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Research report

Sub-chronic treatment with pioglitazone exerts anti-convulsant effects in pentylenetetrazole-induced seizures of mice: The role of nitric oxide

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ABSTRACT

Objectives: Pioglitazone delayed the development of seizure responses and shortened the duration of convulsion of genetically epileptic EL mice. The anti-epileptic effect of pioglitazone was attributed partly through the reduction of inflammatory responses and preventing apoptosis. There are also some reports showing that some pioglitazone effects mediate through nitric oxide. In this study we evaluated sub-chronic pioglitazone effects in two models of intravenous and intraperitoneal pentylenetetrazole-induced clonic seizures in mice.
Materials and methods: Different doses of pioglitazone were administered orally for 10 days in different groups of male mice. L-NAME, a non selective inhibitor of nitric oxide synthase, aminoguanidine, a selective inhibitor of inducible nitric oxide synthase, or L-arginine, a nitric oxide donor, was administered acutely or sub-chronically to evaluate the role of nitric oxide in pioglitazone anti-seizure effects.
Results: We demonstrated that sub-chronic administration of pioglitazone exerted anti-convulsant effects in both models of intravenous and intraperitoneal pentylenetetrazole. Acute and sub-chronic pre-administration of L-NAME prevented the anti-convulsant effect of pioglitazone in both models of intravenous and intraperitoneal pentylenetetrazole. Aminoguanidine did not alter the anti-convulsant effect of pioglitazone in two models of intravenous and intraperitoneal pentylenetetrazole. Both acute and sub-chronic pre-treatment of mice with L-arginine exerted anti-convulsant effect when administered with a non effective dose of pioglitazone in intraperitoneal method. In intravenous method, acute administration of L-arginine with a non-effective dose of pioglitazone enhanced the seizure clonic latency.
Conclusion: Taken together, sub-chronic pioglitazone treatment exerts anti-convulsant effects in intravenous and intraperitoneal pentylenetetrazole-induced seizures of mice probably through induction of constitutive nitric oxide synthase.

1. Introduction

Pioglitazone, a thiazolidinedione derivative drug, is a useful glucose-lowering agent for patients with type 2 diabetes. Through peroxisome proliferator-activated receptors (PPAR)-γ-mediated effects, these drugs not only improve insulin sensitivity and glycemia, but also have beneficial effects on lipid metabolism [43]. PPAR-γ is showed to be highly expressed in various brain regions such as striatum, substantia nigra, cortex and hippocampus [20,28]. Thiazolidinediones have been shown to be beneficial in several cellular and animal models of central nervous system diseases. Their efficacy in improving insulin sensitivity correlates with the reduction of diabetes-induced acute brain damage, since chronic hyperglycemia is a major risk factor for neuropathy and vasculopathy [7]. Treatment with thiazolidinedione significantly reduced the symptoms associated with Parkinson's disease and showed improved protection of dopaminergic neurons [5]. The improved outcomes have been observed in multiple sclerosis [29,46], amyotrophic lateral sclerosis [42] and Alzheimer disease.

In the central nervous system, nitric oxide acts as a diffusible intercellular signaling molecule. Nitric oxide (NO) is synthesized from L-arginine in an NADPH-dependent reaction by NO synthase (NOS). Three different isoforms of NOS have been identified including one inducible (iNOS) and two constitutively expressed (endothelial (eNOS) and neuronal (nNOS)) forms [27]. In seizure susceptibility regulation, NOS substrates or NO donors exert various anti-convulsant [6,45,50] or proconvulsant [30,31,34] effects in different models of seizure. Reported results of nitric oxide

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involvement in seizure vary depending on the seizure model, kind and doses of pharmacological tools used in experiments, and route of drug administration [4].

Based upon the previous studies, the contribution of nitric oxide has been revealed in different functions of thiazolidinediones. While some studies demonstrated that pioglitazone activates NOS [10,14,24], there are also some studies indicating that pioglitazone inhibits the activity of NOS [15,17].

The study of Okada et al. [33] is the first to document pioglitazone's antiepileptic efficacy. They showed that pioglitazone delayed the development of seizure responses and mildly shortened the duration of convulsion of genetically epileptic EL mice. mRNA levels of IL-1β, IL-6 and TNF-α before seizure and mRNA levels of IL-6 and TNF-α after seizure were decreased in the brains of the mice with pioglitazone. From these results they suggested that pioglitazone may have ameliorative effects on epileptic seizure responses partly through the reduction of inflammatory responses in the brain [33]. Abdallah et al. [1] also showed that in acute pentylenetetrazole convulsion, as well as kindled mice, pioglitazone protected against pentylenetetrazole-induced seizures and delayed seizure latency onset which this anti-convulsant effect presumably associated with attenuating inflammation and preventing apoptosis.

The current investigation aimed to evaluate sub-chronic pioglitazone effects in two models of intravenous (IV) and intraperitoneal (IP) pentylenetetrazole-induced clonic seizures in mice. The present study was also designed to determine the role of nitric oxide pathway in the anti-convulsant effect of pioglitazone.

2. Materials and methods

2.1. Chemicals

The following drugs were used throughout the study: pioglitazone, L-N^G-Nitro-L-arginine methyl ester hydrochloride (L-NAME) (a non selective inhibitor of NOS), aminoguanidine (a selective inhibitor of iNOS), L-arginine (a NO donor) and pentylenetetrazole (PTZ). All drugs were purchased from Sigma (USA). Pioglitazone suspension was prepared in 0.5% sodium carboxy methyl cellulose and was administered orally by gavage once in a day for 10 days. L-NAME, aminoguanidine and L-arginine were dissolved in sterile isotonic saline solution and were administered intraperitoneally (IP) in a volume of 10 ml/kg of the mice body weight. The chemical structure of pioglitazone has been shown in Fig. 1. To assess clonic seizure experiments, pentylenetetrazole was administered intravenously (0.5%, IV) while to assess generalized tonic–clonic seizures it was administered IP (85 mg/kg).

2.2. Animals

Male NMRI mice weighing 23–30 g were used throughout this study. Animals were housed in groups of 5–6 and were allowed free access to food and water except for the short time that animals were removed from their cages for testing. All behavioral experiments were conducted during the period between 10:00 a.m. and 13:00 p.m. with normal room light (12-h regular light/dark cycle) and temperature (22 ± 1 °C). All procedures were carried out in accordance with the institutional guidelines for animal care and use (ethical approval number: 3183). Each mouse was used only once, and each treatment group consisted of at least eight animals.

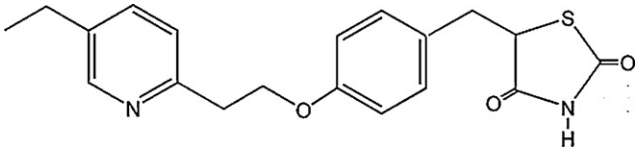

Fig. 1. The chemical structure of pioglitazone.

2.3. Determination of seizure threshold

The primary objective of this study was to examine the role of pioglitazone in modulation of susceptibility to myoclonic seizures induced by pentylenetetrazole that is a standard experimental model of clinical myoclonic petit mal seizures with both face and construct validity [25,48]. Single and repeated injection of the convulsant pentylenetetrazole causes generalized tonic–clonic seizures [2,32,35,39]. Pentylenetetrazole acts as a GABA receptor antagonist to induce seizure [25]. It has been also shown that PTZ induced single and repeated seizures result in increased oxidative damage and lipid peroxidation, and decreased antioxidant defense mechanisms [8]. Modulation of extracellular adenosine levels during PTZ-induced seizures is another mechanism of pentylenetetrazole administration [44]. To assess the seizure susceptibility, we used the more sensitive method of IV administration of PTZ that allows better detection of modulatory effects on convulsive tendency [25]. The threshold of PTZ was determined by inserting a 30-gauge butterfly needle into the tail vein of mice and infusion of PTZ (0.5%) at a constant rate of 0.5 ml/min to unrestrained freely moving animals. Infusion was halted when forelimb clonus followed by full clonus of the body was observed and the dose of PTZ administered (mg/kg of mice weight) was measured as an index of clonic seizure threshold. As such, seizure threshold is dependent on PTZ dose administered and time-related.

To further characterize the effects of pioglitazone on seizure threshold in another seizure model, we examined the effect of different treatment groups on generalized tonic–clonic seizures induced by IP injection of high dose PTZ. This method is an experimental model for grand-mal seizures [21,25]. The method used was as previously reported [11,34]. In brief, acute IP administration of PTZ (85 mg/kg, CD97 for generalized tonic–clonic seizures in the current experiment) was used to evaluate the incidence and latency of generalized tonic–clonic seizures and the incidence of death. Time of observation following PTZ was limited to 30 min and a latency of 1800 s was recorded for experiments in which no generalized seizure occurred.

2.4. Treatments

We used two methods of IV and IP administration of PTZ to assess the seizure susceptibility. The doses were chosen based on previous studies [12,16], pilot experiments and the sensitivity of tests. In the first experiment, pioglitazone was administered orally for 10 days in different groups of male mice. The doses of pioglitazone used were 0.5, 1, 5, 10 and 20 mg/kg in IV method and 5, 10 and 20 mg/kg in IP method.

In other experiments we examined the role of nitric oxide in anti-convulsant effects of pioglitazone. In acute tests, we administered non effective doses of L-NAME, a non selective inhibitor of NOS (5 mg/kg), aminoguanidine, a selective iNOS inhibitor (100 mg/kg), or L-arginine, a nitric oxide donor (60 mg/kg), 30 min before the last dose of pioglitazone on the 10th day, i.e. 60 min before PTZ. For sub-chronic tests, we injected L-NAME (2 mg/kg), aminoguanidine (50 mg/kg) or L-arginine (30 mg/kg), 30 min before each dose of pioglitazone during 10 days. The pioglitazone doses of 1 and 5 mg/kg for IV method and 20 mg/kg for IP method were selected for L-NAME and aminoguanidine experiments. We also selected the dose of 0.5 mg/kg of pioglitazone for IV tests and 10 mg/kg for IP method when we administered L-arginine.