Epilepsy Research (2014) 108, 396—404

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

Glutathione pegylated liposomal methylprednisolone administration after the early phase of status epilepticus did not modify epileptogenesis in the rat

Linda Holtman[a], Erwin A. van Vliet[a,b], Chantal Appeldoorn[c], Pieter J. Gaillard[c], Marco de Boer[c], Rick Dorland[c], Wytse J. Wadman[a,b], Jan A. Gorter[a,b,*]

[a] *Swammerdam Institute for Life Sciences, Center for Neuroscience, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands*
[b] *Epilepsy Institute in The Netherlands Foundation (Stichting Epilepsie Instellingen Nederland, SEIN), Achterweg 5, 2103 SW Heemstede, The Netherlands*
[c] *to-BBB technologies BV, J.H. Oortweg 19, 2333 CH Leiden, The Netherlands*

Received 23 July 2013; received in revised form 2 December 2013; accepted 6 January 2014
Available online 31 January 2014

KEYWORDS
Brain inflammation;
Temporal lobe epilepsy;
Pulse treatment;
Methylprednisolone;
Liposomes

Summary It has been reported that glucocorticoids (GCs) can effectively control seizures in pediatric epilepsy syndromes, possibly by inhibition of inflammation. Since inflammation is supposed to be involved in epileptogenesis, we hypothesized that treatment with GCs would reduce brain inflammation and thereby modify epileptogenesis in a rat model for temporal lobe epilepsy, in which epilepsy gradually develops after electrically induced status epilepticus (SE).

To prevent the severe adverse effects that are inevitable with long-term GC treatment, we used liposome nanotechnology (G-Technology®) to enhance the sustained delivery to the brain. Starting 4 h after onset of SE, rats were treated with glutathione pegylated liposomal methylprednisolone (GSH-PEG liposomal MP) according to a treatment protocol (1× per week; 10 mg/kg) that is effective in other models of neuroinflammation.

Continuous electro-encephalogram (EEG) recordings revealed that SE duration and onset of spontaneous seizures were not affected by GSH-PEG liposomal MP treatment. The number and duration of spontaneous seizures were also not different between vehicle and GSH-PEG liposomal MP-treated animals. Six weeks after SE, brain inflammation, as assessed by quantification of microglia activation, was not reduced by GSH-PEG liposomal MP-treatment. Also, neuronal cell loss and mossy fiber sprouting were not affected.

* Corresponding author at: Swammerdam Institute for Life Sciences, Center for Neuroscience, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands. Tel.: +31 205257893.
E-mail address: j.a.gorter@uva.nl (J.A. Gorter).

0920-1211/$ — see front matter
http://dx.doi.org/10.1016/j.eplepsyres.2014.01.010

Our study shows that the selected GSH-PEG liposomal MP treatment regimen that was administered beyond the acute SE phase does not reduce brain inflammation and development of temporal lobe epilepsy.

Introduction

Molecular studies in temporal lobe epilepsy animal models have indicated that brain inflammation and immune response may be important targets for epilepsy therapy (Gorter et al., 2006; Vezzani et al., 2011a,b). This has been further supported by studies in tissue from epilepsy patients (Aronica and Crino, 2011; van Gassen et al., 2008). Brain inflammation has been observed at the level of brain parenchymal cells and at the level of the endothelial cells that form the blood-brain barrier (BBB) (Ravizza et al., 2008; Vezzani and Granata, 2005). Therefore it has been investigated whether interference with specific inflammatory pathways could provide a therapy for epilepsy. Interference with specific anti-inflammatory drugs in models of epilepsy might suppress seizures (Maroso et al., 2011; Ravizza et al., 2006) and could have an anti-epileptogenic effect (Fabene et al., 2008). However, the complexity and the multitude of inflammatory pathways suggest that targeting of a broad spectrum of inflammatory pathways may be a strategy with therapeutic potential (Vezzani et al., 2011b). The administration of glucocorticoids (GCs), which are known for their broad anti-inflammatory and immunosuppressive features (Avnir et al., 2008; McMaster and Ray, 2007) and which are widely used for several pediatric epilepsy syndromes (Darke et al., 2010; Gupta and Appleton, 2005; Sevilla-Castillo et al., 2009) might also be an option to treat temporal lobe epilepsy (TLE). The exact mechanisms on how they can suppress seizures are not clear, but suppression of inflammation and restoration of BBB integrity have been suggested as possible mechanisms (Gupta and Appleton, 2005; Marchi et al., 2011; Ozkara and Vigevano, 2011; Tischner and Reichardt, 2007). Therefore, we hypothesized that treatment with the synthetic corticosteroid methylprednisolone (MP) will reduce brain inflammation and delay or prevent the development of epilepsy. However, chronic treatment with high doses of corticosteroids can cause irreversible adverse effects, such as glucocorticoid-induced osteoporosis, glaucoma, decreased wound healing and it will increase the risk of infections due to their overall immunosuppressive action (McMaster and Ray, 2007). If the plasma circulation time can be prolonged and the brain delivery of MP can be enhanced, a lower total dosage can be administered while maintaining the effectiveness in the brain. This could limit the side effects that are associated with chronic use of GCs. In the present study, we used a delivery method that is used to enhance the sustained delivery of drugs across the BBB. MP was incorporated in liposomes, which increases the half-life of MP (Gaillard et al., 2012). In addition, to enhance brain delivery of liposomal MP, the liposomes were coated with glutathione, which enables specific transport of the liposomes into the brain by glutathione transporters (Rip et al., 2011). Together, this enhances availability of MP in the brain (Gaillard et al., 2012). These glutathione pegylated (GSH-PEG) liposomes (G-Technology®) filled with MP effectively reduce clinical symptoms in an animal model for multiple sclerosis (Gaillard et al., 2012), which is associated with severe brain inflammation (Goverman, 2009).

We used a rat model for temporal lobe epilepsy (Gorter et al., 2001) in which epilepsy develops after electrically induced status epilepticus (SE). Animals received an exploratory treatment regimen of six weekly administrations of GSH-PEG liposomal MP at a dose that was previously used to reduce clinical signs of experimental autoimmune encephalitis (EAE) and experimental autoimmune uveitis rats and SOD1 G93A mice (unpublished results).

To evaluate the effect of MP on the development of epilepsy rather than the acute SE phase, treatment started beyond the early SE phase. After 6 weeks, when all animals had developed spontaneous seizures, histochemical and immunocytochemical analyses were performed on brain tissue to investigate whether brain inflammation, neuronal cell loss and BBB leakage were affected by GSH-PEG liposomal MP treatment.

We expected that the immunosuppressive action of MP would reduce brain inflammation and BBB leakage, which would delay or prevent the development of epilepsy in this model for temporal lobe epilepsy.

Material and methods

Experimental animals

Adult male Sprague Dawley rats (Harlan Netherlands, Horst, The Netherlands) weighing 300—500 g were used. This study was approved by the University Animal Welfare Committee. All surgery was performed under anesthesia, and all efforts were made to minimize suffering. The rats were housed individually in a controlled environment ($21 \pm 1\,^{\circ}C$; humidity 60%; lights on 08:00 a.m. to 8:00 p.m.; food and water available ad libitum).

Electrode implantation and status epilepticus (SE) induction

In order to record hippocampal EEG and to induce SE, insulated stainless steel electrodes were implanted in the left dentate gyrus and angular bundle, respectively, as described previously (Gorter et al., 2001). SE was characterized by periodic epileptiform discharges (PEDs) of 1—2 Hz and was accompanied by behavioral and electroencephalographic (EEG) seizures, which lasted for several hours. Control animals ($n = 6$) were implanted with electrodes in an identical manner, but they did not experience SE. During the experiments, video-EEG was recorded continuously (24 h/day) until the animals were killed, as described previously (Gorter et al., 2001).