

RESEARCH ARTICLE

Effect of carbamazepine-beta-cyclodextrin inclusion complex on seizure-like events in an in vitro model of temporal lobe epilepsy

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Objective: Pharmacoresistant epilepsy represents a significant global health challenge, necessitating novel therapeutic approaches. Despite advances in antiseizure medications, many patients remain treatment-resistant partially due to complex pharmacokinetic issues. Beta-cyclodextrin, known for enhancing drug solubility and stability, offers potential solutions by forming inclusion complexes, thereby improving anti-seizure medication's efficacy. This study aimed to investigate the effect of beta-cyclodextrin and beta-cyclodextrin-complexed carbamazepine on epileptiform activities, using an in vitro model of temporal lobe epilepsy.

Methods: Seizure-like neuronal activity was induced using the low-magnesium model. Local field potentials were recorded from transverse rat hippocampal slices immersed in epileptogenic artificial cerebrospinal fluid, followed by the administration of either beta-cyclodextrin or carbamazepine, the latter in 100 micromolar concentration.

Results: Beta cyclodextrin, applied alone, significantly reduced the duration of interictal and ictal phases while increasing the frequency of seizure-like events. Carbamazepine exhibited an important anticonvulsant effect, significantly reducing ictal and postictal phase durations. However, the frequency of seizure-like events was increased. Notably, in some of the slices, carbamazepine completely suppressed epileptiform activity.

Conclusions: Beta cyclodextrin had an effect on its own; it shortened seizure durations and increased their frequency. Carbamazepine in complexed form, as used in our study, exhibited anticonvulsant efficacy, emphasizing the feasibility of solubility enhancement by this method. This study provides insights into potential therapeutic strategies for pharmacoresistant temporal lobe epilepsy, improving the pharmacological properties of the drugs. As cyclodextrins emerge as promising excipients for antiepileptic drugs with poor solubility, more effort is needed in order to elucidate the underlying mechanisms of their effects.

Keywords: temporal lobe epilepsy, beta-cyclodextrin, carbamazepine, in vitro epilepsy model, seizure-like events

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Introduction

Epilepsy is a syndrome of various cerebral disorders of the Central Nervous System (CNS), characterized by excessive and synchronized discharges of neuronal populations. It affects approximately fifty million people worldwide; within the spectrum of neurological conditions, epilepsy represents a substantial social and economic burden for healthcare and society [1]. The occurrence of symptoms is highly unpredictable and can be disabling.

Over the past decades, a substantial number of antiseizure medications (ASMs) have been developed. Despite these advancements, a considerable proportion, estimated at 30–40% of individuals with epilepsy, continues to suffer from drug-resistant epilepsy, remaining refractory to conventional pharmacological treatments [2]. The potential causes of pharmacoresistance are manifold and complex, particularly due to an incomplete understanding of underlying mechanisms, representing a significant challenge in the development of drugs that can effectively target the root causes. It must be noted, that the lack of proper un-

derstanding of the underlying mechanisms is one of the reasons why currently all ASMs are actually anticonvulsant therapies that do not significantly modify the process of epileptogenesis [3]. Further complicating the therapy, many of the recognized ASMs exhibit poor or variable pharmacokinetics, including absorption, distribution, solubility, metabolization, and elimination. All these factors can highly affect their efficacy, collectively having a substantial impact on the overall efficacy of these drugs [4,5].

Substantial resources and funding have been directed towards unraveling the molecular and cellular mechanisms associated with the pathophysiology and pharmacodynamic processes of epileptic syndromes [6–9]. However, the practical significance of these studies is still not yet sufficient to dramatically improve the quality of life for patients. The conventional approach for screening potential treatment advancements continues to involve a combination of *in vivo* and *in vitro* animal models [10].

Due to its particular intrinsic circuitry, hippocampus is the brain region with the lowest seizure threshold [11]. *In vitro* models using acute hippocampal slice preparations, which preserve synaptic circuits, are essential for examining both structural and functional features using electro-

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physiological techniques. Extracellular field potential recording on hippocampal slices offers real-time observation of the effects of antiepileptic drugs by influencing neuronal activity through various infusion solutions. Substance doses can be regulated precisely by bypassing the blood-brain barrier, but the influence of other brain structures and endogenous factors are also eliminated, potentially providing different results from *in vivo* conditions [12]. Epileptiform activity (seizure-like events, SLE) in hippocampal sections, commonly induced by perfusing magnesium-free artificial cerebrospinal fluid (0MgACSF), generates synchronized, recurrent epileptiform events through NMDA receptor activation, which can be enhanced by concomitant increase in potassium concentration [13–16]. Pyramidal cell discharges originating in the *cornu Ammonis* (CA) 3 area of the hippocampus propagate to CA1, characterized by repetitive positive deflections (0.3–0.5 Hz, 40–120 ms duration, 2–5 mV amplitude) [17].

Introduced in 1968 as a classic sodium channel blocker antiepileptic, carbamazepine (CBZ) has proven effective against both partial and generalized seizures. Possessing a crystalline structure and hydrophobic nature, this drug exhibits very low solubility in water. Approximately 75–85% of CBZ binds to plasma proteins, while its concentration in cerebrospinal fluid ranges from 17–31% of the serum concentration [18,19]. Belonging to the class of dibenzazepines, CBZ operates by inhibiting voltage-dependent Na⁺ channels, binding to them and maintaining their inactive state [20].

In recent years several studies focused on improving the hydrosolubility of CBZ and other ASMs. Techniques such as cogrinding with microcrystalline cellulose or the creation of nanoparticles stabilized by polyvinylpyrrolidone have demonstrated enhanced solubility [21,22]. Additionally, the preparation of solid lipid nanoparticles containing CBZ has shown promise in increasing the antiepileptic effect of CBZ [23,24]. Beta-cyclodextrin (β CD) is a cyclic oligosaccharide renowned for its ability to form inclusion complexes with various molecules. Through the formation of these complexes, cyclodextrins can increase water solubility, improving drug bioavailability and stability [25]. Moreover, they can influence the release rate and enhance absorption across biological membranes [26].

Previous studies have already examined the combination of CBZ with β CD to enhance solubility and control release in tablet formulations [27]. These investigations primarily focused on demonstrating increased solubility of CBZ through complexation with β CD and evaluating *in vitro* dissolution profiles, resulting in improved bioavailability and controlled-release profiles. However, it is important to note that these and other earlier studies concentrated on aspects such as solubility enhancements, dissolution properties, pharmacokinetics and efficacy, with evaluations often on *in vivo* models. These studies lacked exploration into the impact of CBZ- β CD complex on epileptiform activities. Despite β CD's historical use in studies

focusing on pharmacokinetics and efficacy, its effects on the CNS remain relatively unexplored. Experiments on cellular cultures have revealed its influence on brain capillary endothelial cell permeability and cholesterol mobilization [28]. Furthermore, patch-clamp recordings conducted on cellular cultures have identified β CD as a potent modulator of gamma-aminobutyric acid-A receptors (GABA_AR), potentially influencing inhibitory neurotransmission [29].

To enhance comprehension of β CD's effects and the implications of using β CD-complexed CBZ in treating TLE, we investigated their influence on epilepsy-like neuronal activity in an *in vitro* epilepsy model.

Methods

Animals

All procedures involving animals were performed after approval by the Ethics and Research Committee of the University of Medicine, Pharmacy, Science and Technology of Târgu Mureș (approval number: 119/27.06.2018, 1233/22.12.2020). 13 Male Wistar rat pups aged postnatal day 7–13 were used in this experiment.

Chemicals and solutions:

All chemicals used for preparation of solutions were purchased from Sigma Aldrich, St. Louis, Missouri, USA.

The artificial cerebrospinal fluid (ACSF) was used in three different compositions. To prepare and slice the brain of the animals, we used a *preparing* ACSF (pACSF) containing (mM) NaCl 87, saccharose 75, glucose 25, NaHCO₃ 25, MgSO₄ 7, KCl 2.5, NaH₂PO₄ 1.25, CaCl₂ 0.5. To observe the baseline activity of the slices, a *normal* ACSF (nACSF) solution was used composed of (mM) NaCl 129, NaHCO₃ 21, glucose 10, KCl 3, MgSO₄ 1.8, CaCl₂ 1.6, NaH₂PO₄ 1.23. In order to induce the epileptiform activity, we used a *magnesium-free* ACSF (0MgACSF) which had a similar composition to nACSF, but MgSO₄ was omitted and the concentration of KCl was increased to 5 mM.

Carbamazepine was added at a concentration of 100 μ M to 0MgACSF, in which β CD had been previously dissolved at a 1% concentration. To make sure that the complexation of carbamazepine was effective, the solution was continuously stirred at room temperature for 24 hours prior to the experiment. β CD was also independently tested by adding it to 0MgACSF in 1% concentration to investigate its individual effects on neuronal activity.

Experiments

The rat pups were quickly decapitated and their brain was rapidly removed and transferred into ice-cold nACSF. The frontal lobe and the cerebellum were cut, then the obtained block was fixed with ethyl 2-cyanoacrylate to a vibratome stage (Leica VT1000S, Leica Biosystems, Deer Park, IL, USA) and submerged in ice-cold oxygenated nACSF before being sectioned into 400 μ m thick transverse hip-

pocampal slices. Experiments were performed on these transverse slices, with the hippocampal formation, including the dentate gyrus, CA1, CA2, CA3, and subiculum, excised from the sections using micro-scissors.

The hippocampal slices underwent a 1-hour incubation at 36°C and then were stored at room temperature in an interface-type holding chamber in nACSF bubbled with a carbogen gas mixture (95% O₂-5% CO₂). Slices were individually transferred to a perfusion chamber within a Faraday cage to eliminate ambient electromagnetic contamination. They were then superfused with prewarmed (36°C) and carbonated nACSF at a rate of 3 ml/min using a peristaltic pump (Minipuls 3, Gilson Medical Electronics, Villiers-le Bel, France) and a dual-channel in-line solution heater (TMP 5b, SuperTech, Switzerland).

Local field potential (LFP) was recorded with microelectrodes with resistance between 3 and 8 MΩ, pulled from glass capillaries (TW150F-4; World Precision Instruments (WPI), Sarasota, FL, USA) using a two-stage pipette puller (PUL-2, WPI, Sarasota, FL, USA), filled with nACSF. The tip of the capillary was inserted into the CA3 region of the hippocampus under microscopic guidance using a hydraulic micromanipulator (WR88, Narishige, Tokyo, Japan). Recordings were performed using 5 kHz sampling rate. An amplifier (BioAmp SBA1-v6, SuperTech, Switzerland) with a nominal preamplifier was used (bandwidth 0.16 Hz–2 kHz, 2k gain, and a built-in 50 Hz notch filter). Data were digitized with an A/D card (PCI 6036E, National Instruments, Austin, TX, USA).

Recordings began with a 5-minute accommodation period to observe the baseline activity of sections perfused with nACSF. Subsequently, 0MgACSF was applied, and epileptiform activities, also known as SLEs, were monitored. After five consecutive events, the section was perfused with βCD-complexed carbamazepine dissolved in 0MgACSF, or βCD dissolved in 0MgACSF for the βCD group. After at least five epileptiform discharges or, in their absence, after 20 minutes of exposure, washout with 0MgACSF was performed to observe the reversibility of carbamazepine's effect and to verify tissue viability in case of total disappearance of activity. The protocol was then completed with nACSF solution washout.

Data analysis

The recorded data was stored in .txt format on a computer hard disk for offline analysis and later converted to .smrx format compatible with the program used for data processing (Spike 2 v8.01c, Cambridge Electronic Design). The time of the transitions between the perfused solutions was marked on the recordings, after which the preictal, ictal, postictal and interictal periods characteristic for epileptiform seizures were identified, delimited and measured (Figure 1). To identify these phases, we focused on the frequency variations of the discharges (spikes). Similar to Zhang et al. (2012), preictal phase was considered to be initiated when the frequency increased above 0.2 Hz, postictal phase was delimited from ictal activity when the frequency decreased below 1 Hz, and interictal periods presented a spiking activity below 0.2 Hz [30].

On all slices, duration of the preictal, ictal, postictal and interictal phases were marked and measured and seizure frequency was calculated (Figure 2).

When comparative analysis was performed between control – βCD and βCD+CBZ groups, the inspected parameters were normalized for each slice and the induced changes were calculated as percentage (%) change of the parameters. The mean duration of SLEs measured in the 0MgACSF was considered a reference (100 %). The obtained data were statistically processed using the Kruskal-Wallis and Dunn's multiple comparison tests.

All statistical analysis was performed using GraphPad Prism v. 9.4.1 (Graph Pad Software, San Diego). A p-value of less than 0.05 was considered statistically significant.

Results

In order to observe the effects of βCD on the seizure-like activity, recordings of 8 transversal hippocampal slices were studied, performing measurements, and subsequent statistical analysis on over 90 SLEs. We tested whether βCD at a 1% concentration exerts any influence on epileptiform activities (Figure 3).

To assess the effects of βCD-complexed carbamazepine on seizure-like activity, we included recordings of 9 hippocampal slices in our statistical analysis, performing measurements on over 70 SLEs. We investigated whether

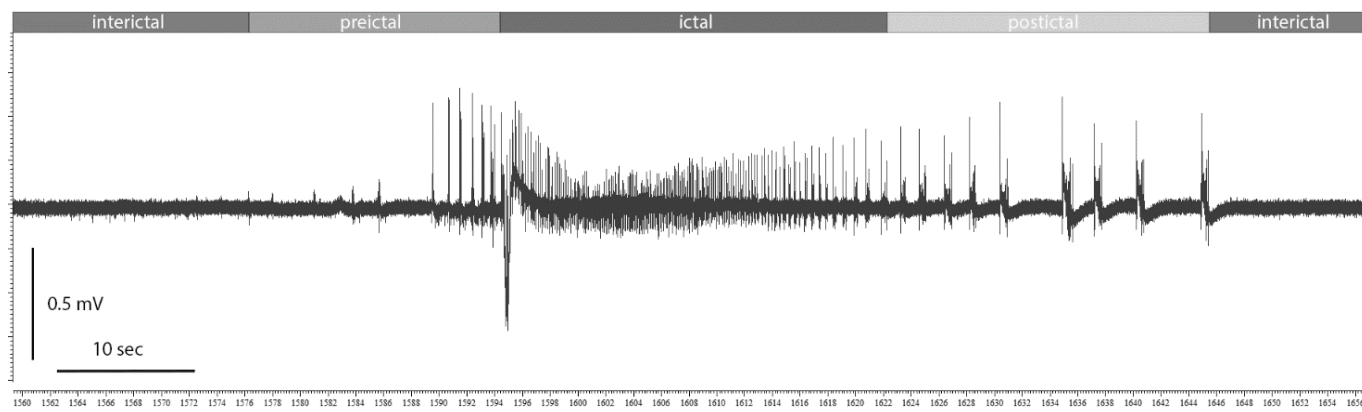


Fig. 1. Seizure-like event with preictal, ictal, postictal, and interictal phases marked according to discharge frequency.

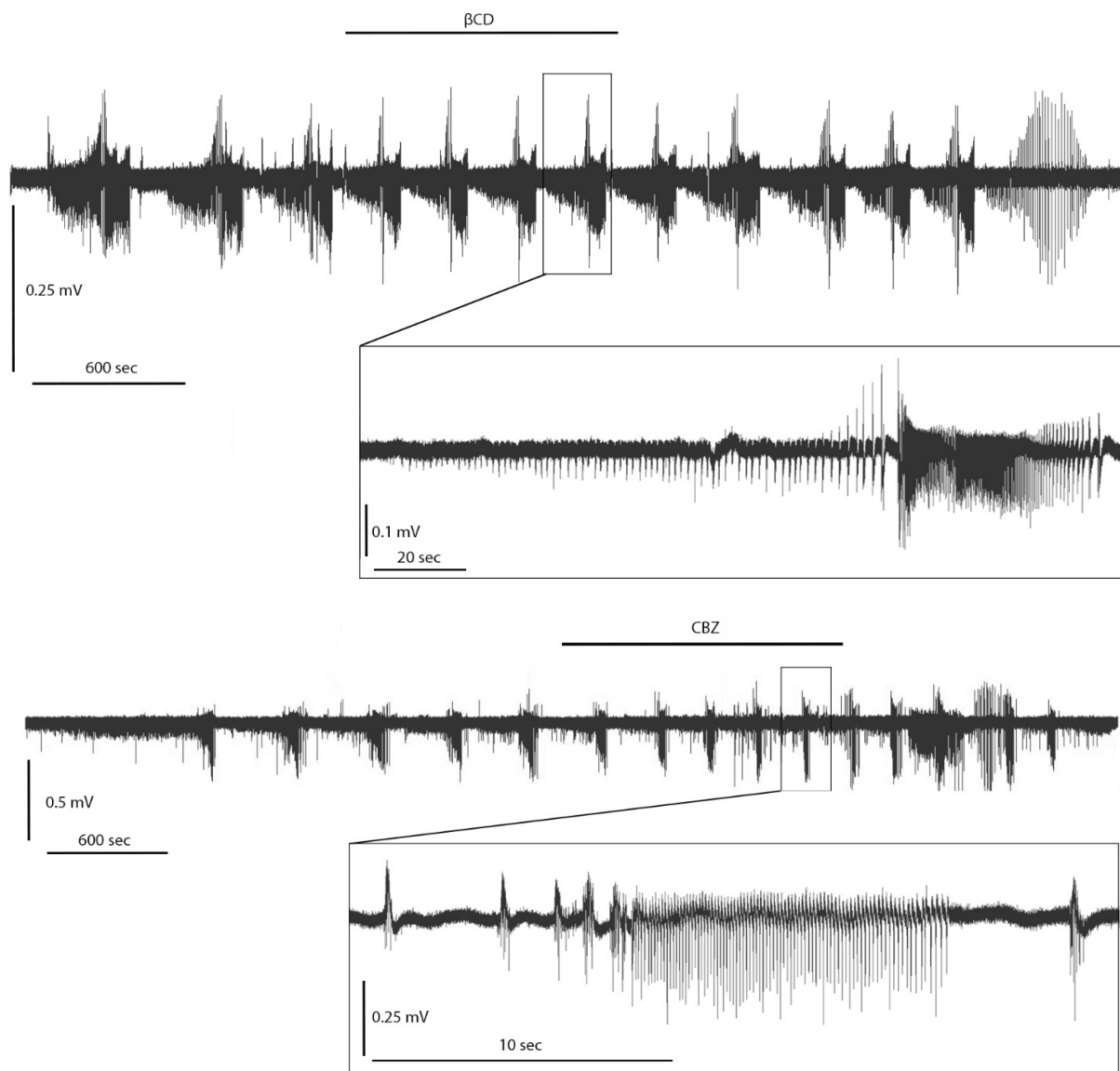


Fig. 2. Seizure-like event with preictal, ictal, postictal, and interictal phases marked according to discharge frequency.

β CD-complexed CBZ at a concentration of 100 μ M exerts any influence on epileptiform activities (Figure 4). Additionally, 9 recordings were excluded from the analysis due to the complete suppression of SLEs following the application of β CD-complexed CBZ.

A side-by-side comparison of the effects of control seizures, β CD and β CD-complexed CBZ at a concentration of 100 μ M reveals statistically significant differences in some of the evaluated parameters (Figure 5).

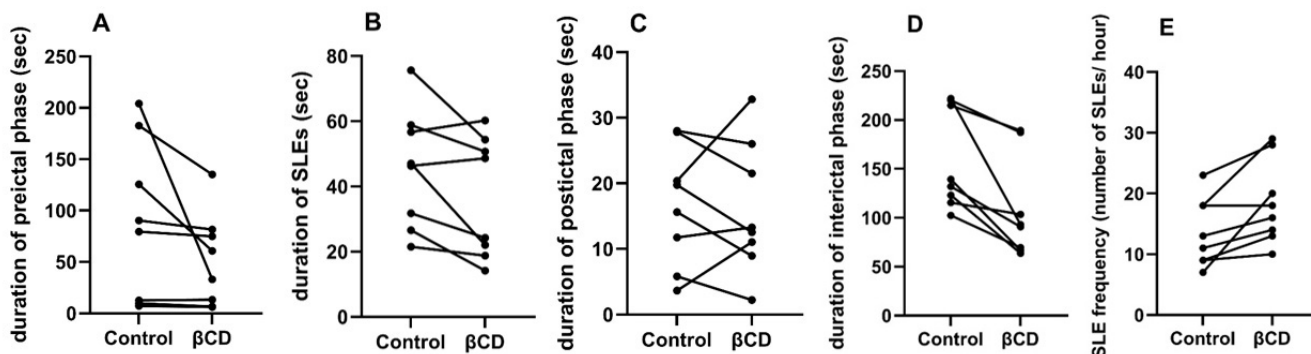


Fig. 3. 0MgACSF induced SLE duration and frequency as well as the duration of interictal periods are significantly influenced by application of β CD at a concentration of 1 %. Each point represents a mean value of all SLEs, calculated for each slice before and after applying β CD (N= 8): A. duration of the preictal phase; B. SLE duration; C. duration of the interictal phase; D. duration of the postictal phase; E. SLE frequency. (β CD Beta-cyclodextrin; SLE seizure-like events; 0MgACSF magnesium-free artificial cerebrospinal fluid).

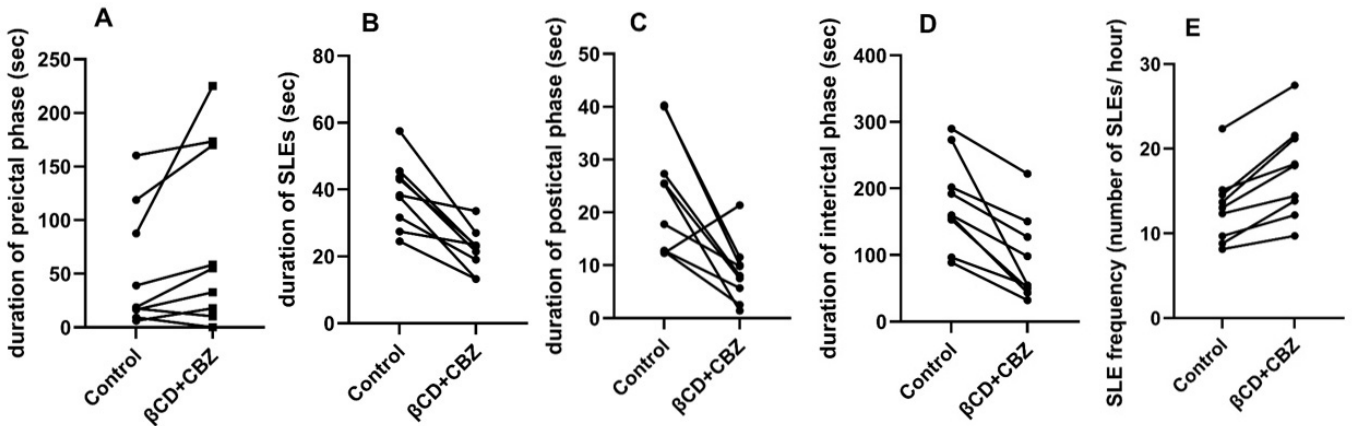


Fig. 4. 0MgACSF induced SLEs are significantly influenced by application of β CD-complexed CBZ in 100 μ M concentration regarding the SLE's duration as well as the duration of postictal and interictal phase and SLE frequency. Each point represents a mean value of all SLEs calculated for each slice before and after applying CBZ (N= 9): A. duration of the preictal phase; B. SLE duration; C. duration of the interictal phase; D. duration of the postictal phase; E. SLE frequency. (β CD Beta-cyclodextrin; CBZ carbamazepine; SLE seizure-like events; 0MgACSF magnesium-free artificial cerebrospinal fluid).

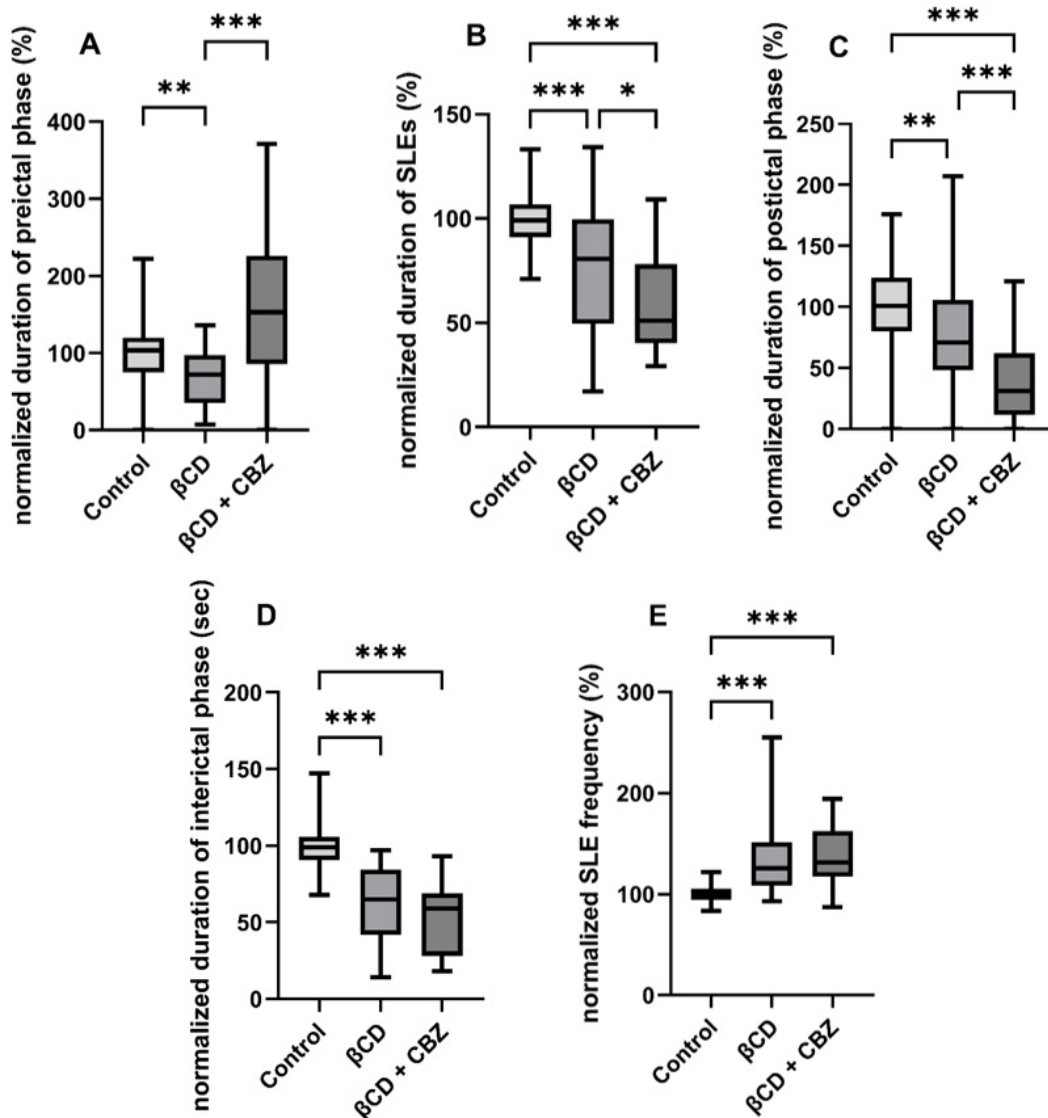


Fig. 5. Side-by-side comparison of the effects of β CD and β CD-complexed CBZ at a concentration of 100 μ M reveals statistically significant differences in the case of every phase of the SLEs. A Boxplots show the normalized distribution of each parameter; whiskers show minimum to maximum values, mean value is shown with continuous line (N= 8-17): A. duration of the preictal phase; B. SLE duration; C. duration of the interictal phase; D. duration of the postictal phase; E. SLE frequency. Asterisk marks statistically significant difference at the 95% level ($p < 0.05$) * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. (β CD Beta-cyclodextrin; CBZ carbamazepine; SLE seizure-like events; 0MgACSF magnesium-free artificial cerebrospinal fluid).

The length of the preictal phase was markedly decreased by β CD (65.14 ± 6.14 %, mean \pm SEM) compared to control (100 ± 5.16 %), and increased by CBZ (164.5 ± 20.81 %) compared to β CD, but there was no significant difference between CBZ and control seizures (Figure 5A).

The duration of the ictal phase was markedly reduced by β CD (76.39 ± 5.25 %) compared to the control group (99.97 ± 1.51 %) (Figure 5B). There was an even greater reduction in the duration of the ictal phase when 1% β CD-complexed CBZ was applied (57.31 ± 3.87 %) compared to control seizures, and it was also significant compared to β CD (Figure 5B).

The length of the postictal period was decreased under the influence of CBZ (37.18 ± 4.51 %) compared to control (99.8 ± 3.83 %) and also compared to β CD (76.78 ± 5.9 %). The reduction of this phase was also significant between β CD and control group (Figure 5C).

We found that 1% β CD significantly reduced the duration of the interictal phase (62.47 ± 2.24 %) compared to the recorded control seizures (99.37 ± 2.02 %). CBZ further reduced this phase (51.19 ± 4.08 %), but it was not significantly shorter than in the case of β CD (Figure 5D).

The frequency of the SLEs was significantly increased after applying 1% β CD on the slices (137 ± 8.94 %) compared to the control group (101 ± 1.09 %) (Figure 5E). Following the application of CBZ on the slices, there was also a notable and statistically significant increase in the frequency of spontaneous epileptiform events (137 ± 4.85 %) compared to the control group (Figure 5E), but the difference was not significant between β CD and CBZ.

As mentioned earlier, it is worth noting that CBZ completely suppressed seizure-like activity in 50% of the slices (these specific recordings were excluded from the statistical analysis). In contrast, no similar effect was observed in the case of β CD applied without the drug.

Discussions

While previous studies explored the hydrosolubility of CBZ and general pharmacokinetics, our research narrows its focus to epileptiform activities induced by 0MgACSF. This approach offers additional information on how β CD and CBZ, individually and in combination, affect SLEs in an *in vitro* model of temporal lobe epilepsy.

Our study aimed to investigate the effects of β CD administered alone and as a carrier for CBZ in *in vitro* epileptiform activities induced by 0MgACSF. The field potential in the pyramidal layer of the CA3 hippocampal region was recorded to assess the impact. It is essential to note that the *in vitro*-induced epileptiform activities, in the lack of altered brain circuits, while not fully mirroring the pathomechanisms of epileptic syndromes, offer supplementary insights to *in vivo* studies.

Several efforts have been made to establish *in vitro* models for pharmacoresistant epilepsies and utilize them as preclinical screening tools, as previously reported [31]. The SLEs induced in immature brain tissue were previ-

ously shown to be resistant to standard ASMs' anticonvulsant effects, including CBZ at a concentration similar to that used in our study, albeit diluted in dimethylsulfoxide (DMSO). Additionally, these ASMs exacerbated SLEs instead of suppressing them [32]. Earlier studies have often interpreted the anticonvulsant effects of ASMs in the context of complete SLE suppression, and various models were labeled pharmacoresistant [32].

The primary objective of our study was to analyze SLEs, focusing on several properties that characterize the pharmacological actions of CBZ.

The concentration of compounds used in experiments can have a crucial role. In our experiments, we consistently employed a concentration of 100 μ M for CBZ. While prior studies have reported that carbamazepine exhibits anticonvulsant effects at this level in low-Mg²⁺ models [33], there is a lack of research on CBZ when complexed with β CD in *in vitro* models. Nonetheless, various studies have indicated β CD's effectiveness as a carrier due to its facilitation of absorption and traversing the blood-brain barrier.

In our analysis of the anticonvulsant action of CBZ, we evaluated several parameters of the SLEs rather than focusing on the total suppression of epileptiform activity. Based on the pattern of modifications, the pharmacological actions of CBZ included the reduction of the duration of SLEs, consistent with previous reports [13,34]. In contrast, it increased preictal length and seizure frequency.

Based on our results, we observed that applying β CD alone to hippocampal slices exerts a moderate impact on epileptiform activities. This is manifested by a reduction in the duration of interictal phases, leading to an increased frequency of spontaneous epileptiform activity. However, these SLEs are significantly shorter than control SLEs. From this perspective, β CD could potentially be a superior choice in *in vitro* experiments, as an excipient for ASMs in comparison to some other known excipients, e.g., DMSO, known to increase the duration and frequency of epileptiform discharges [34], propylene glycol, which may induce epileptic seizures in non-epileptic patients [35] or polyethylene glycol, linked to an increased frequency of seizures in some epileptic patients [36]. The impact of β CD on brain capillary endothelial cell permeability and inhibitory neurotransmission has been demonstrated in earlier studies [28,29].

Despite β CD's impact on SLEs, it demonstrated its potential as an excipient for ASMs with poor solubility. However, it is important to recognize certain limitations, such as the *in vitro* nature of the epilepsy model and the need for further investigations regarding the mechanisms underlying the observed effects. These further inquiries, particularly in conjunction with low-solubility antiepileptic drugs, could provide deeper insights into the pharmacological actions of β CD. Studies focusing on the solubility and enhanced bioavailability of CBZ with β CD affirm its significant potential in practical therapeutic contexts.

Conclusions

Our study investigates the effects of β CD, both independently and as a carrier for CBZ, on in vitro epileptiform activities induced by 0MgACSF, offering complementary insights to in vivo investigations.

We found that β CD alone, administered at a 1% concentration, significantly influenced various aspects of epileptiform activities, suggesting a dual effect on neuronal activity—manifesting both anticonvulsant and proconvulsant effects. Similarly, our investigation of 1% β CD-complexed CBZ at 100 μ M concentration indicated the anticonvulsant potential of the CBZ- β CD complex. Despite a marked increase in preictal phase duration with CBZ application, seizure frequency did not significantly rise compared to β CD alone. The ability of CBZ to completely suppress seizure-like activity in 50% of the slices, while β CD alone did not exhibit a similar effect, indicates that enhancements in solubility, along with potential influences on pharmacokinetics and other underlying mechanisms, likely play a crucial role in enhancing CBZ's anticonvulsant properties.

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Authors' contribution

RJK (Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Resources, Visualization, Writing – original draft)

ACS (Data curation, Formal analysis, Investigation, Visualization)

MS (Data curation, Formal analysis, Investigation)

ZSAN (Data curation, Formal analysis, Investigation)

ASZ (Data curation, Formal analysis, Investigation)

ZSG (Conceptualization, Methodology, Resources, Validation, Writing – review & editing)

TSZ (Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing)

KOK (Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing)

Conflict of interest

Authors declare no conflict of interests.

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