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Chronic mild stress eliminates the neuroprotective effect of Copaxone after CNS injury

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ABSTRACT

Copolymer (Cop)-1, also known as glatiramer acetate, is an active compound of Copaxone, a drug widely used by patients with multiple sclerosis (MS). Copaxone functions in MS through two mechanisms of action, namely immunomodulation and neuroprotection. Because the immune system is suppressed or altered in depressed individuals, and since depression is often associated with neurological conditions, we were interested in examining whether the neuroprotective effect of Copaxone persists under conditions of stress-induced depressive behavior. We exposed mice to unpredictable chronic mild stress for 4 weeks and then treated them with three doses of Copaxone at 3-day intervals, with the last dose given immediately before the mice underwent a crush injury to the optic nerve. Whereas nonstressed mice exhibited a strong neuroprotective response after Copaxone treatment, this effect was completely absent in mice that underwent chronic mild stress. Interestingly, when Copaxone was combined with Prozac, the neuroprotective effect of Copaxone was regained, suggesting that chronic mild stress interferes with the neuroprotective effect of Copaxone. These results may shed a light on mechanism of action of Copaxone and lead to new combined therapies for neurodegenerative and neuroinflammatory disorders.

1. Introduction

Injury to the central nervous system (CNS) results in a continuous spread of neuronal degeneration beyond the initial damage, often culminating in a much larger final infarct zone than could have been predicted from the initial injury (Faulkner et al., 2004; Popovich et al., 1994; Schwartz and Yoles, 1999; Schwartz et al., 1999; Sofroniew, 2000). Among the numerous and complex molecular pathways that underlie this degeneration are the release of toxic compounds from dying cells, impairment of blood supply to the site of injury, gliosis around the injury site, and an inflammatory response (Gillessen et al., 2002; Jones et al., 2004; Lipton, 1993; Popovich et al., 1999; Schwartz et al., 2003). Most of the current therapeutic interventions are aimed at manipulating the functions of individual molecules. However, given the complex nature of the neurodegenerative process, a more global approach may be required in order to achieve substantial neuronal survival.

A little over a decade ago, T cells were found to be capable of inducing neuronal survival after injury (Hauben et al., 2000a,b; Kipnis et al., 2002, 2001; Moalem et al., 1999; Schwartz and Kipnis, 2001). At about the same time it was also proposed that macrophages, if appropriately activated, were beneficial for neuronal survival (Lazarov-Spiegler et al., 1998; Rapalino et al., 1998). After injury to the CNS, neuronal survival in immune-deficient mice is lower than in mice with a normal immune system, and this impaired survival can be largely restored to wild-type levels by passive transfer of T cells from wild-type donors into the immune-deficient recipients (Kipnis et al., 2002, 2001; Yoles et al., 2001).

Copaxone, an FDA-approved drug used by patients with multiple sclerosis (MS), was designed to compete with pathological peptides and displace them from the MHC II groove, thus attenuating their pathological effect (Aharoni et al., 1997; Arnon et al., 1989; Teitelbaum et al., 1997a,b, 1996). While this drug is effective in MS and in experimental autoimmune encephalomyelitis (EAE) (an animal model of MS), its mechanism of action is not yet completely understood and appears to be substantially more complex than was initially anticipated. While Copaxone exerts an immune-modulatory effect, it is also strongly neuroprotective, as shown in numerous animal models of neurological diseases such as CNS trauma, glaucoma, Alzheimer's disease, Parkinson's disease, and others (Angelov et al., 2003; Kipnis and Schwartz, 2002; Kipnis et al., 2000; Liu et al., 2007; Mosley et al., 2007; Schori et al., 2001).

Major depression is associated with immune malfunction (Choudhry et al., 2007; Frank et al., 2002; Leonard, 2000; Miller, 2010; Miller et al., 2009, 1999; Schuld et al., 2003; Soygur et al.,

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2007). The outcome of CNS injury in patients with major depression has not been extensively studied, and it is not yet known whether the immune malfunction associated with major depression results in exaggerated neuronal loss after CNS injury or under conditions of disorders such as MS. Moreover, to the best of our knowledge hardly any studies have addressed the question of whether immune-modulatory drugs whose action is neuroprotective, such as Copaxone, maintain their neuroprotective activities under conditions of chronic stress (animal depression model).

Here we examined the effect of unpredicted chronic mild stress on the survival of neurons after CNS injury and on the neuroprotective effects of Copaxone in such mice. We found that neither acute nor chronic stress had any effect on neuronal survival after optic nerve crush injury. Interestingly, however, whereas Copaxone was significantly effective in promoting neuronal survival after optic nerve injury in normal control mice, in depressed mice this neuroprotective effect was absent. Neuroprotective effect of Copaxone was regained when mice were co-treated with Copaxone and the anti-depressant, Fluoxetine (Prozac).

2. Results

To determine whether neuronal survival after CNS injury is altered by stress, we induced depressive behavior in C57Bl6 mice by exposing them to chronic unpredicted mild stress (Feng et al., 2012; Isingrini et al., 2010) for 4 weeks (Table 1). Both stressed mice and nonstressed controls were weighed weekly, revealing a characteristic progressive loss of growth in the stressed mice (Fig. 1a, $F(1,51) = 6.80$), $p = 0.001$). In addition, weekly examination for escape-like behaviors by means of the tail suspension test showed a substantial reduction in the escape behaviors of the stressed mice over time ($F(5,68) = 11.59$, $p < 0.001$, Fig. 1b). Similar results were obtained with forced swim test ($F(3,24) = 15.07$, $p < 0.001$, Fig. 1c), further demonstrating the depressive phenotype of these mice. We also tested the peripheral immune system in these mice in both the draining (deep cervical) and the nondraining (auxiliary) lymph nodes. As expected, the numbers of TCRβ+ cells and percentage of CD4+ T cells in all tested lymph nodes of the stressed mice were slightly yet significantly reduced (Fig. 1d and e).

After these mice had been exposed to chronic unpredicted mild stress for 4 weeks, we examined their responses to injury. Our hypothesis was that the depressed mice would show signs of exacerbated neuronal degeneration. For this experiment we used two control groups, one consisting of naïve mice (not exposed to stress) and the other comprising acutely stressed mice (exposed to a single stress). The mice in all three groups were subjected to optic nerve crush injury and their neuronal survival was assessed 1 week later. No differences were observed between the groups ($F(2,18) = 0.658$, $p = 0.530$, Fig. 1f), suggesting that the immune suppression detected earlier in the lymph nodes of the chronically stressed mice was not sufficient to impair neuronal survival in this model.

Next, we examined whether therapeutic vaccination with Copaxone (shown in this and other models of neurodegeneration to be neuroprotective) retains its therapeutic effect in the stressed mouse model. To this end, we subjected another group of mice to the 4-week paradigm of chronic unpredicted mild stress, and then injected each mouse with three doses of Copaxone. The first dose was given 6 days before the injury, the next dose 3 days before the injury, and the last on the day of injury. A control group of naïve (nonstressed) mice received similar injections. As expected, Copaxone treatment was highly neuroprotective after optic nerve crush injury in the naïve mice (Fig. 2a and b). In the stressed mice, however, treatment with Copaxone after optic crush injury had no

Table 1
Experimental protocol. Regimen of different chronic mild stressors on each day and behavioral examination of the subjected mice throughout the experiment are presented. Injury induction and endpoint of the experiment are also demonstrated.

	Frequency	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20	Day 21	Day 22	Day 23	Day 24	Day 25	Day 26	Day 27	Day 28	Day 29	Day 30	Day 31	Day 32	Day 33	Day 34	Day 35	Day 36	Day 37	Day 38
1 h of restraining in a 50 mL tube and 24 h of light(no dark period)	every 3 days	X				X			X			X		X				X			X				X				X				X				X			
1 h of tail suspension and 12 h of tilting with little bedding	every 3 days			X			X			X			X		X				X			X				X				X				X				X		
0.5 h of forced swimming	every 3 days				X			X			X			X			X			X			X				X			X				X				X		
Social Change	every 3 days				X			X			X			X			X			X			X				X			X										
Weight	weekly	X							X	X										X			X							X										
Tail Suspension Test (6 min)	weekly	X							X							X				X			X							X										
Forced Swim Test (6 min)	weekly		X							X						X					X			X							X									
Sucrose preference Test	end of 4 week																X										X	X	X											
Fluorogold injection	on day 28																													X										
Optic nerve crush	on day 28																																X							
Retinat's detachment	on day 38																																							X