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Minocycline fails to exert antiepileptogenic effects in a rat status epilepticus model

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ABSTRACT

The tetracycline antibiotic minocycline can exert strong anti-inflammatory, antioxidant, and anti-apoptotic effects. There is cumulating evidence that epileptogenic brain insults trigger neuroinflammation and anti-inflammatory concepts can modulate the process of epileptogenesis. Based on the mechanisms of action discussed for minocycline, the compound is of interest for intervention studies as it can prevent the polarization of microglia into a pro-inflammatory state.

Here, we assessed the efficacy of sub-chronic minocycline administration initiated immediately following an electrically-induced status epilepticus in rats. The treatment did not affect the development of spontaneous seizures. However, minocycline attenuated behavioral long-term consequences of status epilepticus with a reduction in hyperactivity and hyperlocomotion. Furthermore, the compound limited the spatial learning deficits observed in the post-status epilepticus model. The typical status epilepticus-induced neuronal cell loss was evident in the hippocampus and the piriform cortex. Minocycline exposure selectively protected neurons in the piriform cortex and the hilus, but not in the hippocampal pyramidal layer.

In conclusion, the data argue against an antiepileptogenic effect of minocycline in adult rats. However, the findings suggest a disease-modifying impact of the tetracycline affecting the development of behavioral co-morbidities, as well as long-term consequences on spatial learning. In addition, minocycline administration resulted in a selective neuroprotective effect. Although strong anti-inflammatory effects have been proposed for minocycline, we could not verify these effects in our experimental model. Considering the multitude of mechanisms claimed to contribute to minocycline's effects, it is of interest to further explore the exact mechanisms underlying the beneficial effects in future studies.

1. Introduction

Epileptogenic brain insults can trigger microglia activation, resulting in an enhanced formation and release of pro-inflammatory mediators (Allan and Rothwell, 2001; Ravizza et al., 2011; Walker and Sills, 2012). As a direct consequence, a vicious circle can be induced with an impact on astrocyte function, increased formation of reactive oxygen species and nitric oxide, and subsequent neuronal damage (Devinsky et al., 2013). Cellular components from damaged neurons can then further enhance microglia activation states (Devinsky et al., 2013; Hanisch and Kettenmann, 2007; Xu et al., 2013). These events critically contribute to epileptogenesis and associated co-morbidities (Ravizza et al., 2013; Vezzani et al., 2013).

Both, genetic and pharmacological targeting strategies, have shown some success in experimental rodent studies with a delay in the formation of a hyperexcitable network in the kindling model of temporal lobe epilepsy or in post-status epilepticus models (Maroso et al., 2011; Noe et al., 2013; Ravizza et al., 2011). The vast majority of the strategies specifically targeted single pro-inflammatory mediators such as cyclooxygenase-2 (COX-2), IL-1β, and TNFα. Respective approaches might efficaciously prevent detrimental effects of one enzyme or cytokine, however, other mediators might still maintain the vicious circle of glial activation and neuronal damage. Therefore, there is an interest in evaluating strategies, which have a less specific anti-inflammatory mechanism of action and affect the overall microglia functional state. Minocycline can efficaciously inhibit the polarization of microglia to a pro-inflammatory state (Kobayashi et al., 2013), and might thus, serve as an interesting compound for broader targeting approaches. Disease-modifying effects have been demonstrated for minocycline in different rodent models of neurological diseases

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including amyotrophic lateral sclerosis, cerebral ischemia, traumatic brain injury, Parkinson's disease, and Huntington's disease (Kobayashi et al., 2013; Sanchez Mejia et al., 2001; Wang et al., 2003; Wu et al., 2002; Yrjanheikki et al., 1999). In these models minocycline efficaciously reduced the lesion size and associated neurological deficits. These effects are probably related to the attenuation of glial activation and of the transcription of downstream pro-inflammatory mediators including COX-2, iNOS, NAPDH-oxidase and P38 MAPK (Kim and Suh, 2009). It has been reported that minocycline partially suppresses the production of inflammatory molecules (IL-6, TNFα and IL-1β) by inhibition of the nuclear translocation of NF-$_K$B (Kobayashi et al., 2013; Pang et al., 2012). Previous studies revealed an acute anticonvulsant effect of minocycline in seizure and epilepsy models (Beheshti Nasr et al., 2013; Wang et al., 2012). However, there is a lack of studies which addressed the question whether minocycline can exert preventive disease-modifying or antiepileptogenic effects in chronic epilepsy models with development of spontaneous seizures. Two studies have assessed respective effects with status epilepticus induction in the early postnatal phase (Abraham et al., 2012; Kwon et al., 2013). When administered alone minocycline had no impact on status epilepticus-associated hippocampal neuronal injury and development of spontaneous seizures in rat pups (Kwon et al., 2013). In a "two-hit" model with repeated induction of status epilepticus in mice pups, minocycline treatment following the first seizure event abrogated the increased susceptibility to the second chemoconvulsant exposure (Abraham et al., 2012). The authors concluded that this finding might indicate an antiepileptogenic potency of minocycline, however it requires further assessment.

In general, the effects observed in the early postnatal phase cannot be extrapolated to the adult phase, considering that the consequences of status epilepticus and the response to pharmacological intervention differ tremendously between these stages of life (Baram, 2012). Therefore, we aimed to thoroughly evaluate the effects of minocycline treatment initiated following electrically-induced status epilepticus in adult rats. The impact on development of spontaneous seizures, behavioral alterations, spatial learning, and neurodegeneration was assessed.

2. Material and methods

2.1. Animals

Experiments were performed in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/EU) and following approval by the Government of Upper Bavaria (license number: 55.2-1-54-2532-173-11). All efforts were made to reduce the number of animals and minimize pain or discomfort of the rats used in this study. Female Sprague Dawley rats weighing 200–224 g were obtained from Harlan Laboratories, An Venray, The Netherlands. Animals were housed under controlled environmental conditions (24–25 °C, 50–60% humidity, 12 h dark/light cycle) with freely available tap water and food.

2.2. Electrode implantation

A teflon-isolated bipolar stimulation- and recording-electrode was stereotactically implanted into the right anterior basolateral nucleus of the amygdala (BLA), as previously described (Pekcec et al., 2008). This sub-region of the amygdala is highly sensitive to electrical stimulation resulting in the development of a self-sustained status epilepticus (Brandt et al., 2003b). The stereotactic coordinates of the tip of the electrode were ap −2.2, lat +4.7 and dv −8.5 in mm relative to Bregma. Implantation was carried out at least one week after the arrival of the animals. Rats were anesthetized by an intraperitoneal injection of chloral hydrate (360 mg/kg). Thirty minutes prior and 24 h following implantation animals received a subcutaneous injection of Meloxicam (Metacam® 5 mg/ml, Boehringer Ingelheim Vetmedica GmbH, Ingelheim/Rhein, Germany; 1 mg/kg). Bupivacain 0.5% (Bupivacain Jenapharm® 0.5%, Mibe GmbH, Brehna, Germany) was subcutaneously injected for local anesthesia. Antibiotics (Marbofloxacin, Marbocyl®, Vétoquinol, Ravensburg, Germany; 1 mg/kg) were subcutaneously administered twice daily (8.00 a.m. and 4.00 p.m.) for eight days to prevent post-operative infections.

2.3. Electrical induction of a self-sustained status epilepticus

At least six weeks after electrode implantation, a self-sustained status epilepticus (SE) was induced in 33 rats. The time interval was chosen based on a previous study, which revealed that a period of several weeks following implantation increases the sensitivity of the implanted area to seizure induction (Bankstahl et al., 2014), therefore facilitating the induction of a SE. Animals were stimulated via the BLA electrode as described previously (Brandt et al., 2003b; Pekcec et al., 2008) with a peak pulse intensity of 700 μA. The stimulus lasted for 25 min and consisted of 100 ms trains (frequency of 2/s and intra-train pulse frequency of 50/s) of 1 ms alternating positive and negative square-wave pulses. An Accupulser A310C stimulator connected with a stimulus isolator (A365, World precision Instruments, Berlin, Germany) was used for stimulation. Following the end of the stimulation, the development and duration of SE were monitored by 5 min EEG recording and 3.35 h behavioral observation. After the termination of the electrical BLA stimulation, the behavioral seizure activity was used to classify the SE type. Therefore, seizure severity was classified according to the grading system by Racine (Racine, 1972) and used to divide SE into three different types: type 1 (partial SE) was characterized by non-convulsive seizure activity and stereotypies (e.g., sniffing); type 2 (partial SE with generalized seizures) by predominantly partial seizure activity interrupted by occasional episodes of generalized convulsive (stage 4/5) seizures and type 3 (generalized convulsive SE) by generalized seizure activity. Four hours after the induction of SE, animals received an intraperitoneal injection of diazepam (Diazepam-ratiopharm®, ratiopharm GmbH, Ulm, Germany; 10 mg/kg). This time point for diazepam injection was chosen based on a study, which evaluated several experimental conditions in the model, showing that diazepam administered at this relatively late time point does not exert major effects on epileptogenesis (Brandt et al., 2003a). Behavioral observation and EEG analyzes were used to verify the efficacy of diazepam treatment. In case of ongoing seizure activity, the application of this dose of diazepam was repeated until motor seizure activity was completely suppressed and EEG was normalized to baseline activity. Animals exhibiting type I SE were excluded from further analyzes (seizure monitoring, behavioral and immunohistological analyzes), whereas rats with type II and III SE were randomly distributed to different treatment groups [vehicle-treated control rats ($n=13$), vehicle-treated SE rats ($n=14$), minocycline-treated control rats ($n=13$), minocycline-treated SE rats ($n=14$)]. The mean number of diazepam applications did not differ between the treatment groups. Electrode-implanted control animals were not stimulated, but subjected to all handling and experimental procedures including the diazepam injection.

2.4. Treatment with minocycline

The study was designed to test for an antiepileptogenic effect. Therefore, animals received the first administration of either minocycline (minocycline hydrochlorid, Sigma, Saint Louis, USA) or vehicle solution (aqua ad injectabilia, B. Braun Melsungen AG,