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Research article

Stiripentol efficacy against status epilepticus and associated mortality in mice

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ABSTRACT

Stiripentol (STP, Diacomit©) is an antiseizure medication indicated for Dravet syndrome, a rare developmental and epileptic encephalopathy characterized by drug-resistant seizures, including status epilepticus (SE). SE is a life-threatening event that may lead to increased risk of morbidity and mortality. Here, we evaluated the effect of STP on SE and SE-associated mortality using a CBA mouse model induced by systemic administration of methionine sulfoximine (MSO), an irreversible inhibitor of glutamine synthetase. MSO induces convulsions, prolonged seizure (SE) and death, with an increase of blood ammonia level. A single acute intraperitoneal pretreatment with 200–300-400 mg/kg of STP significantly inhibited the number of seizures, SE occurrence and death in MSO-treated animals in a dose-dependent manner. Regarding blood ammonia level, STP significantly reduced by 41 % the hyperammonemia induced by MSO. In conclusion, our results show protective effects of STP to reduce and or suppress the occurrence of SE as well as its associated mortality in mice.

1. Introduction

Stiripentol (STP, Diacomit©) is an antiseizure medication indicated in association with sodium valproate or clobazam as adjunctive therapy of refractory generalized tonic-clonic seizures in patients with Dravet syndrome, a rare developmental and epileptic encephalopathy characterized by drug-resistant seizures, including status epilepticus (SE).

In a recent retrospective review covering the last 30 years, STP has been described to significantly prevent the occurrence of SE and mortality in Dravet syndrome, particularly when initiated from 6 months of age [1]. Moreover, in three case reports, STP has been shown to stop SE in a total of 8 patients [2,3].

Few preclinical studies were done on the potential protective effect of STP regarding the SE occurrence in epilepsy. One can mention two studies that used the pilocarpine-induced SE rat model [4,5]. STP significantly prevented the occurrence of SE when administered 1h before SE induction [4]. In addition, STP was able to terminate SE when established, up to 45 min after SE start (at a time when benzodiazepines were no more effective [5].

To further explore potential protective effects of STP on SE occurrence, we studied its action in a preclinical model. In this model, methionine sulfoximine (MSO), an irreversible inhibitor of glutamine synthetase, was used to induce SE in CBA mice. MSO induces an accumulation of brain glycogen and hyperammonemia through an increase of gluconeogenesis and inhibition of glutamine synthetase, and its effects culminate with convulsions and death [6,7]. Repetitive tonic-clonic and tonic seizures can be observed after the 5th hour

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post-injection and can last up to 24h. These repetitive seizures, sometimes longer than 5 min (status epilepticus), may lead to death [8].

Here, we evaluated the protective effect of STP on this preclinical murine model of SE and compared its efficacy to the reference compound sodium valproate, recommended as first-line drug in Dravet syndrome [9,10] and known to be effective against MSO-induced seizures [11,12].

2. Results

2.1. STP prevents MSO-induced seizures and SE-associated death

We first studied the dose-effect relationship of intraperitoneal MSO administration in mice (Fig. 1A). At 40 mg/kg, MSO did not induce convulsion and mortality. Starting from 50 mg/kg, MSO induced SE that appeared 5h after administration, with 90 % mortality at the 8th hour. At 60 and 80 mg/kg, MSO induced convulsions more rapidly, from 4th and 3rd hour respectively, with 90–100 % mortality. The following studies were done with the minimum MSO dose inducing seizures and death (50 mg/kg).

In mice receiving the vehicle of STP, administration of MSO at 50 mg/kg induced SE with a progressive increase of the Racine score starting from 0 during the first 4h and up to the value of 5.5 ± 0.2 and percentage of 84 % death at the 8th hour (Fig. 1B). Single (30 min pre-treatment) administration of 200, 300 and 400 mg/kg i.p. STP dose-dependently and significantly inhibited MSO-induced seizures with Racine scores of 5.1 ± 0.5, 3.1 ± 0.4 and 1.4 ± 0.6, respectively. Moreover, percentages of death were 70 %, 30 % and 0 %, respectively. Likewise, VPA at 300 mg/kg significantly reduced Racine score with value of 0.7 ± 0.2, and strongly reduced death, similarly to STP 400 mg/kg. There was no significant difference between the effects of STP at 400 mg/kg and VPA at 300 mg/kg on the percentages of death or Racine score.

Electrophysiological evaluation of MSO-induced SE clearly showed prolonged seizures 5h after MSO administration (Fig. 2A). STP decreased the total number of seizures (Fig. 2B) and increased the latency to seizure onset (Fig. 2C). EEG analysis also showed that only isolated spikes occurred in STP-treated animals, without prolonged seizure (Fig. 2D).

2.2. STP reduces hyperammonaemia induced by MSO

Another effect of MSO is a strong and significant increase of blood ammonia level. Eight hours after MSO administration, ammonia levels were increased up to 763 ± 71 μM vs 184 ± 12 μM in the control group (Fig. 3A). Pretreatment by STP 300 mg/kg significantly reduced MSO-induced hyperammonemia level by 41 % to a value of 448 ± 65 μM. In the same way, VPA 300 mg/kg significantly diminished MSO-induced hyperammonemia level by 47 % to a value of 402 ± 34 μM. We also evaluated the proper effect of each drug (Fig. 3B), blood ammonia level was measured in mice not treated with MSO. STP 300 mg/kg did not change the blood ammonia levels (176 ± 14 μM) as compared to the control group (179 ± 11 μM), whereas VPA 300 mg/kg significantly increased blood ammonia level by 31 % (235 ± 12 μM).

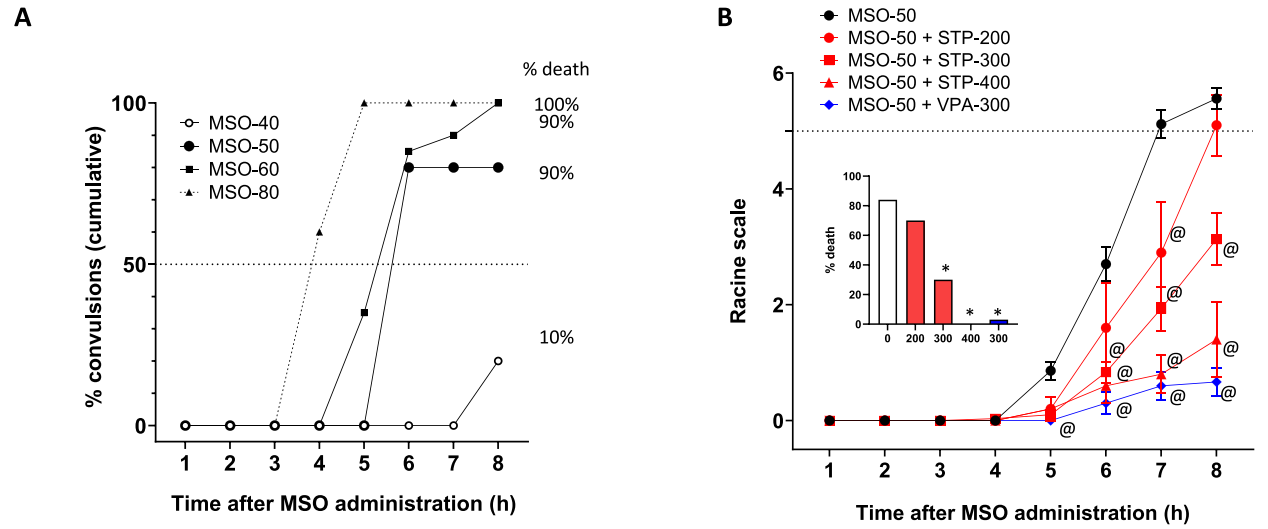

Fig. 1. Convulsions induced by methionine sulfoximine (MSO) in CBA mice. (A) Percentages of convulsion and death induced by MSO administration (intraperitoneal route) in mice (n = 10/group, except for n = 20 at 60 mg/kg). (B) Effect of stiripentol (STP) or sodium valproate (VPA) on Racine score and percentage of death after MSO administration (50 mg/kg) in mice (n = 50 for MSO alone, n = 10 for groups receiving STP at 200 and 400 mg/kg and n = 20 for groups receiving STP or VPA at 300 mg/kg). STP or VPA were administered intraperitoneally 30 min before MSO. @ $p < 0.05$, ANOVA statistical test with repeated measures of treated groups (STP or VPA) compared to MSO group about the Racine score. *$p < 0.05$, Fisher exact test of treated groups (STP or VPA) compared to MSO group about death.

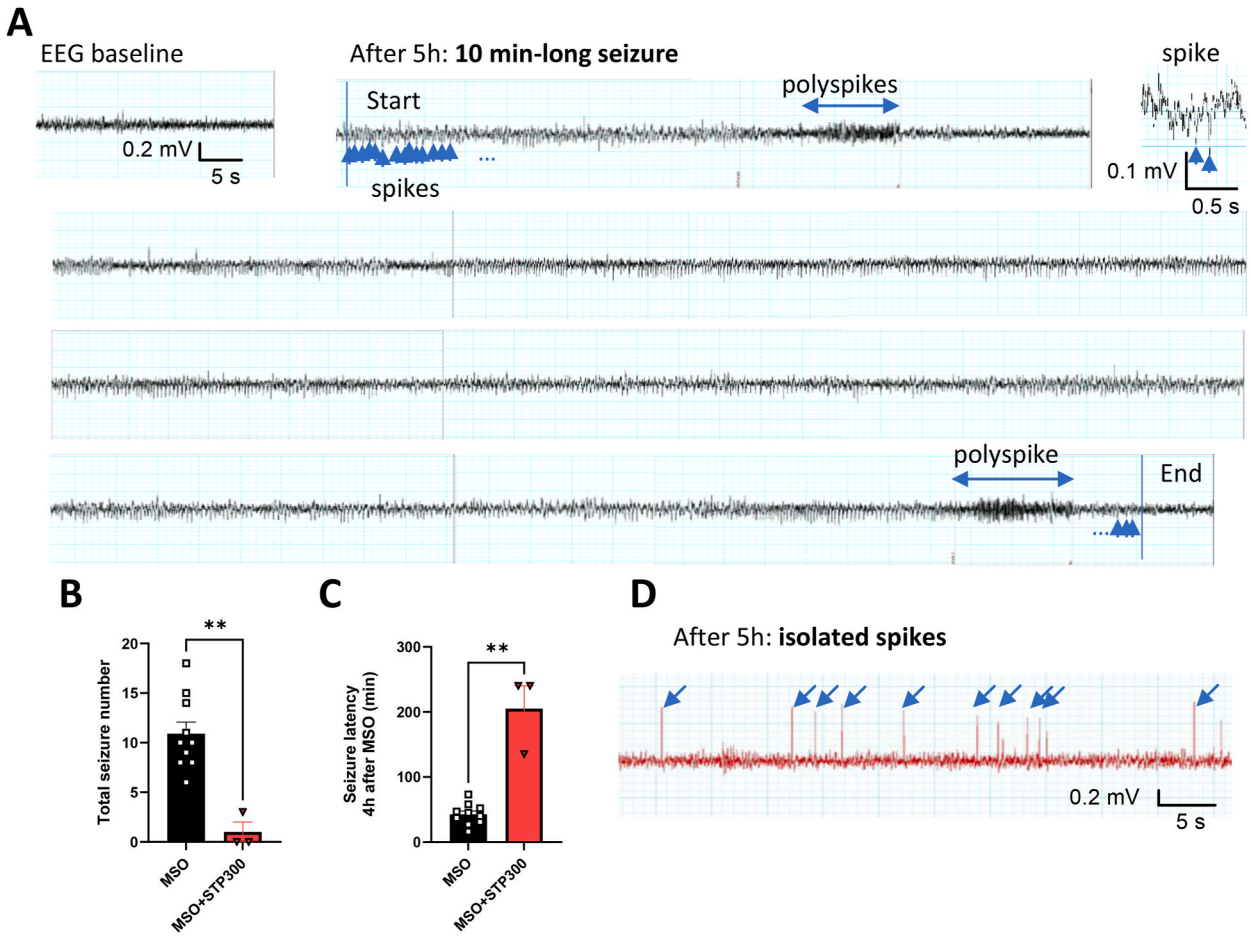

Fig. 2. Electro encephalographic recordings of the MSO-induced seizures and effect of STP. (A) Example of prolonged seizure 5h after MSO administration, with continuous spiking, and some polyspikes activity. (B–C) Effect of stiripentol (STP) on total seizures number (B) and latency to seizure onset 4h post MSO-administration (C) (n = 3 to 10, each dot represents an individual mouse. No seizures were detected before 4h). (D) Example of isolated spikes seen in STP-treated animal, 5 h after MSO administration. **p < 0.01Mann Whitney test of STP-treated group compared to MSO group.

3. Discussion

In our study, a single methionine sulfoximine (MSO) administration (from 50 mg/kg), induced seizures, death and hyperammonemia in CBA/J mice, in accordance with previous results [8,13]. In this preclinical model of SE, pretreatment by STP 300 mg/kg i.p. significantly reduced seizures and the occurrence of death, suggesting a potent protective effect. Another antiseizure medication used in Dravet syndrome, sodium valproate at 300 mg/kg, significantly inhibited seizures and death, in accordance with previous results [11,12].

STP possesses several mechanisms of action, which could be implicated in its effects against MSO-induced seizures [14]. The first one is the positive allosteric modulation of GABA-$_A$ receptors leading to an increase of GABAergic neurotransmission [15,16]. STP potentiates GABA-$_A$ receptors containing gamma subunits or delta subunits [15]. This mechanism is particularly interesting during SE, as gamma-containing GABA-$_A$ receptors, but not delta-ones, are internalized 15 min following prolonged seizure [17]. Gamma-containing GABA-$_A$ receptors are sensitive to benzodiazepines which loose efficacy following SE whereas STP may conserve its antiseizure efficacy due to its continuing action on delta-containing receptors. STP also modulates calcium and sodium channels and thereby regulates neuronal excitability [18,19]. Another potential mechanism of STP is the regulation of glucose energy metabolism and inhibition of lactate dehydrogenase [20]. MSO alters brain glucose metabolism with accumulation of brain glycogen and inhibition of glutamine synthetase [6,7]. These mechanisms of action require different concentrations of STP, the inhibition of lactate dehydrogenase requiring higher concentrations as compared with effect on GABAergic neurotransmission or ion channels [14].

MSO inhibition of glutamine synthetase, an enzyme involved in the ammoniac detoxification, induces hyperammonia [21]. In our study, a single MSO administration strongly and significantly increased ammonia level. Pretreatment by STP 300 mg/kg i.p. significantly reduced MSO-induced hyperammonemia, without any proper/intrinsic effects on ammonia levels in mice without MSO, indicating a potential protective effect. In the same protocol, VPA 300 mg/kg also significantly reduces MSO-induced

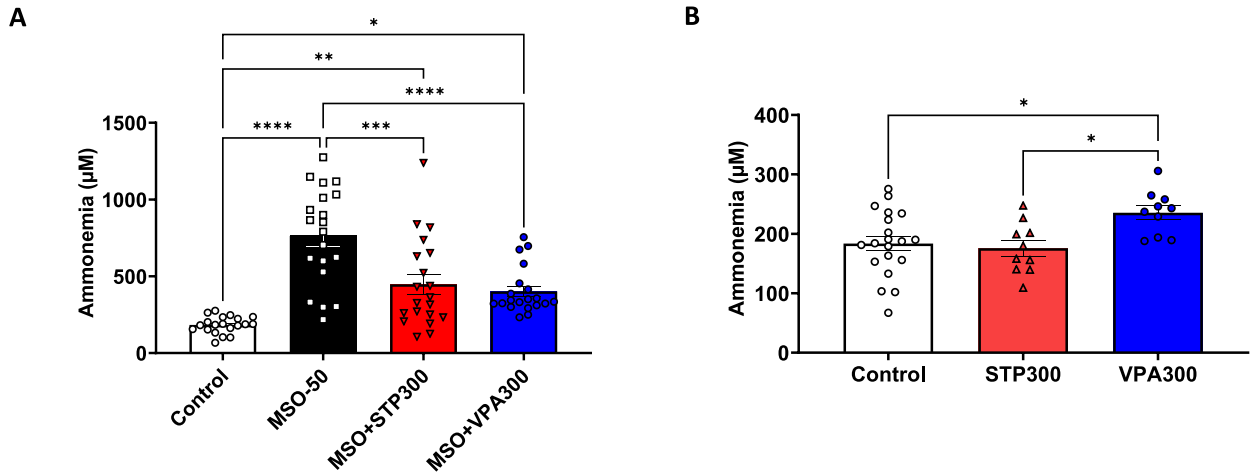

Fig. 3. Blood ammonia level in CBA mice. Each dot represents an individual mouse. (A) Effect of stiripentol (STP) or sodium valproate (VPA) on ammonemia after methionine sulfoximine (MSO) administration (intraperitoneal route) in mice (n = 20). STP or VPA were administered intraperitoneally 30 min before MSO (B) Effect of STP or VPA on ammonemia in mice (n = 10 to 40). *p < 0.05; **p < 0.01; ***p < 0.001, ANOVA statistical test comparison between groups.

hyperammonemia, but contrarily to STP, VPA significantly increased ammonia level by itself, as already shown in rats [22] or during chronic therapy [23,24]. For example, 8 days of intraperitoneal administration of VPA at 200 and 400 mg/kg in rats significantly increased ammonia level from 68 µM to 159 and 215 µM, respectively [25].

Regarding SE, therapeutic combination of STP with other antiseizure medications, essentially sodium valproate and clobazam, has demonstrated efficacy to markedly reduce the frequency or prevent these prolonged seizures in children and adults [1,26–28]. Our results confirm potential protective effects of STP to reduce and or suppress the occurrence of SE in MSO-treated mice. Moreover, and contrary to VPA, STP does not increase blood ammonia level, suggesting a safer profile.

Hyperammonemia, whether symptomatic or asymptomatic, is a known adverse effect of valproate treatment, as well as other antiseizure medication, such as topiramate [29]. Elevated ammonia levels can cause life-threatening valproate-induced hyperammonemia encephalopathy [30]. A less severe hyperammonemia may induce antiseizure medication intoxication, with lethargy, headaches, confusion and agitation [24]. A recent study with Dravet syndrome patients treated with valproate and clobazam in association with STP described an increased ammonia level [31]. Hyperammonemia was improved by carnitine treatment which allowed the continuation of antiseizure medication. Our data suggest that the hyperammonemia observed in the latter study was more likely due to VPA rather than STP treatment.

Future studies will evaluate the mechanisms of action of STP explaining its effects against SE-related mortality. Nevertheless, as shown in this study, the reduction of death rate by STP was dose-dependent and related to its antiseizure effects.

4. Conclusion

Our results show protective effects of STP to reduce and or suppress the occurrence of SE as well as its associated mortality in mice. Moreover, STP does not increase ammonia levels in CBA mice.

5. Experimental procedures

5.1. Animals

Adult male CBA/JRj mice (Janvier, France) weighing 23–27 were used. They were housed in a temperature (22 ± 2 °C) and humidity (50 ± 20 %) controlled room, under a 12 h light-dark cycle (lights on at 7:00 a.m.), with ad libitum access to food and tap water. All procedures were conducted in accordance with EU regulations and approved by French minister (APAFIS #5229).

5.2. Products

Methionine sulfoximine (Sigma, ref M5379) was solubilized in 0.9 % NaCl. STP (batch 197) and sodium valproate (Sigma, ref P4543) were suspended in 5 % Tween 80 solution (0.9 % NaCl). All drugs were administered intraperitoneally (i.p.) using a volume of administration of 10 mL/kg. STP or VPA were administered 30 min before MSO administration.

5.3. MSO-induced SE

The method was adapted from Picard et al. [12]. After intraperitoneal administration of MSO, mice were carefully observed for 8 h (non-blinded measurement). During the first 4 h, mice generally did not display any behavioural sign. Starting from the 4th hour, tonic and clonic seizures appeared and then disappeared progressively. Mice behaviour was evaluated according to the Irwin scale [32], and to the Racine scale [33] with the following stages: 0, no abnormality; 1, activity with period of immobility; 2, stereotypy with head nodding and tremor; 3, head myoclonus and forelimb clonus; 4, head myoclonus and complete clonic seizure; 5, generalized seizure with running and falling; 6, death. The number of seizures, their time of onset and the associated mortality were recorded for each mouse. To measure ammonia levels, blood samples were taken under isoflurane anaesthesia through cardiac puncture at the end of the study (8th hour) or just after cardiac arrest or euthanasia. The number of mice was 10 or 20 per group for the first experiment involving the dose-effect relationship of convulsions induced by MSO. For the second experiment regarding pretreatment effect of STP or VPA, the number of mice was 10 or 20 per group, except for the group receiving only MSO with 50 mice.

5.4. EEG recordings and analysis

Ten mice treated only by MSO and three mice treated by STP and MSO were implanted with electrodes to record EEG during MSO induced seizures with a telemetry system provided by DSI (ref HD-XO2). One week before MSO administration, mice were anesthetized with isoflurane and placed in a stereotaxic frame. After incision of the skull, electrodes were implanted bilaterally on the parietal cortex and fixed with surgical glue, and the transducer was set up subcutaneously on the back. EEG was recorded at 500 Hz without filter from mice placed in recording cages. The Racine score was measured continuously starting from the 4th hour following MSO administration to explore potential correlation between EEG recordings and behaviour.

LabChart (Ad Instruments) was used to extract examples of seizure, and to perform spectral analysis.

5.5. Ammonia assay

Blood was collected in 1.3 mL S-Monovette tube containing EDTA (Sarstedt). Samples were centrifugated at 2000 g during 10 min at 4 °C (Beckman Allegra centrifuge). Plasma was recovered in 1 mL microtube (Brand). Ammonia levels were measured by the colorimetric assay with an Abcam kit ammonia assay (Ab83360) at 570 nm. The number of mice was 20 per group for the experiment involving the effect of STP or VPA on MSO-induced hyperammonemia. For the experiment regarding the effect by STP or VPA on ammonemia, the number of mice was 10–40 per group.

5.6. Statistical analysis

Results are expressed in mean ± SEM. The minimum number of animals per group was 10 to perform relevant statistical analysis. The Fisher exact test was used to determine significant differences concerning the percentages of convulsions and mortality at the level of 5 % (Sigma Plot). ANOVA was used to determine significant differences between groups concerning the ammonia levels at the level of 5 %. For the Racine score, ANOVA with two factors (treatment and time) with repeated measures on time was used, and significance differences between groups was determined at the level of 5 % (Holm-Sidak test).

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Ethic declaration

Authors report that the study and the results are from our original research.

The manuscript was not submitted to another journal.

Generative AI and AI-assisted technologies were not used to write the manuscript.

The studies involve the use of animals, and the authors ensure that the manuscript contains a statement that all procedures were performed in compliance with relevant laws and institutional guidelines, and that the appropriate institutional committee(s) have approved them.

Data availability statement

The data that has been used is confidential.

CRediT authorship contribution statement

P. Girard: Writing – original draft, Methodology, Conceptualization. **A. Bacq:** Writing – original draft, Methodology, Formal analysis. **P. Cloarec:** Investigation, Formal analysis. **C. Lesueur:** Investigation, Formal analysis. **M. Verleye:** Writing – review & editing, Conceptualization. **V. Castagné:** Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Girard reports financial support was provided by Biocodex SA. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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