Early polytherapy for benzodiazepine-refractory status epilepticus

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ARTICLE INFO

ABSTRACT

The transition from single seizures to status epilepticus (SE) is associated with malaptive trafficking of synaptic gamma-aminobutyric acid ($GABA_A$) and glutamate receptors. The receptor trafficking hypothesis proposes that these changes are key events in the development of pharmacoresistance to antiepileptic drugs (AEDs) during SE, and that blocking their expression will help control drug-refractory SE (RSE).

We tested this hypothesis in a model of SE induced by very high-dose lithium and pilocarpine (RSE), and in a model of SE induced by sc soman. Both models are refractory to benzodiazepines when treated 40 min after seizure onset. Our treatments aimed to correct the loss of inhibition because of SE-associated internalization of synaptic $GABA_A$ receptors ($GABA_A$R), using an allosteric $GABA_A$R modulator, sometimes supplemented by an AED acting at a nonbenzodiazepine site. At the same time, we reduced excitation because of increased synaptic localization of NMDA and AMPA (?-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartate) receptors (NMDAR, AMPAR (?-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, N-methyl-D-aspartate receptors)) with an NMDAR channel blocker, since AMPAR changes are NMDAR-dependent.

Treatment of RSE with combinations of the $GABA_A$R allosteric modulators midazolam or diazepam and the NMDAR antagonists dizocilpine or ketamine terminated RSE unresponsive to high-dose monotherapy. It also reduced RSE-associated neuronal injury, spatial memory deficits, and the occurrence of spontaneous recurrent seizures (SRS), tested several weeks after SE. Treatment of soman-induced SE also reduced seizures, behavioral deficits, and epileptogenesis. Addition of an AED further improved seizure outcome in both models. Three-dimensional isobolograms demonstrated positive cooperativity between midazolam, ketamine, and valproate, without any interaction between the toxicity of these drugs, so that the therapeutic index was increased by combination therapy.

The midazolam–ketamine–valproate combination based on the receptor trafficking hypothesis was far more effective in stopping RSE than the midazolam–fosphenytoin–valproate combination inspired from clinical guidelines for the treatment of SE. Furthermore, sequential administration of midazolam, ketamine, and valproate was far less effective than simultaneous treatment with the same drugs at the same dose.

These data suggest that treatment of RSE should be based at least in part on its pathophysiology. The search for a better treatment should focus on the cause of pharmacoresistance, which is loss of synaptic $GABA_A$R and gain of synaptic glutamate receptors. Both need to be treated. Monotherapy addresses only half the problem. Improved pharmacokinetics will not help pharmacoresistance because of loss of receptors. Waiting for one drug to fail before giving the second drugs gives pharmacoresistance time to develop. Future clinical trials should consider treating both the failure of inhibition and the runaway excitation which characterize RSE, and should include an early polytherapy arm.

This article is part of the Special Issue "Proceedings of the 7th London-Innsbruck Colloquium on Status Epilepticus and Acute Seizures"

Published by Elsevier Inc.

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1. Introduction: the receptor trafficking hypothesis of seizure-induced pharmacoresistance

Many drugs are now available to treat status epilepticus (SE). Most of them are effective when given early in the course of SE [1]. As a matter of fact, a few minutes' gain in delivering benzodiazepines during early SE results in a measurable difference in outcome [2]. However, when treatment is delayed, seizures become refractory to benzodiazepines and other antiepileptic drugs (AEDs) [3–5]. This pharmacoresistance increases as the internalization of synaptic GABA$_A$R, [6–8] and the migration of NMDAR and AMPAR toward synapses progresses [9,10].

At present, we cannot block the seizure-associated acceleration of receptor trafficking, but we can block its consequences. If the receptor trafficking hypothesis is correct, this should reduce pharmacoresistance and improve outcome.

It also suggests that the search for a better monotherapy may be futile, because it is unlikely to correct the maladaptive changes in both GABA and glutamate networks which cause pharmacoresistance, unless one practices "polytherapy with a single drug". It also suggests that the current practice of waiting for one drug to fail before trying the next one [11] may give pharmacoresistance time to develop, and should be reevaluated.

We tested these hypotheses in a model of SE induced by very high-dose lithium and pilocarpine [12], and in a model of soman-induced SE [13]. The results support the receptor trafficking hypothesis. Methods have been published [14,15] and will not be described in this brief review.

2. Drug combinations based on the receptor trafficking hypothesis stop RSE seizures

2.1. Choice of drugs

The loss of synaptic GABA$_A$ receptors and the increase in synaptic NMDAR and AMPAR induced by SE are proconvulsant and maintain seizure activity. Both need to be treated. Benzodiazepines penetrate the brain rapidly and allosterically enhance the GABA$_A$R response. They are essential to the initial treatment of SE. Midazolam and diazepam penetrate the brain more rapidly than lorazepam, and were selected for that reason.

When treatment is delivered late, there may not be enough GABA$_A$Rs left in synapses to fully restore inhibition, and an additional AED which enhances inhibition at a nonbenzodiazepine site provides additional benefit [15]. The choice of the best AED for that purpose would require too much space for this brief review. Valproate is one of several effective candidate drugs and was used in of some of the experiments described here.

Dizocilpine (MK-801), a selective ligand of the NMDAR channel, was used in proof-of-principle experiments. Since it is too toxic for human use, we also used ketamine, an NMDAR ligand which has additional mechanisms of action [16], but is effective when injected iv or im [17], and has a long record of safe human use. Since AMPAR changes are NMDAR-dependent [10], use of an NMDAR blocker addresses both AMPAR and NMDAR changes.

2.2. Results: pilocarpine-induced RSE

Dizocilpine monotherapy (1 mg/kg) reduced seizure number and mortality, but did not terminate SE, cumulative seizure time, time to the first seizure-free minute, or Hjorth function [18], a measure of seizure severity correlated to Electroencephalogram (EEG) power diazepam monotherapy (5–20 mg/kg) reduced mortality but did not stop SE, or reduce seizure number, time to the first seizure-free minute, cumulative seizure time, or Hjorth function. However, the combination of diazepam with dizocilpine was very effective in stopping SE,

eliminating mortality and reducing SE duration over 100-fold compared to the untreated and diazepam-treated groups (Fig. 1A).

The combination of low-dose ketamine (10 mg/kg) with moderate dose diazepam (5 mg/kg) reduced the duration of SE six-fold, and the number of seizures 9-fold compared to controls, diazepam (20 mg/kg), and diazepam (5 mg/kg) animals (p < 0.001), and also reduced the delay to the first seizure-free minute of EEG, Hjorth function, and EEG power integral over the first hour posttreatment. Hjorth function remained low 6 h posttreatment, indicating that seizures did not recur. EEG power immediately before treatment was the same in all groups [14].

2.3. Results: soman-induced SE

Rats treated with midazolam (3 mg/kg) 40 min after SE had greater 24-h survival compared to rats treated with saline or with 1 mg/kg midazolam. EEG power integral increased in all GD-exposed rats during untreated SE. During the first hour after treatment, midazolam or ketamine monotherapy reduced EEG power compared to vehicle, but by 6 h after exposure, there was no difference between these groups (data not shown). Rats treated with 30 mg/kg ketamine + 3 mg/kg midazolam or with 30 mg/kg ketamine + 3 mg/kg midazolam + 90 mg/kg valproic acid after 40 min of GD-induced SE had significantly reduced EEG power integral during the 1-h and the 6-h time periods after treatment, compared to the midazolam monotherapy group.

3. Drug combinations based on the receptor trafficking hypothesis reduce the long-term consequences of RSE

3.1. Neuronal injury

After pilocarpine-induced refractory SE (RSE), no neuronal injury was detected in any ketamine-treated animals in CA3 (Fig. 2A) or CA1 (Fig. 2B), suggesting an NMDAR-dependent mechanism of injury during RSE in hippocampal pyramidal cells. Rats treated with the midazolam–ketamine–valproate combination, which stopped RSE rapidly, showed remarkable reduction of neuronal injury, but rats treated with triple-dose ketamine alone, which did not terminate RSE, showed equally good neuroprotection. Thus, this appeared to be related to the neuroprotective properties of ketamine, rather than to the ability of NMDAR antagonist-containing combinations to stop RSE. This suggests that the early use of ketamine for neuroprotection, regardless of or in addition to its ability to stop seizures, might be a useful strategy in RSE. This neuroprotection is remarkable because signs of neuronal injury have been documented within 20 min of seizure onset [19], but is not universal. Ketamine neuroprotection was less complete in dentate hilus [20], confirming that seizure-induced neuronal injury is both cell-type- and seizure-model-specific.

A neuroprotective effect of the midazolam–ketamine combination was observed 5 weeks after soman-induced SE in hippocampus, thalamus, lateral amygdala, and piriform cortex, while animals treated with midazolam or ketamine alone had significant neuronal loss in those areas (personal communication).

3.2. Spontaneous recurrent seizures

Treatment of pilocarpine-induced RSE with the midazolam + ketamine combination prevented epileptogenesis. No rat treated with that combination displayed any spontaneous recurrent seizures (SRS) (dual therapy: 0 SRS; n = 10; p < 0.0001 vs valproate controls). We did not have an untreated SE group, because no rat in that group survived long enough to be tested. All rats which received 270 mg/kg of valproate, which increased long-term survival but did not alter the severity of SE, developed SRS (4.6 ± 1 SRS per week; n = 7). Some rats treated with double-dose ketamine or double-dose midazolam developed SRS (p < 0.05 vs dual therapy), but differences with dual therapy
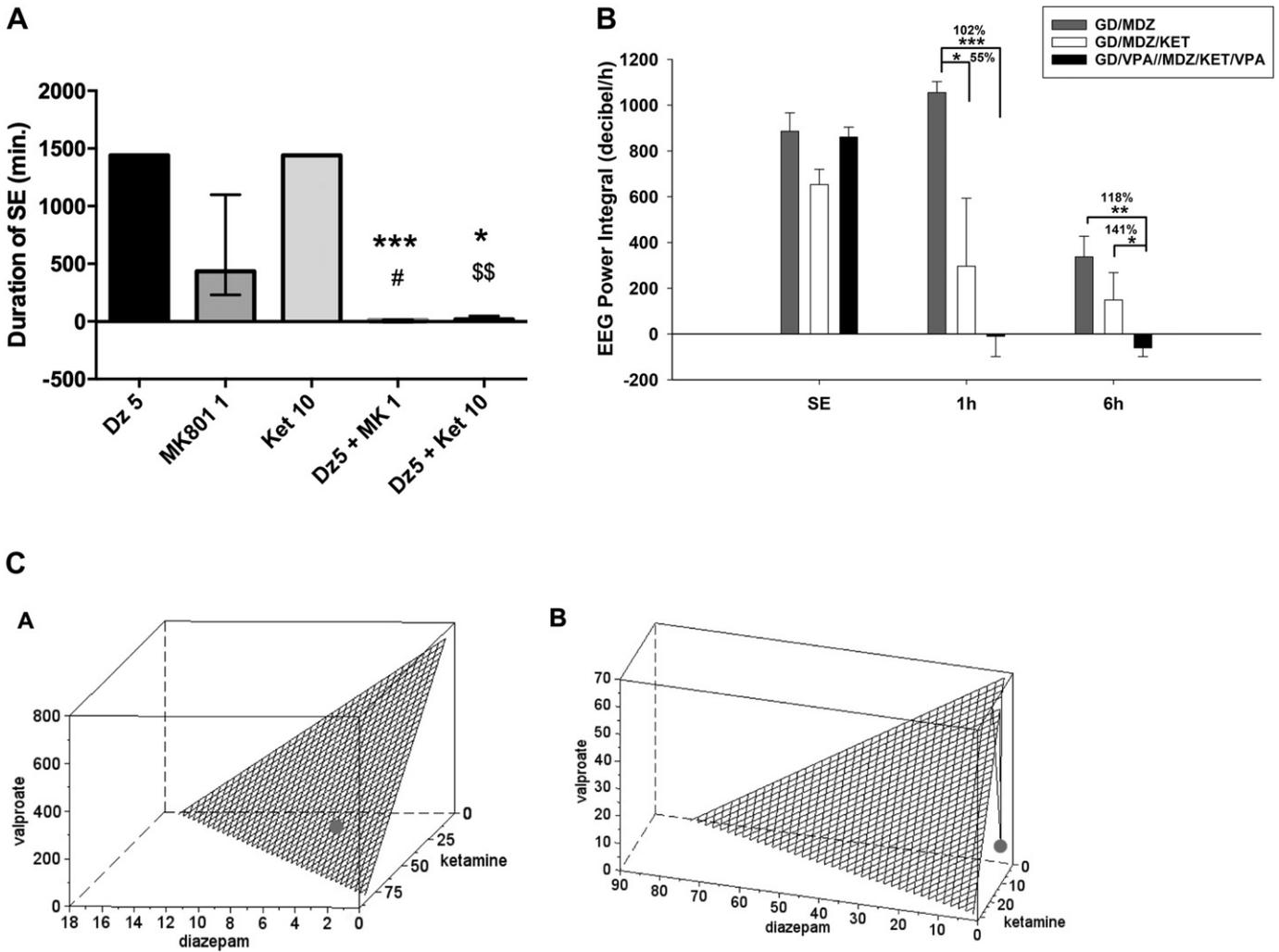
Fig. 1. A. Response of high-dose lithium–pilocarpine RSE to monotherapy with the GABA$_A$R agonist diazepam or the NMDAR antagonists dizocilpine or ketamine, and to combination therapy with GABA$_A$R agonist coupled with NMDAR antagonists. Cholinergic RSE was terminated by GABA$_A$R agonist – NMDAR antagonist combinations, and failed to stop with monotherapy, even at high dose (diazepam 20 mg/kg, not shown). Dz5: diazepam 5 mg/kg; MK1: dizocilpine 1 mg/kg; Ket 10: ketamine 10 mg/kg; Dz5 + MK1: diazepam 5 mg/kg combined with dizocilpine 1 mg/kg; Dz5 + Ket 10: diazepam 5 mg/kg combined with ketamine 10 mg/kg. *, **, ***: $p < 0.05$, 0.01, or 0.001 compared to diazepam 5 mg/kg. #, ##: $p < 0.05$ or 0.01 compared to dizocilpine 1 mg/kg. $, $$, $$$: p, 0.05, 0.01, or 0.001 compared to ketamine 10 mg/kg. Values are median ± interquartile range. Kruskall–Wallis with Dunn's multiple comparisons. B. Results of treating sc soman-induced SE with benzodiazepine monotherapy, with a GABA$_A$R agonist-NMDAR antagonist combination, or with a GABA$_A$R agonist-NMDAR antagonist-AED combination, 40 min after seizure onset. EEG power before treatment (SE) was similar in all experimental groups. Treatment with midazolam 3 mg/kg (GD/MDZ/SAL) or with ketamine 30 mg/kg (not shown) reduced EEG power slightly compared to untreated animals. The midazolam 3 mg/kg/ketamine 30 mg/kg combination (GD/MDZ/KET) and the midazolam 3 mg/kg/ketamine 30 mg/kg/valproic acid combination reduced the EEG power integral much better than midazolam or ketamine monotherapy. ***$p < 0.001$; **$p < 0.01$; $p < 0.05$. Data shown are mean ± S.E.M. These results suggest that GABA$_A$R agonist-NMDAR antagonist and GABA$_A$R agonist-NMDAR antagonist-AED combinations are efficacious in reducing EEG seizure severity against soman-induced SE.

were not significant. We do not know to what extent these results reflect a reduction in SE severity and duration versus a true antiepileptogenic action (Fig. 2C).

After soman-induced SE, 83% of untreated rats (VEH) that survived beyond 1 week developed SRS, as expected [21], versus 80% in the midazolam-treated group and 75% in the ketamine-treated group, and 25% of rats treated with the midazolam–ketamine combination had SRS (Fig. 2D). The latter was significantly lower than the midazolam monotherapy group ($p < 0.05$).

3.3. Spatial memory deficits

In the pilocarpine-induced RSE model, SE-associated loss of spatial memory in the Morris water maze [22] (Fig. 2E) was impaired in the valproate group (n = 7) compared to sham (no SE) controls (n = 8). In the acquisition test, the midazolam + ketamine group (n = 10) was undistinguishable from sham (no SE) controls and performed

better than the valproate group (n = 7; $p < 0.0001$), the midazolam group (n = 10; $p < 0.05$), and the ketamine group (n = 10; $p < 0.01$) by Kruskal–Wallis/Dunnet testing [14].

In soman-induced SE (Fig. 2F), spatial memory deficits were evident in the groups treated with midazolam (GDMDZ) or ketamine (KETMDZ) monotherapy, while rats treated with a combination of midazolam and ketamine (GDMDZKET) were not statistically different from untreated, no SE controls (no GD).

These observations show that combining a GABA$_A$R agonist and an NMDAR antagonist reduces not only the severity of SE but also its long-term consequences as well.

4. Drug combinations based on the receptor trafficking hypothesis are synergistic and have a high efficacy/toxicity ratio

The efficacy/toxicity ratio is the key to therapeutic success. If an increase in therapeutic potency is matched by an increase in potency of
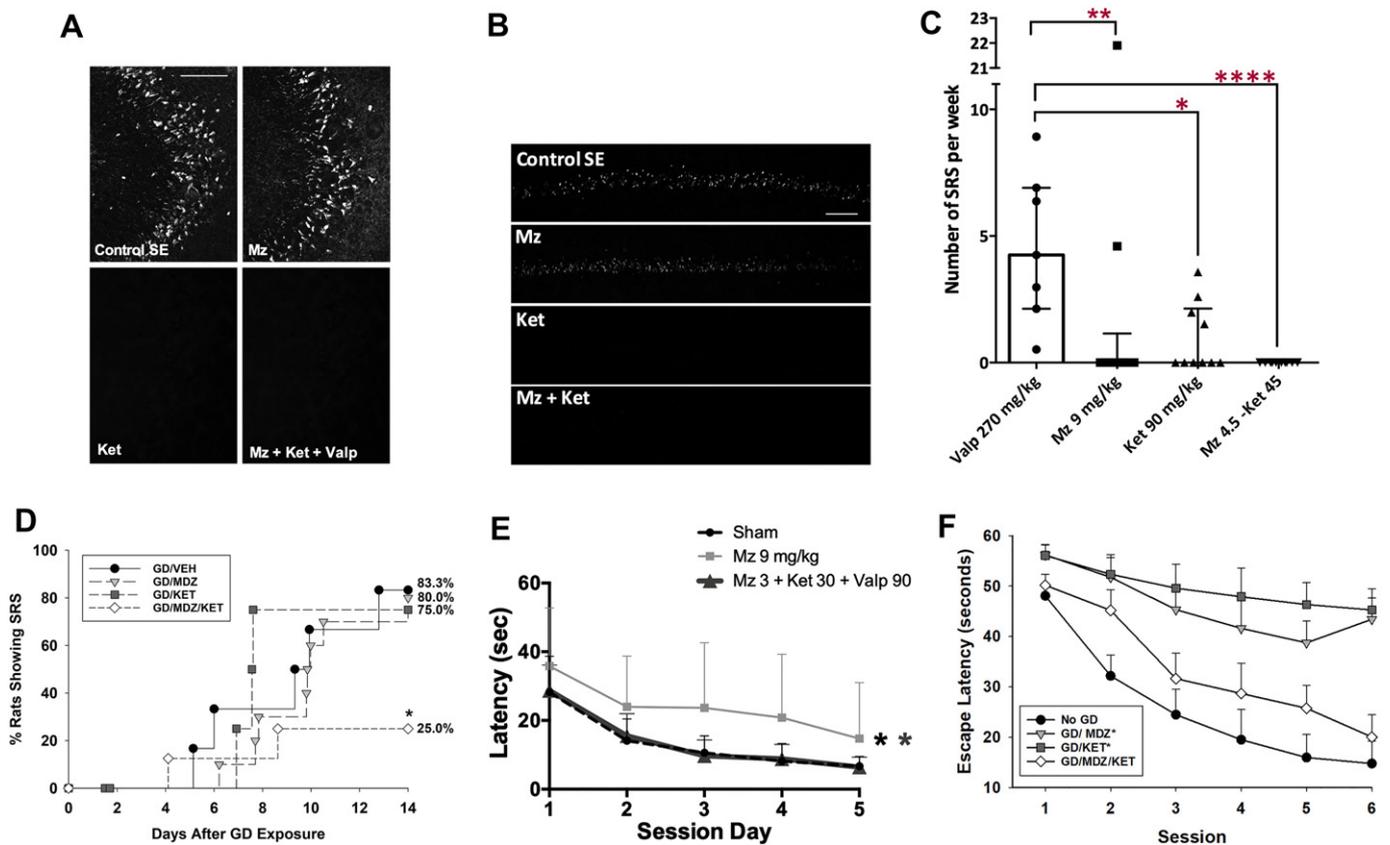
Fig. 2. A. Untreated SE controls showed extensive neuronal injury in CA3, 48 h after SE. Animals treated with midazolam or valproate monotherapy showed no significant reduction of neuronal injury. Polytherapy with combinations of midazolam/ketamine or midazolam/ketamine/valproate at 40 min after seizure onset showed nearly complete protection from neuronal injury, but so did ketamine monotherapy. $*p < 0.05$; $**p < 0.01$ by Kruskal–Wallis analysis followed by Dunn's test. From Niquet et al. [14]. B. Results in CA1 were similar to CA3, with nearly complete neuroprotection in the polytherapy and in the ketamine monotherapy groups. In the dentate hilus (not shown), neuroprotection was less complete. Neuronal injury measured by unbiased stereology showed only an approximately 50% reduction in Fluoro-Jade positive cells in all three treatment groups. C. Midazolam–ketamine dual therapy reduces epileptogenesis. Graph showing the number of spontaneous recurrent seizures (SRS) per week, ≥6 weeks after high-dose lithium–pilocarpine SE. The group treated with midazolam–ketamine dual therapy had no SRS. We used valproate monotherapy as our control group because of high mortality in the untreated control group. $*p < 0.05$, $**p < 0.01$, and $****p < 0.0001$ versus valproate (Kruskal–Wallis followed by Dunn's test). From Niquet et al. [14]. D. SRS after soman-induced SE. Treatment with the midazolam–ketamine combination reduced the incidence of SRS during the first 2 weeks after SE, compared to midazolam monotherapy ($p < 0.05$). E. Treatment of lithium–pilocarpine SE with the midazolam–ketamine combination reduced behavioral deficits. Performance in the Morris water maze (MWM) shows the latency to reach the hidden platform (y-axis) on each testing day (x-axis). Acquisition was slower in the group treated with triple-dose midazolam monotherapy than in "no SE" controls. The group treated with midazolam + ketamine + valproate was indistinguishable from "no SE" controls. $**p < 0.01$ versus valproate 270 mg/kg (by Kruskal–Wallis followed by Dunn's test). Data are presented as mean ± standard error of the mean (SEM). $*p < 0.05$ versus Mz4.5 + Ket45, $**p < 0.01$ versus Mz4.5 + Ket45, and $****p < 0.0001$ versus Mz4.5 + Ket45 by two-way analysis of variance (ANOVA). From Niquet et al. [14]. F. After sc soman SE, spatial memory acquisition in the MWM was slower in rats treated with midazolam or ketamine monotherapy than in animals treated with combination therapy or in "no SE" controls.

toxic adverse effects, nothing is gained. We build isobolograms [23–25] for both seizure-reducing therapeutic efficacy of drug combinations based on the receptor-trafficking hypothesis, and for their toxic side effects (impairment of motor function and impairment of consciousness). The results suggest that the midazolam–ketamine–valproate combination potentiates the therapeutic response without potentiating toxicity, so that the therapeutic index [26] is improved by switching from mono- to polytherapy. Fig. 1C shows that toxicity was simply additive between the three drugs of the combination, while the therapeutic response showed positive cooperativity between drugs and was synergistic. As a result of that synergy, this three-drug combination delivered a greater therapeutic response than monotherapy for the same level of drug-induced toxicity. The mechanism of that synergy is unknown, since the drugs involved have no known direct interaction at the molecular level, and the effect is probably too rapid to be due to pharmacokinetic interactions. This synergism was powerful enough to overcome pharmacoresistance: the diazepam + ketamine + valproate combination stopped seizures at subanesthetic doses while anesthetic doses of diazepam (20 mg/kg) did not.

5. Drug combinations based on the receptor trafficking hypothesis are more effective at stopping RSE than clinical guideline-recommended combinations of AEDs

5.1. Therapeutic efficacy

We compared a drug combination based on the receptor trafficking hypothesis to a drug combination suggested by current evidence-based clinical guidelines. American Epilepsy Society (AES) guidelines recommend benzodiazepine monotherapy followed by an AED (e.g., fosphenytoin), then by another AED (e.g., valproate), or anesthesia [11]. A combination of midazolam (3 mg/kg), fosphenytoin (50 mg/kg), and valproic acid (90 mg/kg), which follows AES guidelines, was far less effective at stopping RSE than the combination of midazolam (3 mg/kg), ketamine (30 mg/kg), and valproic acid (90 mg/kg), which targets seizure-induced changes in $GABA_A$ and glutamate receptors. Drugs were delivered simultaneously in both groups, 40 min after EEG seizure onset. The combination based on the receptor trafficking hypothesis was far more effective in reducing the number of posttreatment seizures (Fig. 3A), the EEG power integral over the first hour
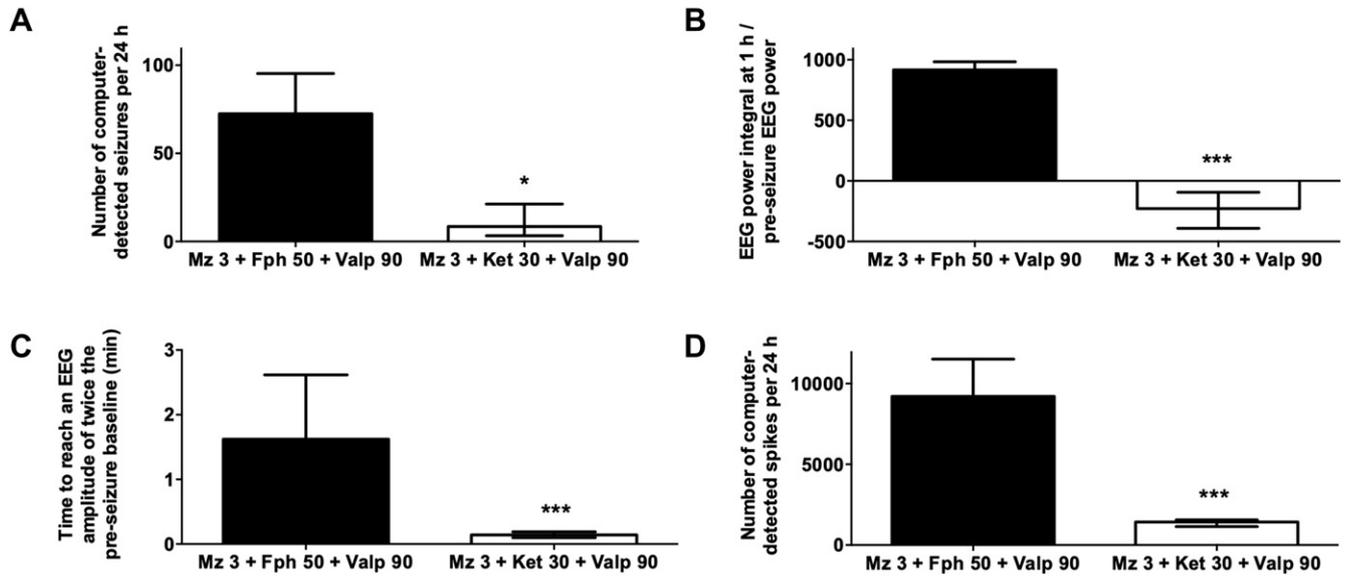
Fig. 3. The midazolam–ketamine–valproate combination, which targets seizure-induced changes in GABA$_A$ and glutamate receptors, was more effective than the midazolam–fosphenytoin–valproate combination, which follows American Epilepsy Society guidelines [11]. The graphs show the number of computer-detected seizures per 24 h (A), the ratio of EEG power integral over the first hour posttreatment to initial EEG power at baseline (B), the time needed to reach an EEG amplitude of twice the preseizure baseline (C), and the number of computer-detected spikes per 24 h (D). The combination of 3 mg/kg midazolam, 30 mg/kg ketamine, and 90 mg/kg valproate (n = 10) was far more potent than the combination of 3 mg/kg midazolam, 50 mg/kg fosphenytoin, and 90 mg/kg valproate. *p < 0.05 or ***p < 0.001 by Mann–Whitney analysis. Fosphenytoin is expressed in phenytoin equivalents.

posttreatment (Fig. 3B), the time needed for EEG amplitude to decline to twice the preseizure baseline (Fig. 3C), and the number of posttreatment spikes for 24 h (Fig. 3D) than the drug combination which followed AES guidelines.

5.2. Relevance of doses used in rodents to clinical SE

Receptor properties are similar in both species, and benzodiazepine pharmacoresistance has been observed clinically [2,27], but circuitry and brain size are quite different in rats and humans, and the heterogeneity of clinical SE may limit the applicability of conclusions drawn from any animal model to clinical situations. However, this type of comparison is routinely done in cancer chemotherapy. Oncology drugs are compared across species of vastly different size by using body surface area rather than body weight as a denominator, and this method is recommended by the FDA (Food and Drug Administration) to estimate the initial human dose in chemotherapy trials [28–31]. Comparing our 250–300 g rats to 70 kg humans by that method suggests that the doses of valproate, fosphenytoin, and levetiracetam used in this study are at or below the "human equivalent dose" (HED) derived from the clinical literature [1, 11,32], while the midazolam dose used is slightly higher than the HED and the ketamine dose is higher than the HED [1] but is lower than the dose used in patients with RSE who responded to ketamine [17].

6. Timing of drug delivery is key: early simultaneous use of drug combinations is far more effective than sequential use of the same drugs at the same dose

Standard practice in treating SE is sequential polytherapy, in which we wait for the first drug to fail before giving the second drug, and for the second drug to fail before giving the third drug [11]. This has the advantage of minimizing drug delivery and drug toxicity in responders, but the disadvantage of delaying delivery of the second drug by at least 30 min, and delivery of the third drug by at least 1 h. A study of prehospital treatment of SE [2] suggests that even a few minutes' delay in treatment can result in differences in the likelihood of developing full-blown SE. In order to mimic clinical situations where drugs are only injected after the previous treatment fails, we treated SE with the same drugs at the same dose in two groups of rats. In one group, the

three drugs were injected simultaneously. In the second group, the second drug was injected 30 min after the first, and the third drug was delivered 30 min after the second drug (Fig. 4A). Simultaneous polytherapy was far more effective than sequential polytherapy in reducing the posttreatment EEG power integral during the first hour (Fig. 4B), or the first 6 h after treatment; in reducing the time needed for EEG amplitude to decline to twice the preseizure baseline (Fig. 4C) and in reducing the number of posttreatment seizures (not shown). By those measures, sequential polytherapy was not significantly different from high-dose benzodiazepine monotherapy, matching the clinical experience where the second and third drugs used in sequential polytherapy have a low success rate [27]. This difference in outcome is compatible with increasing pharmacoresistance, associated with seizure-induced increases in receptor trafficking, during the delay between sequential drug injections. Fig. 3 confirms that principle in the treatment of experimental RSE.

7. Conclusions

Traditional treatments of SE fail to overcome pharmacoresistance and lead to RSE in about 30% of patients, yet clinical guidelines continue to rely on a very small number of empirical trials, and pay little attention to pathophysiology. Our improving knowledge of the mechanisms of seizure-induced pharmacoresistance should prompt us to question this tradition. Status epilepticus triggers key changes in GABA and glutamate receptors, yet we continue to treat it with a single GABAergic drug, leaving changes in excitatory networks untreated. We also continue to delay administration of the second drug until the first one has failed, and administration of the third drug until the second one has failed, ignoring evidence that pharmacoresistance increases with time and with seizure burden. Replacing a benzodiazepine (diazepam) with another benzodiazepine with better pharmacokinetics (midazolam) is bound to fail because it does not address the main reason for benzodiazepine pharmacoresistance, which is the loss of synaptic GABA$_A$R. Clinical trials comparing monotherapies are bound to show only minor differences between drugs, since each one treats only part of the problem.

We tested the hypothesis that decreases in synaptic GABA$_A$R and increases in synaptic glutamate receptors are key elements in the development of pharmacoresistance and in the initiation and maintenance

A

Simultaneous Mz + Ket + Valp

Implant —7d— Lithium —16h— Pilo + Scop. m.b. EEG start — 1st SZ —40min— Scop. + Mz + Ket + Valp —24 h— EEG end

Sequential Mz + Valp + Ket

Implant —7d— Lithium —16h— Pilo + Scop. m.b. EEG start — 1st SZ —40min— Scop. + Mz —30min— Valp —30min— Ket —24 h— EEG end

B — EEG power integral 1 h — Mz 9 mg/kg, Simultaneous Mz+Valp+Ket, Sequential Mz+Valp+Ket — **** , ****

C — Time to reduce EEG amplitude to twice the pre-seizure baseline (min) — Mz 9 mg/kg, Simultaneous Mz+Valp+Ket, Sequential Mz+Valp+Ket — ** , ***
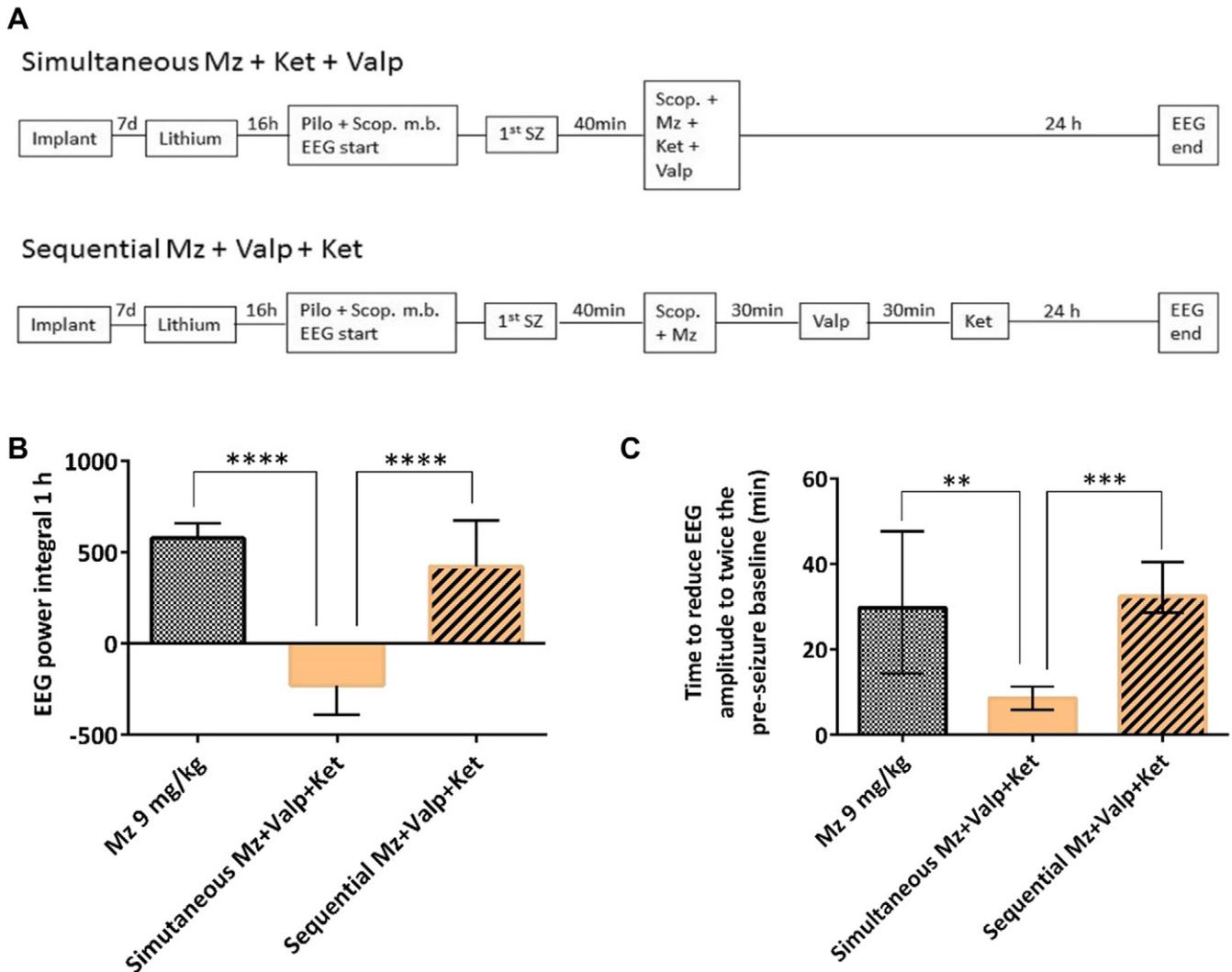
Fig. 4. A. Experimental flow: In the simultaneous group, the combination of midazolam 3 mg/kg, ketamine 30 mg/kg, and valproate 90 mg/kg was administered simultaneously 40 min after seizure onset. In the sequential group, the same drugs at the same dose were injected 30 min apart, starting 40 min after seizure onset. B–C. The graphs show the ratio of EEG power integral over the first hour posttreatment to initial EEG power at baseline (B), and the time needed for EEG amplitude to decline to twice the preseizure baseline without EEG bursts (C), indicating seizure termination. Simultaneous polytherapy (n = 10) was far more effective than sequential monotherapies (n = 8–9) or higher-dose midazolam (n = 10) in reducing EEG power, and in stopping SE. * $p < 0.05$, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ by ANOVA followed by Tukey's multiple comparison or by Kruskal–Wallis analysis followed by Dunn's test.

of SE. Our results suggest that changes in both $GABA_A$ and glutamate networks need to be treated to overcome seizure-induced pharmacoresistance. In the treatment of RSE, drug combinations which include a $GABA_A$R agonist and a NMDAR antagonist are far more effective at stopping seizures than higher-dose monotherapy, or than other drug combinations, supporting a key role of $GABA_A$ and glutamate receptor trafficking in the pathophysiology of SE. This is true in soman-induced SE as well. Drug combinations based on the receptor trafficking hypothesis not only terminate RSE, but also reduce or abolish some of its long-term consequences: neuronal injury, spatial memory deficits, and epileptogenesis. Unlike benzodiazepines, they work when treatment is delayed by 40 min after seizure onset. They show positive cooperativity between drugs, so that their therapeutic index is improved by their synergistic interaction.

We compared a drug combination inspired by the receptor trafficking hypothesis to a combination inspired by standard clinical guidelines. Late treatment with the midazolam–ketamine–valproate combination was more potent than the midazolam–fosphenytoin–valproate combination, showing that not all triple therapies show synergism, and suggesting that simultaneously targeting $GABA_A$R and glutamate receptor changes is a valid therapeutic strategy. The simultaneous administration of the three drugs was far more

efficient in stopping seizures than the standard practice of injecting the drugs sequentially, suggesting that timing of treatment is essential, and that an early polytherapy arm should be included in future clinical trials.

Acknowledgment

References

[1] Chen JWY, Wasterlain CG. Status epilepticus: pathophysiology and management in adults. Lancet Neurol 2006;5:246–56.

[2] Silbergleit R, Durkalski V, Lowenstein D, Conwit R, Pancioli A, Palesch Y, et al. Intramuscular versus intravenous therapy for prehospital status epilepticus. N Engl J Med 2012;366(7):591–600.

[3] Morrisett RA, Jope RS, Snead 3rd OC. Effects of drugs on the initiation and maintenance of status epilepticus induced by administration of pilocarpine to lithium-pretreated rats. Exp Neurol 1987;97:193–200.

[4] Kapur J, Macdonald RL. Rapid seizure-induced reduction of benzodiazepine and Zn2 + sensitivity of hippocampal dentate granule cell GABAA receptors. J Neurosci 1997;17:7532–40.

[5] Mazarati AM, Baldwin RA, Sankar R, Wasterlain CG. Time-dependent decrease in the effectiveness of antiepileptic drugs during the course of self-sustaining status epilepticus. Brain Res 1998;814:179–85.

[6] Naylor DE, Liu H, Wasterlain CG. Trafficking of GABA(A) receptors, loss of inhibition, and a mechanism for pharmacoresistance in status epilepticus. J Neurosci 2005;25:7724–33.

[7] Goodkin HP, Joshi S, Mtchedlishvili Z, Brar J, Kapur J. Subunit-specific trafficking of GABA(A) receptors during status epilepticus. J Neurosci 2008;28:2527–38.

[8] Niquet J, Baldwin R, Suchomelova L, Lumley L, Eavey R, Wasterlain CG. Treatment of experimental status epilepticus with synergistic drug combinations. Epilepsia 2017;58(4):e49–53.

[9] Naylor DE, Liu H, Niquet J, Wasterlain CG. Rapid surface accumulation of NMDA receptors increases glutamatergic excitation during status epilepticus. Neurobiol Dis 2013;54:225–38.

[10] Rajasekaran K, Todorovic M, Kapur J. Calcium-permeable AMPA receptors are expressed in a rodent model of status epilepticus. Ann Neurol 2012;72(1):91–102.

[11] Glauser T, Shinnar S, Gloss D, Alldredge B, Arya R, Bainbridge J, et al. Evidence-based guideline: treatment of convulsive status epilepticus in children and adults: report of the guideline Committee of the American Epilepsy Society. Epilepsy Curr 2016;16:48–61.

[12] Tetz LM, Rezk PE, Ratcliffe RH, Gordon RK, Steele KE, Nambiar MP. Development of a rat pilocarpine model of seizure/status epilepticus that mimics chemical warfare nerve agent exposure. Toxicol Ind Health 2006;22:255–66.

[13] Schultz MK, Wright LK, de Araujo Furtado M, Stone MF, Moffett MC, Kelley NR, et al. Caramiphen edisylate as adjunct to standard therapy attenuates soman-induced seizures and cognitive deficits in rats. Neurotoxicol Teratol 2014;44:89–104.

[14] Niquet J, Baldwin R, Norman K, Suchomelova L, Lumley L, Wasterlain CG. Midazolam-ketamine dual therapy stops cholinergic status epilepticus and reduces Morris water maze deficits. Epilepsia 2016;57:1406–15.

[15] Niquet J, Baldwin R, Norman K, Suchomelova L, Lumley L, Wasterlain CG. Simultaneous triple therapy for the treatment of status epilepticus. Neurobiol Dis 2017;104:41–9.

[16] Zanos P, Moaddel R, Morris PJ, Georgiou P, Fischell J, Elmer GI, et al. NMDAR inhibition-independent antidepressant actions of ketamine metabolites. Nature 2016;533(7604):481–6.

[17] Gaspard N, Foreman B, Judd LM, Brenton JN, Nathan BR, McCoy BM, et al. Intravenous ketamine for the treatment of refractory status epilepticus: a retrospective multicenter study. Epilepsia 2013;54:1498–503.

[18] Hjorth B. Principles for transformation of scalp EEG from potential field into source distribution. J Clin Neurophysiol 1991;8:391–6.

[19] Fujikawa DG. The temporal evolution of neuronal damage from pilocarpine-induced status epilepticus. Brain Res 1996;725(1):11–22 [PMID: 8828581].

[20] Lopez-Meraz ML, Wasterlain CG, Rocha LL, Allen S, Niquet J. Vulnerability of postnatal hippocampal neurons to seizures varies regionally with their maturational stage. Neurobiol Dis 2010;37:394–402.

[21] de Araujo Furtado M, Lumley LA, Robison C, Tong LC, Lichtenstein S, Yourick DL. Spontaneous recurrent seizures after status epilepticus induced by soman in Sprague-Dawley rats. Epilepsia 2010;51(8):1503–10.

[22] Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 1984;11(1):47–60.

[23] Chou TC. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev 2006;58(3):621–81.

[24] Tallarida RJ. Drug synergism and dose-effect data analysis. Boca Raton, FL: Chapman & Hall/CRC; 2000.

[25] Tallarida RJ. An overview of drug combination analysis with isobolograms. J Pharmacol Exp Ther 2006;319:1–7.

[26] Muller PY, Milton MN. The determination and interpretation of the therapeutic index in drug development. Nat Rev Drug Discov 2012;11(10):751–61.

[27] Treiman DM, Meyers PD, Walton NY, Collins JF, Colling C, Rowan AJ, et al. A comparison of four treatments for generalized convulsive status epilepticus. Veterans Affairs Status Epilepticus Cooperative Study Group. [see comments] N Engl J Med 1998;339:792–8.

[28] Pinkel D. The use of body surface area as a criterion of drug dosage in cancer chemotherapy. Cancer Res 1958;18:853–6.

[29] FDA, editor. Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers; 2005.

[30] Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB J 2008;22(3):659–61.

[31] Kaestner SA, Sewell GJ. Chemotherapy dosing part I: scientific basis for current practice and use of body surface area. Clin Oncol (R Coll Radiol) 2007;19:23–37.

[32] Gezalian MM, Sen-Gupta I, Chen JWY, Wasterlain CG. Management of status epilepticus. Fed Pract 2014;31(S5):24–9.