahl, 1999). Thus, abolishment of NF-κB signaling by gene silencing or specific antagonists is accompanied by serious side effects due to interfering NF-κB mediated anti-apoptosis mechanisms (Sun et al., 2013). Although, it has been reported that NF-κB blocking in astrocytes promoted oligodendrogenesis (Raasch et al., 2011; Bruck et al., 2012), NF-κB activation increased transcription of anti-apoptotic genes and decreased mature OLGs apoptosis (Graham and Gibson, 2005).

In CNS, activation of microglia/macrophages and astrocytes are important components of the lesion environment that can impact demyelination process (Murta et al., 2012). These cells cause an immune response to accelerate and further promote remyelination, and eventually resolve the inflammatory response (Tanaka and Yoshida, 2014; Hibbits et al., 2012). However, prolonged reactive gliosis is not able to block the progression of Cup lesion (Buschmann et al., 2012). It has been demonstrated that activation of astrocytic NF-κB can lead to OLG damage; on the contrary, attenuation of NF-κB signaling has a protective effect on myelin loss, pro-inflammatory response, and reactive gliosis (Raasch et al., 2011; Bruck et al., 2012; Brambilla et al., 2005).

Triptolide (TP, PG490) is one of major bioactive components of *Tripterygium wilfordii* Hook f. (TWHf) and affects the cell fate via anti-inflammatory property and attenuation of NF-κB signaling (Liu, 2011; Liu et al., 2012; Ma et al., 2007; Kiviharju et al., 2002; Zheng et al., 2013). Triptolide is a diterpenoid triepoxide (small hydrophobic molecule) that can easily pass cell membranes. Previous studies showed that TP regulates inflammatory responses in macrophages (Premkumar et al., 2011; Wu et al., 2006), ameliorates important neuropathological and behavioral changes in animal model of Alzheimer's disease (Cheng et al., 2014), and suppresses neuroinflammation in both astrocytes and microglia (Gong et al., 2008, Dai et al., 2006).

In the current study, we explored the effects of different TP doses, as a classic NF-κB inhibitor, on inflammatory responses, glial activation, apoptosis markers, and behavioral evaluations in the Cup-induced MS model. As female mice were partially resistant to the demyelination (Gudi et al., 2014), therefore, we used male mice. The aim of the present study was to shed new light on the mechanisms that may affect the dynamic cross-talk between survival and death in OLGs after inhibition of NF-κB signaling in glial cells.

2. Methods

2.1. Animals and induction of toxic demyelination

Old male C57BL/6 mice (7–8 weeks old) with body weight ranging from 18 to 20 g were purchased from Pasteur Institute, Tehran, Iran. The animals had free access to food and water and were maintained on a 12-h light/dark cycle at room temperature (20–22 °C). Demyelination was induced by feeding a diet containing 0.2% (w/w) Cup mixed into ground standard rodent chow for 6 weeks. The 6-week Cup period was chosen because it can result in maximum toxic-induced demyelination, greatly increased neurogliosis (astrogliosis and microgliosis), and the detectable autonomous repair (oligodendrogenesis) during lesion expansion (Praet et al., 2014). All animal manipulations were carried out according to the Ethical Committee for the use and care of laboratory animals of Tehran University of Medical Sciences (TUMS). Every possible effort was made to minimize the number of animals used and their suffering.

2.2. The study design

The degree of demyelination in the Cup model may be variable, thus, determining the time of demyelination and remyelination is difficult. Our previous work demonstrated that the best time for cellular and molecular monitoring of active demyelination and activation of autonomous repair is between weeks 5 to 6 of Cup administration (Sanadgol et al., 2017b). Accordingly, the best time for performing therapeutic interventions is during the last week of model induction (week 6) in which the minimum changes in lesion are measureable. Therefore, one hundred twenty mice were divided randomly into four groups (Fig. 1A and B): (i) control group received normal powdered chow for 6 weeks and intraperitoneal (i.p.) injection of 1:10 ratio of dimethyl sulfoxide (DMSO) and 1× (final concentration of 10 mM $PO4^{3-}$, 137 mM NaCl, and 2.7 mM KCl) phosphate buffered saline (PBS, pH 7.2) solution as vehicle was performed every day in the last week; (ii) healthy groups which were divided into three (a, b and c) subgroups (12 mice per subgroup) that received normal powdered chow for 6 weeks and three doses of TP (125, 250, or 500 μg/kg body weight/day; i.p) dissolved in vehicle during the last week; (iii) Cup group were fed with powdered chow mixed with 0.2% Cup for 6 weeks with i.p. injection of vehicle every day during the last week; (iv) treatment groups that were divided into three (a, b and c) subgroups (12 mice per subgroup) which treated with three doses of TP (125, 250, or 500 μg/kg body weight/day; i.p) dissolved in vehicle during the last week of Cup feeding period. The dosages used for TP administration were selected based upon previous studies (Cheng et al., 2014; Jin et al., 2015). As Cup-induced demyelination is detectable after six weeks, predominantly in the thick band of nerve fibers that divides the cerebral cortex lobes into left and right hemispheres called corpus callosum (CC), this area of brain was used for all of cellular and molecular evaluations. All measurements were performed by a blinded observer to group assignments.

2.3. Evaluation of motor function by open-field test

Animals were placed in the center of an open-field box (50 × 50 cm), and their movement was tracked over a 5-min period by a video camera (IKEGAMITSUSHINKI, Japan) placed above the box. The EthoVision tracking system (Noldus Information Technology Co., Wageningen, Netherlands) was used to evaluate motor function by measuring the total traveled distance (TD, cm), movement velocity (cm/s) and duration traveled in the central zone (DC, *sec*). The DC/TD ratio was also calculated.