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Simultaneous triple therapy for the treatment of status epilepticus

Jerome Niquet [a,b,*], Roger Baldwin [b], Keith Norman [b], Lucie Suchomelova [b], Lucille Lumley [c], Claude G. Wasterlain [a,b,d]

[a] Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA
[b] Epilepsy Research Laboratory (151), Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, CA, USA
[c] US Army Medical Research Institute of Chemical Defense (USAMRICD), 2900 Ricketts Point Rd., Aberdeen Proving Ground, MD 21010, USA
[d] Brain Research Institute, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

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ABSTRACT

Early maladaptive internalization of synaptic $GABA_A$ receptors ($GABA_AR$) and externalization of NMDA receptors (NMDAR) may explain the time-dependent loss of potency of standard anti-epileptic drugs (AED) in refractory status epilepticus (SE). We hypothesized that correcting the effects of changes in $GABA_AR$ and NMDAR would terminate SE, even when treatment is delayed 40 minutes. SE was induced in adult Sprague-Dawley rats with a high dose of lithium and pilocarpine. The $GABA_AR$ agonist midazolam, the NMDAR antagonist ketamine and the AED valproate were injected 40 min after SE onset in combination or as monotherapy. The midazolam-ketamine-valproate combination was more efficient than triple-dose midazolam, ketamine or valproate monotherapy or higher-dose dual therapy in reducing several parameters of SE severity. Triple therapy also reduced SE-induced acute neuronal injury and spatial memory deficits. In addition, simultaneous triple therapy was more efficient than sequential triple therapy: giving the three drugs simultaneously was more efficient at stopping seizures than the standard practice of giving them sequentially. Furthermore, midazolam-ketamine-valproate therapy suppressed seizures far better than the midazolam-fosphenytoin-valproate therapy, which follows evidence-based AES guidelines. These results show that a treatment aimed at correcting maladaptive $GABA_AR$ and NMDAR trafficking can reduce the severity of SE and its long-term consequences.

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1. Introduction

Pharmacoresistance to benzodiazepines and other drugs (Kapur and Macdonald, 1997; Mazarati et al., 1998) remains a challenge in the treatment of status epilepticus (SE), a life-threatening condition which affects 150,000–200,000 patients per year in the USA and is responsible for 22,000–42,000 deaths yearly (DeLorenzo et al., 1996). The incidence of SE increased from 3.5 to 12.5/100,000 between 1979 and 2010 (Dham et al., 2014). Benzodiazepine monotherapy, which is recommended for initial treatment of SE, fails to stop seizures in 35–69% of cases (Glauser et al., 2016; Holtkamp et al., 2005; Mayer et al., 2002; Treiman et al., 1998).

Studies in experimental models of SE show that early maladaptive internalization of synaptic $GABA_A$ receptors ($GABA_AR$) may explain the loss of benzodiazepine potency (Goodkin et al., 2008, 2005; Kapur and Macdonald, 1997; Mazarati et al., 1998; Naylor et al., 2013, 2005). The drugs may stop the seizures in the early stage of SE by binding to $GABA_AR$, but progressively lose potency when $GABA_AR$ are inactivated by internalization into endosomes. At the same time, glutamatergic excitation, driven by migration of NMDAR subunits toward synapses (Naylor et al., 2013), is increasing runaway excitation and excitotoxicity. We hypothesized that polytherapy aimed at correcting the consequences of receptor trafficking should reduce SE severity (Niquet et al., 2016b). Indeed, combinations of a $GABA_AR$ agonist and an NMDAR antagonist, such as diazepam and ketamine (Martin and Kapur, 2008) or midazolam and ketamine (Niquet et al., 2016a) have been successful in treating experimental SE and may be synergistic. However, when treatment is delayed, the reduction of the number of synaptic $GABA_AR$ makes it difficult to fully restore inhibition with benzodiazepines, and another AED acting at a non-benzodiazepine site is needed to restore the balance between excitation and inhibition. In the present study, we treated 40 min after seizure onset, and combined midazolam and ketamine with the AED valproate. We also studied the timing of drug delivery, since recent studies suggest that it is a major determinant of pharmacoresistance (Silbergleit et al., 2012), and compared AES guideline-inspired combinations to our combination, which is based on the receptor-trafficking hypothesis. Our results show that the simultaneous administration of midazolam, ketamine and valproate

* Corresponding author at: Epilepsy Research Laboratory, VA Greater Los Angeles Healthcare System, 11301 Wilshire Boulevard, Building 114, Room 139, West Los Angeles, CA 90073, USA.
E-mail address: jniquet@ucla.edu (J. Niquet).
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is more efficient in stopping seizures than triple dose monotherapy, higher-dose dual therapy, sequential triple therapy, or the midazolam-fosphenytoin-valproate combination.

2. Methods

2.1. Animals

Male Sprague-Dawley rats (200–300 g, mean 249 g; Charles River, MA) were housed in a temperature- and humidity-controlled room with 12 h light-dark cycles (7 am–7 pm) and had free access to food and water. All experiments were conducted with the approval and in accordance with the regulations of the Institutional Animal care and Use Committee of West Los Angeles VA Medical Center.

2.2. Induction of SE, monotherapy and dual therapy

Rats were administered lithium chloride (5 mEq/kg; #L-0505 Sigma, St. Louis MO, USA) subcutaneously and, 16 h later, SE was induced with i.p. pilocarpine hydrochloride (320 mg/kg; #P6503 Sigma). Only lithium/pilocarpine-treated rats displaying behavioral/EEG seizures were used. All rats received scopolamine methyl bromide (1 mg/kg; i.p., #S8502; Sigma), a muscarinic antagonist that does not cross the blood-brain barrier, at the same time as pilocarpine, to decrease peripheral cholinergic effects such as pulmonary secretions. Seizures occurred 7.6 ± 2.7 min after pilocarpine injection, so that time from pilocarpine injection to mono or dual therapy was approximately 48 min. All animals subsequently received scopolamine (10 mg/kg i.p.; #S1013; Sigma) to remove the original seizure trigger without stopping SE, and sham injection (control SE group), one drug (monotherapy), a combination of two drugs (dual therapy) or a combination of three drugs (triple therapy) i.p. 40 min after EEG seizure onset to make sure that pharmacoresistance and self-sustaining seizures were well established. Drugs for monotherapy groups included midazolam (9 mg/kg; Caraco Pharmaceutical Laboratories Ltd), ketamine (90 mg/kg; #RL3760 Hospira), sodium valproate (270 mg/kg; #P4543 Sigma). Dual therapy groups included combination of 4.5 mg/kg midazolam with 45 mg/kg ketamine. Triple therapy groups included combination of 3 mg/kg midazolam with 30 mg/kg ketamine and 90 mg/kg valproate, or 3 mg/kg midazolam with 30 mg/kg ketamine and 100 mg/kg levetiracetam (UCB Pharmaceuticals), or 3 mg/kg midazolam with 50 mg/kg fosphenytoin (Parke-Davis), and 90 mg/kg valproate. The doses used were determined in preliminary experiments when we delayed treatment to 40 min after seizure onset, instead of after the second stage 3 seizure, see (Niquet et al., 2017), which required a higher therapeutic dose. The midazolam dose is lower than the anesthetic doses (25 mg/kg) used in other models of SE (Kofke et al., 1993) but similar to the 1–5 mg/kg used against sarin-induced SE (Chapman et al., 2015). The fosphenytoin dose was selected because of the effectiveness of phenytoin in the perforant path stimulation model of SE (Mazarati et al., 1998). The ketamine dose was higher than the dose (10 mg/kg) which stopped perforant path stimulation SE (Mazarati and Wasterlain, 1999), but similar to the monotherapy dose (100 mg/kg) which stopped hippocampal stimulation-induced or chemically-induced SE (Borris et al., 2000; Fujikawa, 1995) or the dose (50 mg/kg) which stopped SE when combined with diazepam (Martin and Kapur, 2008). Levetiracetam was previously used in perforant path SE (Mazarati et al., 2004) groups, and triple dose of drugs compared to triple therapy groups to compensate for the number of drugs used. In the sequential monotherapy group (Fig. 4), 3 mg/kg midazolam, 90 mg/kg valproate and 30 mg/kg ketamine were injected sequentially 30 min apart. For long-term behavioral studies, a sham group, which did not receive drug treatment and was not exposed to SE, was added.

Rats not capable of coordinated walking and movement 16 h after SE were injected SC (10 ml/kg) with 5% glucose twice per day until capable of coordinated movement or until euthanasia at three days. Water moistened food pellets and or gelatin cubes were placed in the cage in Petri dishes. Euthanasia criteria consisted of failure to achieve coordinated movement three days after SE. Animals were euthanized if showing a weight loss of 5% sustained over two days after the coordination criterion had been achieved.

2.3. Implantation of electrodes

Under isoflurane anesthesia, the animals were implanted with stainless steel skull screws to serve as recording electrodes. Two electrodes were used for bipolar recording and were located 3 mm anterior to lambda and 4 mm left and right of the medial suture. The third electrode served as reference and was located 1 mm anterior to bregma and 1 mm to the right of the mid-line defined by the medial suture. The electrodes were connected to a tri-polar connector (Plastics One, VA) and dental cement was used to cover the electrodes so that only the connector was exposed. Animals were used one to two weeks after electrode implantation. The BioPac Systems MP150 was used to record digital EEG using a BioPac UM100A preamplifier. Sampling rate was 200 Hz.

2.4. Acute video-EEG monitoring

Recording was started before pilocarpine injection and was continuous for 24 h which included an initial pre-pilocarpine segment of EEG, the development of SE, drug treatment, and the overnight recovery period (Fig. 1A). The EEGs were processed offline to detect seizures and spikes using Stellate Systems Harmony software (Natus) with default parameters: amplitude threshold 2.7, minimum frequency 3 Hz, maximum coefficient of variation 40% for seizure detection, and a spike amplitude threshold of 6 for spike detection.

Outcome measures were the ratio of EEG power at T time divided by the average baseline EEG power before pilocarpine; the number of seizures per 24 h, the number of spikes per 24 h; the time needed for EEG amplitude to fall for the first time below 2 times the pre-pilocarpine EEG amplitude and be free of semi-periodic spikes or sharp waves for at least 1 min, and the time in SE after treatment, as previously described (Niquet et al., 2016a; Suchomelova et al., 2006). EEG outcome measures for midazolam, ketamine, valproate, and midazolam-ketamine therapy have been previously published (Niquet et al., 2016a).

2.5. Tissue preparation for detection of acute neuronal injury

The animals were anesthetized with an overdose of pentobarbital (100 mg/kg i.p.) 48 h after induction of SE. Then, the animals underwent transcardiac perfusion with 4% phosphate-buffered formaldehyde (#P-6148 Sigma). Brains were kept in situ at 4 °C overnight, after which they were removed and postfixed in the same perfusate for 2–3 h. Subsequently, brains were kept in PB 0.1 M containing 30% sucrose for 48–72 h. Floating sections (30 μM thickness) were obtained using a sliding microtome. Coronal sections were mounted, dried, incubated in potassium permanganate solution (0.06%; w/v) for 15 min, washed and incubated in fluoro-jade B staining solution for 30 min. After 3 rinses, slides were dried overnight at room temperature, cleared three times in xylene and coverslipped with Permount medium. In CA1 and CA3 areas and in the hilus of the dentate gyrus, the number of injured cells was counted by unbiased stereology using the optical dissector method. The first series of one in five sections were stained with fluoro-jade B, and the analysis was performed using a microscope (Olympus AX70) with a motorized stage connected to a computer running the Stereo Investigator software (MBF Bioscience). A counting frame of 45 × 45 μm was randomly positioned in a sampling grid of 70 × 120 μm. In the other areas (frontoparietal, entorhinal, and piriform cortices, thalamus, and amygdala), distribution of fluoro-jade B-positive cells was scored as follows: 0, no injury; 1: 1–30 positive cells per field; 2: 31–60 positive cells per field; 3: 61–100 positive cells per field; 4: >100 positive cells