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Constipation enhances the propensity to seizure in pentylenetetrazole-induced seizure models of mice

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ABSTRACT

Epilepsy is characterized by spontaneous recurrent seizures and represents one of the most frequent neurological diseases, affecting about 60 million people worldwide. The cellular and neurocircuit bases of epilepsy are poorly understood. Constipation is a common gastrointestinal disorder characterized by symptoms such as straining, hard stool, and infrequent defecation. Population-based studies have shown that the prevalence of constipation is up to 30% of the population in developed countries. The causal link between seizure and constipation is a common belief among patients and physicians, but there are no scientific data to support this association. The current investigation evaluated the effects of constipation induced by loperamide (a peripheral μ-opioid receptor agonist without effect on central nervous system receptors) and clidinium (a quaternary amine antimuscarinic agent with reduced central nervous system effects) on two different seizure models of mice: (1) myoclonic, clonic, and generalized tonic seizures and death induced by intraperitoneal administration of pentylenetetrazole and (2) clonic seizure threshold induced by intravenous infusion of pentylenetetrazole. We demonstrated that the measured intestinal transit (%intestinal transit) decreased after loperamide or clidinium treatment for 3 days. Constipation in mice which was induced by loperamide or clonidine caused a decrease in threshold to clonic seizure in the intravenous pentylenetetrazole seizure model. Moreover loperamide- or clidinium-induced constipation decreased latencies to, clonic, and tonic seizures and death in the intraperitoneal pentylenetetrazole model of mice. Serum ammonia levels were slightly elevated in both loperamide- and clidinium-treated mice. In conclusion, loperamide- or clidinium-induced constipated mice are more prone to seizure which might confirm the belief of patients and physicians about constipation as a trigger of seizure.

1. Introduction

Epilepsy is one of the most common neurological disorders affecting roughly 0.5–1% of the population worldwide [1]. Epilepsy is characterized by recurrent seizures. During the seizures, there is abnormal excessive firing of neurons in the brain resulting in diverse symptoms such as staring, muscle stiffness (tonic movements), muscle spasms (clonic movements), and impaired consciousness. Epilepsy can develop at any age, but the incidence of epilepsy is highest during the first years of life and after the age of 65 years [2]. The cellular and neurocircuit bases of epilepsy are poorly understood, and seizures in 20-25% of patients respond poorly to existing medications [3].

Comorbid health conditions are common among people with epilepsy. Proposed explanations for this association include the possibility that first, epilepsy (including its treatment) causes the comorbid condition; second, the comorbid condition (including its treatment) causes epilepsy; or third, a common pathogenic mechanism mediates the co-occurrence of epilepsy and the comorbid condition. It is unlikely that a single explanation will suffice for all of the epilepsy comorbid conditions [4]. Common examples of comorbidity of epilepsy with other diseases include cardiac and respiratory disorders, stroke, dementia, and migraine. Alzheimer's disease and migraine not only are more common persons with epilepsy but also are risk factors for the development of seizures, suggesting a bidirectional association and shared disease mechanisms [5]. Gastrointestinal disorders are also common among people with epilepsy. Higher prevalences of stomach/intestinal ulcers [5–7] and bowel disorders (Crohn's disease/colitis) have been observed in patients with epilepsy [7].

Constipation is a common gastrointestinal disorder characterized by symptoms such as straining, hard stool, and infrequent defecation. Population-based studies have shown that the prevalence of constipation is up to 30% of the population in developed countries [8,9]. Constipation is defined medically as fewer than three stools per week and severe constipation as less than one stool per week. It occurs when the colon absorbs too much water [10]. Constipation is a debilitating condition affecting both physical functioning and emotional functioning.

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It is one of the most frequent complaints related to the lower gastrointestinal tract. Its prevalence is higher in females [11,12] and increases progressively with age [11–13] in both genders. While some patients have a systemic disease responsible for their constipation such as metabolic, neurogenic, or endocrine disorder(s) (hypercalcemia, hypothyroidism, diabetes, multiple sclerosis, Parkinson's disease, and scleroderma), anatomical malformation (e.g., anal stenosis and imperforate anus), previous pelvic surgery, and vaginal or cesarean childbirth, for many patients, the constipation is regarded as "idiopathic". Indeed, in children, 90% of those with constipation have no recognizable underlying organic cause [14].

Several patients believed that constipation can be a triggering factor for seizures which is asserted by some physicians [38,39]. The website of Epilepsy Research UK states that constipation can lead to a buildup of toxins in the system and may lead to an increase in seizures [40]. Despite the belief of patients and physicians, to our knowledge, there is no scientific paper about the role of constipation as a triggering factor for seizure. The current investigation aimed to evaluate the effects of constipation induced by loperamide or clonidine on two different seizure models of mice: (1) myoclonic, clonic, and generalized tonic seizures and death induced by intraperitoneal administration of pentylenetetrazole (PTZ) and (2) clonic seizure threshold induced by intravenous infusion of pentylenetetrazole. We also evaluated serum ammonia levels in both loperamide-treated mice and clidinium-treated mice.

2. Materials and methods

2.1. Reagents

The drugs loperamide (a peripheral μ-opioid receptor agonist without effect on central nervous system receptors), clidinium (a quaternary amine antimuscarinic agent with reduced central nervous system effects), gum acacia, and pentylenetetrazole (PTZ) were purchased from Sigma (USA). All drugs were freshly prepared in a physiologic saline solution to such concentrations that requisite doses were administered in a volume of 10 ml/kg of the mice body weight. Loperamide and clidinium were administered orally by gavage, and pentylenetetrazole was injected intraperitoneally (ip) or intravenously dependent on the method of inducing seizure. In the intravenous PTZ model, pentylenetetrazole was prepared in saline as a 0.5% solution and administered as an intravenous infusion.

2.2. Subjects

Male NMRI mice weighing 20–25 g at the time of the experiments were used throughout this study. The animals were housed in standard polycarbonate cages in a temperature-controlled room (23 ± 2 °C) on a 12-h light/dark cycle with free access to food and water and acclimated at least 2 days before the experiments. The protocol for this project was approved by the ethics committee of the university, and all experiments were performed according to the institutional guidelines for animal care and use. The experiments took place between 10 AM and 3 PM, and all possible measures were taken to minimize animals' discomfort during the procedures. Each treatment group consisted of at least six animals.

2.3. Behavioral seizure evaluation

2.3.1. Intravenous PTZ-induced seizure

The intravenous pentylenetetrazole infusion test is a very appropriate test for evaluation of the anticonvulsant activities of different compounds [15–17]. The clonic seizure threshold was determined by inserting a 30-gauge dental needle into the lateral tail vein of the mouse. The needle was then secured to the tail with a narrow piece of adhesive tape. With the mouse moving freely, the PTZ solution was infused into the tail vein at a constant rate of 0.5 ml/min using an infusion pump (Harvard, USA), which was connected to the dental needle by polyethylene tubing. Infusion was halted when forelimb clonus followed by full clonus of the body was observed. The minimum dose of PTZ (mg/kg mouse weight) needed to induce a clonic seizure was measured as an index of clonic seizure threshold.

2.3.2. Intraperitoneal PTZ-induced seizure

Generalized tonic–clonic seizure induced by near maximal intraperitoneal pentylenetetrazole is a distinct model related to generalized tonic–clonic seizures [18]. In this model, pentylenetetrazole (85 mg/kg, CD97 for generalized tonic–clonic seizures in the current experiment) was administered with a single intraperitoneal injection to evaluate the latency for the onset of myoclonic, clonic, and tonic seizures and death following seizures [19,20]. Immediately after the injection of PTZ, animals were transferred to an open field (50 cm in diameter) and monitored for the appearance of convulsion or death for 30 min. Following the administration of 85 mg/kg of PTZ, time latencies for the first myoclonic, clonic, and tonic seizures and death were measured. Latencies were calculated as a time between PTZ injections and the onset of these stages.

2.4. Induction of constipation in mice and measurement of intestinal transit

Different doses of loperamide and clidinium were selected to induce constipation in mice. Intestinal transit was measured with a charcoal meal according to procedures described by Puig and Pol [21]. In order to make the animals' intestines free of stool, they were deprived of food 18 h before the experiments in special cages with a floor made of metal grids (to prevent them from coprophagia) with free access to water. On the test day, each mouse received intragastrically 0.25 ml of a suspension of 10% vegetable charcoal in 5% gum acacia and was sacrificed 20 min afterward; the stomach and small intestine were totally removed, and, to let the intestine be fully straightened, the mesentery was completely separated, avoiding stretching. Then, both the length of the small intestine from the pyloric sphincter to the ileocecal junction and the distance traveled by the charcoal from the beginning of the small intestine to the most distal part of the charcoal bulk were measured in centimeters, and the intestinal transit (%intestinal transit) was calculated as the percentage of the distance traveled by the charcoal (CHARC) relative to the total length of the small intestine (INTS).

$$\%\ \text{transit} = (\text{CHARC}/\text{INTS}) \times 100$$

2.5. Treatments

In experiment 1, animals in different groups received oral administration of saline, loperamide (1.25, 2.5, and 5 mg/kg), or clidinium (0.25, 0.5, and 1 mg/kg) for 3 days. On the third day, 30 min after the last dose of loperamide or clidinium, each mouse received intragastrically vegetable charcoal in acacia and was sacrificed 20 min afterward to measure %intestinal transit and to find if these doses of drugs could induce constipation.

Experiment 2 was performed to evaluate the effect of constipation on inducing seizure. The separate groups of animals which received the same doses of drugs in experiment 1 for 3 days were used for this experiment. Pentylenetetrazole was administered intravenously or intraperitoneally 30 min after the last dose of saline, loperamide, or clidinium.

Experiment 3 was carried out to assess the effect of chronic treatment with loperamide or clidinium on serum ammonia levels. The separate groups of animals which received the same doses of drugs for 3 days were used for this experiment. After inducing anesthesia, we immediately collected blood samples from the heart of mice and centrifuged 10 min at 1500 ×*g* and 4 °C for serum preparation; then serum ammonia levels were determined in different groups [22].