Journal of Neuroimmunology 258 (2013) 32–40

Contents lists available at SciVerse ScienceDirect

Journal of Neuroimmunology

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

Nasal administration of drugs as a new non-invasive strategy for efficient treatment of multiple sclerosis

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ARTICLE INFO

Article history:
Received 30 October 2012
Received in revised form 24 January 2013
Accepted 19 February 2013

Keywords:
Multiple sclerosis
Experimental autoimmune encephalomyelitis
Nasal drug administration
Glatiramer acetate
Cannabidiol
Neurogenesis

ABSTRACT

We investigated the efficiency of nasal drug administration as a new non-invasive treatment strategy for MS. Glatiramer Acetate (GA) and GA–Cannabidiol (CBD) combination administered in nasal delivery system (NDS) resulted in a statistically significant decrease of clinical scores and inflammatory cytokine expression in experimental autoimmune encephalomyelitis (EAE) mice. Even a suboptimal dose of Prednisolone in NDS was effective in preventing the clinical signs of the disease. Neuron regeneration was observed in the hippocampus of EAE mice treated with GA-CBD in NDS. This work shows that nasal administration improved drug efficiency and stimulates further research for a non-invasive strategy for MS.

1. Introduction

To improve MS therapy efficiency and patient compliance, replacement of the invasive parenteral mode of administration was widely investigated. As a result, Fingolimod, the first orally administered drug for Relapsing Remitting MS (RRMS) was approved by FDA in September 2010 (Killestein et al., 2011). However, its use is associated with a number of adverse effects, demanding long-term safety data (Hohfeld et al., 2011).

The goal of this work was to study nasal administration of a number of drugs in a nasal delivery system (NDS) as a new treatment strategy for MS. For more than two decades, the nasal route is being extensively investigated as an alternative to oral and parenteral administration of drugs for treatment of systemic and central nervous system ailments (Chien, 1992; Illum, 2000, 2003, 2004). However, the systemic and brain absorption of many drugs administered by the nasal route are poor and request specially designed carriers (Hinchcliffe and Illum, 1999; Arora et al., 2002). In previous work, Touitou and her group (Duchi et al., 2011) have shown that NDS, designed for nasal administration, could improve pain therapy and shorten the onset of action.

We have tested here the effect of GA, Prednisolone and a new combination of GA-CBD administered nasally in NDS in the experimental model for MS–EAE.

2. Materials and methods

Animals received the following: Prednisolone (PRE, Sigma, Israel), GA (Teva Pharmaceutical Industries, Israel) and Cannabidiol (CBD, a gift from Prof. Rafael Meshulam from the School of Pharmacy, the Hebrew University of Jerusalem, Israel).

NDS, the nasal delivery carrier used in this study is made of soy phospholipid (Phospholipon 90 G, Phospholipid, US) containing 94% phosphatidylcholine, lysophosphatidylcholine and Vitamin E.

2.1. Induction of EAE and treatment

All experiments on animals were carried out in full compliance with the protocol approved by the joint ethics committee (IACUC) of the Hebrew University and Hadassah Medical Center for animal welfare. The Hebrew University is an AAALAC International accredited institute.

Female C57Bl/6J mice, 6–7 weeks of age, were obtained from Harlan (Harlan, Israel). Mice were housed in cages with free access to food and water and maintained on a 12 h light/dark cycle at room temperature.

Mice were immunized according to a previously described method (Lehmann et al., 1997; Stuve et al., 2008).

Mice were observed daily for the appearance of neurological symptoms and scored arbitrary on a scale from 0 to 6 as follows: 0 = no

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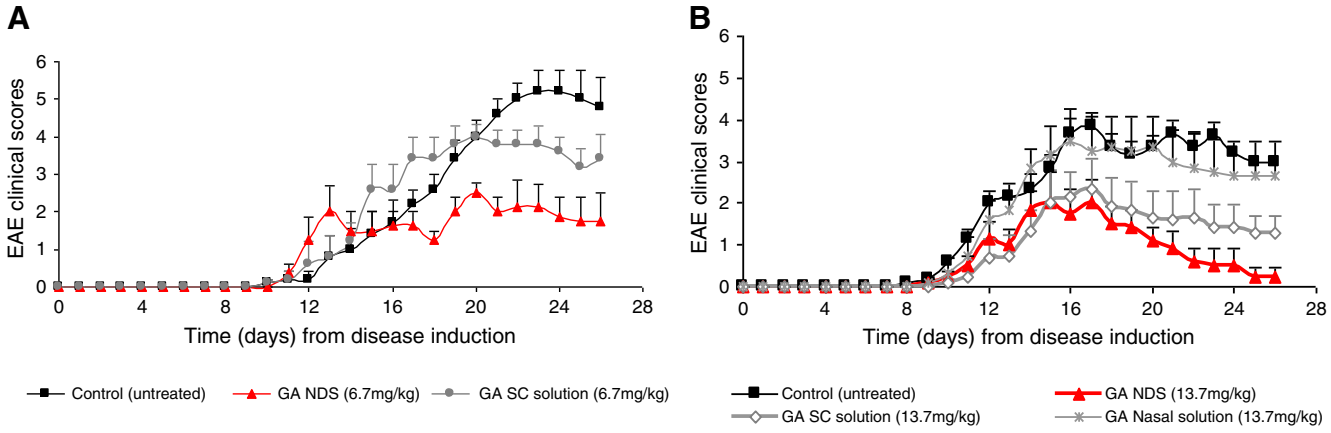
http://dx.doi.org/10.1016/j.jneuroim.2013.02.013

Table 1
EAE mice treatment groups and the mice number used.

Drug	Dose (mg/kg/day)	Administration mode	System	Number of animals	
				Immunized	Developed EAE and clinically evaluated
GA	6.7	Nasally acute	NDS	5	4
		SC acute	Solution	5	5
	13.7	Nasally acute	NDS	6	6[a,b]
		SC acute	Solution	6	6
		Nasally acute	Solution	7	6[a,b]
CBD	6.7	Nasally acute	NDS	5	5[b]
		SC acute	Solution	5	4
CBD + GA	6.7 + 6.7	Nasally acute	NDS	5	5[b]
		SC acute	Solution	5	4
Prednisolone	1.5	Nasally preventive	NDS	6	5[a,b]
		SC preventive	Solution	6	6[b,c]

* Acute treatment–drug administration from the day the first clinical sign appeared; Preventive treatment - drug administration from the day of disease inoculation.
** Two mice were chosen randomly on day 23 or 26 post EAE induction from each experimental group and sacrificed. Then, cerebellum and the spinal cords were removed for cytokine gene expression and histology analysis, respectively.
[a] Two mice from this group were used for evaluation of histopathological changes in the nasal mucosa following system application.
[b] Two mice from this group were used for evaluation of gene expression profiles following system application.
[c] Two mice from this group were used for evaluation of histopathological changes in the spinal cord and cerebellum following system application.

neurological signs; 0.5 = distal limb tail; 1 = limp tail, 2 = loss of righting reflex (the mice have difficulty to turn over after being laid down on the back, but no observed locomotive difficulties); 3 = ataxia, hind limb paresis/paralysis (hind limbs are dragged); 4 = paralysis of the hind legs 5 = quadriplegia, full paralysis (immobility) and 6 = death of the animal (Ovadia and Paterson, 1982). The scoring was carried out on a daily basis by two different observers (one of them blinded).



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Dose (mg/kg/d)	Group	Incidence & mortality	Mean maximal score [a]	Group mean score [b]	Clinical score ≥1, Days [c]	Mean clinical score on day 26	Inhibition % [d]
6.7	NDS	4/5 (0)**	3.00±0.41*	1.70±0.37	12.50±1.76	1.75±0.75*	39.10
	SC injection	5/5 (1)	4.60±0.40	2.61±0.34	13.00±0.95	3.40±0.68	6.45
	Control	5/5 (3)	5.40±0.40	2.79±0.34	12.20±0.97	4.80±0.80	-----
13.7	NDS	6/6 (0)	2.67±0.49*	1.13±0.33*	7.67±1.80*	0.25±0.20*	55.51
	SC injection	6/6 (0)	3.00±0.58	1.42±0.40	8.67±2.04*	1.30±0.40*	44.10
	Nasal solution	6/7 (1)	3.83±0.60	2.44±0.54	13.33±0.92	2.67±0.83	3.94
	Control	6/6 (0)	4.50±0.50	2.54±0.35	15.33±0.99	3.00±0.50	---

Fig. 1. Nasal administration of 6.7 or 13.7 mg/kg/days GA in NDS reduces the clinical score of the disease in EAE mice. Mean clinical scores (mean ± SE) of MOG-induced EAE mice receiving (A) 6.7 mg/kg/days GA nasally in NDS or by subcutaneous injection; or (B) 13.7 mg/kg/days GA in NDS nasally or in solution nasally or by SC injection, on the first day of the clinical manifestation of the disease. (C) Clinical parameters of EAE in mice treated with GA in NDS or in solution given nasally or subcutaneously. *$p < 0.05$ compared to control untreated mice. n, 5–7, for each treatment group.