Epilepsy Research (2014) **108**, 1719—1727

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

Low doses of ethanol markedly potentiate the anti-seizure effect of diazepam in a mouse model of difficult-to-treat focal seizures

Sabine Klein[a,b], **Marion Bankstahl**[a,b], **Martina Gramer**[a,b], **Maria Hausknecht**[a,b], **Wolfgang Löscher**[a,b,*]

[a] *Department of Pharmacology, Toxicology, and Pharmacy, University of Veterinary Medicine Hannover, Hannover, Germany*
[b] *Center for Systems Neuroscience, Hannover, Germany*

Received 26 June 2014; received in revised form 17 September 2014; accepted 1 October 2014
Available online 15 October 2014

KEYWORDS
Epilepsy;
GABA receptors;
Benzodiazepines;
HPLC, high performance liquid chromatography;
Pharmacoresistance;
Kainate

Summary Ethanol is commonly used as a solvent in injectable formulations of poorly water-soluble drugs. The concentrations of ethanol in such formulations are generally considered reasonably safe. It is long known that ethanol can potentiate central effects of sedatives and tranquillizers, particularly the benzodiazepines, most likely as a result of a synergistic interaction at the $GABA_A$ receptor. However, whether this occurs at the low systemic doses of ethanol resulting from its use as solvent in parenteral formulations of benzodiazepines is not known. In the present study we evaluated whether a commercial ethanol-containing aqueous solution of diazepam exerts more potent anti-seizure effects than an aqueous solution of diazepam hydrochloride or an aqueous emulsion of this drug in the intrahippocampal kainate model of temporal lobe epilepsy in mice. Spontaneous epileptic seizures in this model are known to be resistant to major antiepileptic drugs. Administration of the ethanol-containing formulation of diazepam caused an almost complete suppression of seizures. This was not seen when the same dose (5 mg/kg) of diazepam was administered as aqueous solution or emulsion,

Abbreviations: BZD, benzodiazepine; EEG, electroencephalogram; SE, status epilepticus; SRS, spontaneous recurrent seizures.

* Corresponding author at: Department of Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine Hannover, Bünteweg 17, D-30559 Hannover, Germany. Tel.:+49 511 856 8721; fax: +49 511 953 8581.
E-mail address: wolfgang.loescher@tiho-hannover.de (W. Löscher).

http://dx.doi.org/10.1016/j.eplepsyres.2014.10.002

although all three diazepam formulations resulted in similar drug and metabolite concentrations in plasma. Our data demonstrate that ethanol-containing solutions of diazepam are superior to block difficult-to-treat seizures to other formulations of diazepam. To our knowledge, this has not been demonstrated before and, if this finding can be translated to humans, may have important consequences for emergency treatment of acute seizures, series of seizures, and initial treatment of status epilepticus in patients.

Introduction

Benzodiazepines (BZDs) such as diazepam or lorazepam are drugs of first choice for emergency treatment of acute seizures, series of seizures, and initial treatment of status epilepticus (SE) (Schmidt and Wilensky, 2008). In addition, several BZDs, including clobazam and clonazepam, are in clinical use for prolonged treatment of chronic refractory epilepsy, mostly as add-on medication; however, loss of efficacy during prolonged treatment has limited their usefulness (Löscher and Schmidt, 2006).

For emergency treatment of seizures, BZDs such as diazepam are i.v. administered as solution or emulsion (Engel, 2013). Diazepam is only slightly soluble in water but highly soluble in ethanol, so that ethanol is commonly used as a cosolvent in aqueous solutions (Jouyban et al., 2009). However, ethanol is not a neutral cosolvent but, as BZDs, rapidly penetrates into the brain after systemic administration, where, among other effects, it potentiates the inhibitory activity of the neurotransmitter GABA through an effect at the $GABA_A$ receptor (Davies, 2003; Olsen et al., 2007). BZDs, too, act at the $GABA_A$ receptor to potentiate GABA, which explains that BZDs and ethanol share many pharmacological properties and show a well-known synergism in their action when administered together (Olsen et al., 2007). Synergistic interactions between ethanol and BZDs such as diazepam have been reported for several behavioral paradigms in rodents (e.g., Milner, 1968; Linnoila et al., 1979; Van Gorder et al., 1985; Hu et al., 1987; Bach-Rojecky and Samarzija, 2005; Pietrzak and Czarnecka, 2005) and humans (Curry and Smith, 1979; Costa and Guidotti, 1996), including anxiolytic, sedative and hypnotic effects, locomotor coordination, vestibular and visual functions, psychomotor and cognitive performance, muscle strength, and body temperature. Similar to BZDs, ethanol also exerts anti-seizure activity, but only few studies examined whether ethanol potentiates the anti-seizure effect of BZDs (Rastogi and Ticku, 1986; Nutt and Lister, 1988). In order to avoid possible synergistic or additive interaction between ethanol and BDZs, the amount of ethanol used to dissolve BDZs is very small in commercial solutions. However, to our knowledge it is not known whether this small amount of ethanol used as a cosolvent in aqueous BZD solutions may potentiate the anti-seizure effect of the BZD.

This prompted us to compare the anti-seizure effect of different commercial and experimental formulations of diazepam that either contain ethanol or not in a mouse model of difficult-to-treat focal seizures. In this model, kainate is injected into the hippocampus of mice, resulting in limbic SE that induces the development of highly frequent spontaneous recurrent focal seizures, which are resistant to major clinically used antiepileptic drugs (Riban et al., 2002). The high frequency of seizures in this model allows studying the acute anti-seizure effect of drugs on difficult-to-treat spontaneous seizures (Riban et al., 2002). In addition to study the anti-seizure effect of diazepam with or without ethanol in this model, we determined whether the different formulations of diazepam differed in their pharmacokinetics by determining plasma concentrations of diazepam and its active metabolites in mice.

Materials and methods

Animals

FVB/N mice were obtained from Taconic (Ejby, Danmark) at an age of 6 weeks. Female animals were used in all experiments to allow comparison with previous experiments of our group (Gröticke et al., 2008; Töpfer et al., 2014). Animals were housed under controlled conditions (ambient temperature 22—24 °C, humidity 30—50%, lights on from 6:00 a.m. to 6:00 p.m.) and adapted to the laboratories for at least 2 weeks before being used in the experiments. Food (Altromin 1324 standard diet; Altromin, Lage, Germany) and water were freely available.

Experiments were performed according to the EU council directive 86/609/EWG and the German Law on Animal Protection (''Tierschutzgesetz''). Ethical approval for the study was granted by an ethical committee (according to §15 of the Tierschutzgesetz) and the government agency (Lower Saxony State Office for Consumer Protection and Food Safety; LAVES) responsible for approval of animal experiments in Lower Saxony (reference number for this project: 09/1769). All efforts were made to minimize both the suffering and the number of animals.

Intrahippocampal kainate model in mice

In this model, SE is induced by intrahippocampal injection of kainate, which induces spontaneous recurrent seizures (SRS) in the weeks following SE (Riban et al., 2002). For this purpose, mice were anesthetized with chloral hydrate (400 mg/kg i.p.) and kainate (0.21 μg in 50 nl saline) was stereotaxically injected into the right CA1 area of the dorsal hippocampus as described previously (Gröticke et al., 2008). Kainate was slowly injected over 60 s with a 0.5 μl microsyringe at the following stereotaxic coordinates: AP,—1.8 mm; L,—1.6 mm; and V,—2.0 mm, respectively (Paxinos and Franklin, 2001). After injection, the needle of the syringe was maintained in situ for additional 2 min to limit reflux along the injection track. For electroencephalographic