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SHORT RESEARCH ARTICLE

The Effects of the Peroxisome-Proliferator Activated Receptor-alpha Agonist, Fenofibrate, on the Antioxidant Capacity of the Brain in Pentylenetetrazol Kindling Seizures in Mice

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Sarahian N et al. Reactive Oxygen Species 7(19):47–54, 2019; ©2019 Cell Med Press
http://dx.doi.org/10.20455/ros.2019.807
(Received: June 24, 2018; Revised: September 1, 2018; Accepted: September 3, 2018)

ABSTRACT | It has been demonstrated that peroxisome-proliferator activated receptor-alpha (PPARα) has a potent neuroprotective role in various pathological events of the nervous tissue. Since oxidative damage is associated with development of seizure, we aimed to examine whether the PPARα agonist, fenofibrate, exerts protective effects against the repeated seizures in pentylenetetrazol (PTZ) kindling model in mice through improving the brain antioxidant capacity. The experiment was carried out in two groups of mice (each group, n = 12): PTZ-kindled mice and fenofibrate-treated kindled mice. Repetitive intraperitoneal injections of PTZ (65 mg/kg) once every 48 h were used to achieve the kindling seizures till day 21. The mice were administered orally fenofibrate (30 mg/kg/day) during the test. Latency and the brain activities of catalase and superoxide dismutase (SOD) as well as the brain content of reduced glutathione (GSH) were determined at termination of the experiment. The latency following the last injection of PTZ was considerably decreased in untreated kindled mice (49 ± 8 s), whereas fenofibrate treatment prevented this reduction in kindled mice (105 ± 16 s). Treatment with fenofibrate significantly increased the GSH content in kindled mice (20.22 ± 9.87 nmol/mg protein) compared to untreated kindled mice (5.37 ± 0.84 nmol/mg protein), ($p < 0.05$). Likewise, treatment with fenofibrate considerably increased the activities of catalase and SOD in kindled mice compared to untreated kindled mice by 78% and 55%, respectively. In view of the critical protective role of antioxidants in seizures, the findings of the present study suggested that the PPARα agonist, fenofibrate, might modulate the seizure behaviors in the PTZ kindling model in mice through improving the brain antioxidant capacity.

KEYWORDS | Antioxidant capacity; Fenofibrate; Pentylenetetrazol; Oxidative stress; PPARα agonist; Reactive oxygen species; Seizure

ABBREVIATIONS | GSH, reduced glutathione; NBT, nitroblue tetrazolium; PBS, phosphate-buffered saline; PPARα, peroxisome-proliferator activated receptor-alpha; PTZ, pentylenetetrazol; ROS, reactive oxygen species; SOD, superoxide dismutase

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CONTENTS

1. INTRODUCTION

Seizures generally result from an imbalance of excitation and inhibition due to a defect in inhibitory neurotransmission, particularly dysfunction of GABAergic inhibition [1, 2]. Additionally, excessive generation of reactive oxygen species (ROS) has been implicated in the development of seizures [3]. Under normal physiological conditions, ROS are generated to maintain a redox balance and are destructed by the brain antioxidant defense system [3, 4]. There is evidence indicating that the antioxidant capacity of the brain is weakened in several animal models of seizure, and antioxidant therapy may lessen the lesions induced by oxidative stress [4–6]. It has been reported that lipid peroxidation and oxidative damage in accompany with mitochondrial dysfunction are observed following epileptic seizures [7, 8]. ROS accumulation caused by the seizures also induces oxidative damage to susceptible targets including proteins, lipids, and DNA [9, 10]. Consequently, the injuries induced by ROS may also aggravate the development of seizures [11]. In line with this notion, there is evidence showing that treatment with antioxidant substances may partially prevent the occurrence of seizures [7, 12]. Oxidative damage contributes to enhanced neuronal hyperexcitability by modifying the excitatory ion channels and inactivation of the energy-dependent glutamate transporters [3]. Therefore, there is a close relation between oxidative stress and seizure that has been frequently reported in experimental models and genetic studies [8, 13].

Previously, the robust neuroprotective functions of peroxisome-proliferator activated receptor-alpha (PPARα) have been demonstrated in various pathological events of the central nervous system [14–17]. It has been reported that the agonists of this receptor such as fenofibrate have anti-inflammatory and antioxidant properties [15, 18]. PPARα activation also improves the endothelial function and vascular tight junction integrity in a variety of pathophysiological states of the brain [14]. Treatment with the PPARα agonist, fenofibrate, decreased the infarct volume in cerebral ischemia [17]. Fenofibrate also increased the activities of antioxidant enzymes catalase and superoxide dismutase (SOD) in the ischemic brain [18, 19]. The neuroprotective roles of PPARα and its agonist, fenofibrate, have been demonstrated in Alzheimer's and Parkinson's diseases through clinical and basic research [20, 21]. PPARα activation exerted a protective effect against beta-amyloid-induced neurodegeneration and inhibited beta-amyloid-induced pro-inflammatory responses [20, 21]. Treatment with fenofibrate also preserved cognitive function and locomotor activity in a toxin-induced model of Parkinson’s disease through preventing dopaminergic neurons loss and dysfunction [14, 20]. It has been

demonstrated that these effects were mediated in part by decreased oxidative stress and pro-inflammatory mediators, suggesting that the PPARα agonist, fenofibrate, has anti-inflammatory and antioxidant properties [14, 16].

According to previous findings, the brain antioxidant capacity is weakened following the repetitive seizures [3, 6, 22]. Since the antioxidant properties of PPARα agonists have been demonstrated in several pathological events of the nervous tissue, we aimed to evaluate whether the PPARα agonist, fenofibrate, exerts protective effects against the seizures in the pentylenetetrazol kindling model in mice through improvement of the brain antioxidant defense system.

2. MATERIALS AND METHODS

2.1. Animals

All experimental protocols used in the current study were approved by the Institutional Animal Ethics Committee of the University of Baqiyatallah Medical Sciences (Tehran, Iran). Adult male NMRI mice, weighting about 23 ± 7 gram (aging 7–8 weeks old), were employed in the current study. The mice acclimatized in the institutional animal house and were given standard chow and water ad libitum. The animals were housed under controlled conditions of light exposure (12 h light/dark cycle), temperature (22–24˚C), and humidity (40–60%).

2.2. Drugs and Chemicals

Pentylenetetrazol (PTZ) was purchased from Sigma-Aldrich (Steinheim, Germany). Fenofibrate was obtained from Hakim Pharmaceutical (Tehran, Iran). PTZ was dissolved in normal saline and the solution was injected to animals, intraperitoneally. Fenofibrate was suspended in 0.5% w/v of carboxy methyl cellulose (CMC) and the solution was administrated to the animals via oral gavage.

2.3. Experimental Design and Grouping

Twenty-four mice were randomly divided into untreated kindled mice and fenofibrate-treated kindled mice (each group, n = 12). In both groups, the repeated intraperitoneal injections of PTZ (65 mg/kg) once every 48 h were used for induction of seizures until day 21 [23, 24]. The mice were administered orally fenofibrate (30 mg/kg/day) five days before induction of seizures until day 21. Also, untreated kindled mice were administered the CMC solution without fenofibrate in the same volume.

2.4. Determination of the Latency of Seizures

Immediately after PTZ injection, the treated and untreated kindled mice were transferred to a round open field, and the latency of seizures was determined. The time elapsed after injection of PTZ until appearance of the seizure behaviors, such as facial twitches/myoclonus and clonus of one fore-raw or bilateral myoclonus, was recorded as the latency.

2.5. Brain Tissue Preparation

Brains were quickly removed under deep anesthesia for evaluation of the antioxidant parameters. After homogenization of the brains in ice-cold phosphate-buffered saline (PBS), the homogenates were centrifuged at 14000 *g* at 4ºC for 15 min. Then, the supernatants were used to determine the brain contents of reduced glutathione (GSH) as well as the activities of superoxide dismutase (SOD) and catalase. The method of Bradford was used to quantify the protein content of each hemisphere for data calculation [25].

2.6. Measurement of GSH

The GSH levels of the brain were assessed according to the method of Tietz [26]. First, the cellular protein was precipitated by adding sulfosalicylic acid (5%). After centrifugation at 2,000 *g* for 10 min, the supernatant was removed and GSH level was assayed as follows: 100 µl of the protein-free supernatant of the tissue lysate, 100 µl of 0.04% 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) in 0.1% sodium citrate, and 800 µl of 0.3 mM Na_2HPO_4 were mixed. After 5 min, the absorbance was recorded at 412 nm. A standard curve was performed with GSH concentrations between 1 and 100 µM [27]. The GSH levels of the brain were calculated as nmol/mg protein.

2.7. Brain Activity of Catalase

The method of Aebi was used to determine the activity of catalase in the brain tissue homogenate [28].

First, the homogenate was incubated in the reaction mixture that contained 0.1 ml homogenate and 0.85 ml potassium phosphate buffer (50 mM and pH 7.0) at room temperature for 10 min. Then, the reaction was started by adding 0.05 ml H_2O_2 (30 mM prepared in 50 mM potassium phosphate buffer, pH 7.0). A decrease in the absorbance was recorded by a spectrophotometer at 240 nm for 3 min. Specific activity of catalase in the brain tissue was calculated as units/mg protein. One unit of catalase was defined as 1 µmol H_2O_2 decomposed per min.

2.8. Brain Activity of SOD

The SOD activity was measured using the method of Winterbourn et al. [29], based on the capability of SOD to inhibit the reduction of nitroblue tetrazolium (NBT). First, potassium phosphate buffer (0.067 M, pH 7.8) was added to 0.1 M EDTA containing 0.3 mM sodium cyanide, 1.5 mM NBT, and 0.1 ml of sample. Then, to initiate the reaction, riboflavin (0.12 mM) was added to each sample. After a 12 min-incubation, the absorbance of samples was recorded spectrophotometrically at 610 nm for five min. The amount of enzyme needed to induce 50% inhibition was taken as 1 unit [27]. The SOD activity in the brain tissue was calculated as units/mg protein.

2.9. Statistical Analysis

The SPSS (v.21, Chicago, IL, USA) was used for statistical analysis. The t-test was used to analyze the data between two groups (treated and untreated kindled mice). All data were expressed as mean ± SEM. A $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Effect of Fenofibrate on Latency of Kindled Mice

Treatment with fenofibrate did not significantly affect the body weight changes during experimental period (**Figure 1**). As shown in **Figure 2**, the values of latency after first injection of PTZ for untreated and treated kindled mice were 116 ± 14 s and 111 ± 4 s, respectively; there was statistically no significant difference between them. The latency was considerably decreased in untreated kindled mice (49 ± 8 s) following the last injection of PTZ, whereas fenofibrate prevented the reduction of latency in kindled mice (105 ± 16 s) ($p < 0.001$).

3.2. Effect of Fenofibrate on GSH Levels of the Brain

The GSH levels of the brain in kindled mice at termination of the experiment are shown in **Figure 3A**. The mean value of brain GSH levels was 5.37 ± 0.84 nmol/mg protein for untreated kindled mice. Treatment with fenofibrate significantly increased the GSH levels of the brain in kindled mice (20.22 ± 9.87 nmol/mg protein) compared to untreated kindled mice ($p < 0.05$).

3.3. Effect of Fenofibrate on Catalase Activity of the Brain

Figure 3B shows the catalase activity of the brain at termination of the experiment. The mean value of catalase activity in the brain of untreated kindled mice was 0.17 ± 0.01 units/mg protein. Fenofibrate significantly increased the catalase activity in the brain of kindled mice (0.78 ± 0.36 units/mg protein) compared to untreated kindled mice ($p < 0.001$).

3.4. Effect of Fenofibrate on SOD Activity of the Brain

As shown in **Figure 3C**, the SOD activity in the brain of untreated kindled mice was 1.08 ± 0.20 units/mg protein at termination of the experiment. Treatment with fenofibrate considerably increased the activity of SOD in the brain of kindled mice (2.40 ± 0.94 units/mg protein) compared to untreated kindled mice, but the difference was not statistically significant ($p = 0.103$).

4. DISCUSSION

Recently, the numerous reports have demonstrated the neuroprotective effects of the PPARα agonist, fenofibrate, in a variety of neurodegenerative diseases [16, 17]. In the present study, we showed that fenofibrate acts as an anticonvulsant in PTZ kindling model of seizure by preventing the reduction of seizures latency. Treatment with fenofibrate considerably enhanced the activities of antioxidant enzymes

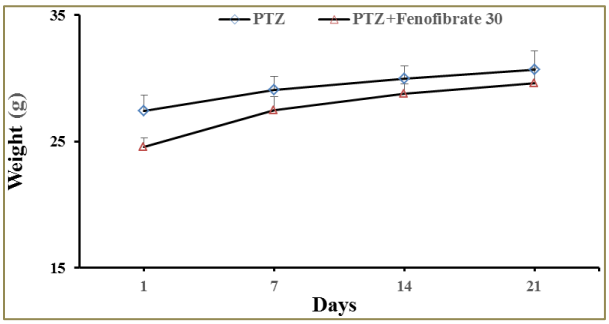

FIGURE 1. The representative changes of body weight of untreated and treated kindled mice during the experiment. Body weight of kindled mice (both groups) gradually increased during the period of the test. All values are presented as mean ± SEM (n = 12).

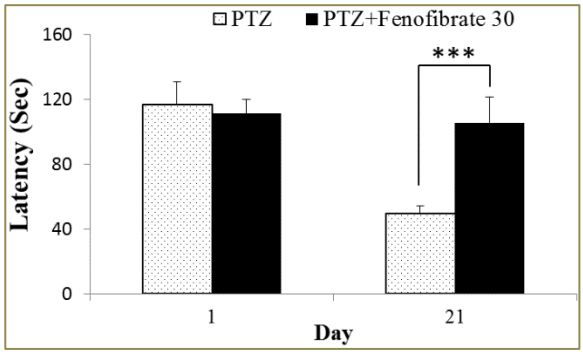

FIGURE 2. Latency in untreated kindled mice (PTZ) and treated kindled mice at the beginning and termination of the experiment. All values are presented as mean ± SEM (n = 12). ***, $p < 0.001$ at day 21.

(SOD and catalase) in the brain of kindled mice compared to untreated kindled mice. Fenofibrate also significantly increased the GSH levels of the brain in kindled mice. Since oxidative damage plays a critical role in the pathophysiology of the seizure-induced brain damage [7, 11], it is suggested that the anticonvulsive effects of fenofibrate may correlate with the capability of this agonist to potentiate the antioxidant capacity of the brain in PTZ kindling model of seizure in mice.

A number of clinical and experimental studies have demonstrated that the increased free radical generation after occurrence of seizures results in membrane lipid peroxidation and oxidative damage [30, 31]. Also, low plasma levels of antioxidants have been reported in patients with status epilepticus, suggesting that seizures induce oxidative damage [30]. Additionally, long-term treatment with certain antiepileptic drugs such as valproic acid increases free radical formation in people [32]. Since the antioxidant imbalance is the main mechanism of free radical accumulation and oxidative damage after seizures [33], improving the antioxidant capacity of the brain would prevent the seizures-induced free radical accumulation and oxidative damage. Our results indicated that treatment with fenofibrate considerably enhanced the antioxidant capacity of the brain in kindled mice. The antioxidant functions of fenofibrate and PPARα activation have been reported in the various pathophysiological states of the nervous tissue [16, 17]. Therefore, it is suggested that fenofibrate may act as a neuroprotectant during seizure periods by enhancing the antioxidant capacity of the brain. Indeed, our findings revealed that fenofibrate prevented reduction of the latency of the kindling seizures.

Based on our results, the brain GSH content of fenofibrate-treated kindled mice significantly increased compared with untreated mice. GSH is a key antioxidant with several cellular functions. It directly reacts with different oxygen and nitrogen free radicals [34]. Also, GSH acts as an essential cofactor for critical antioxidant enzymes, such as glutathione peroxidase that converts hydrogen peroxide to H_2O [35]. Furthermore, GSH protected the neurons against calcium-induced cell death and glutamate-mediated neurotoxicity [36, 37]. The GSH levels of the brain were shown to be decreased during the seizures [38]. Our results suggested that fenofibrate might potentiate the brain antioxidant system by increasing the GSH levels during the kindling seizures. Previously, Prigol et al. reported that GSH protected against seizure incidents by decreasing oxidative damage and antagonizing the glycine and glutamate modulatory locations in the NMDA receptor [37].

Pronounced alterations of antioxidant enzymes such as SOD and catalase have been reported following status epilepticus [39]. According to previous findings, the catalase activity of cerebral cortex was noticeably decreased following experimentally in

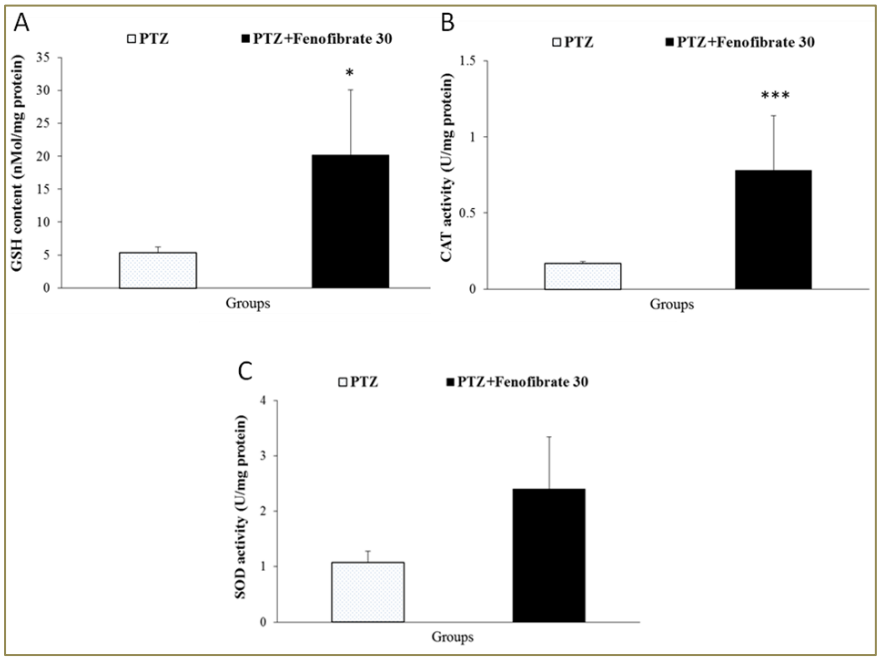

FIGURE 3. Effects of fenofibrate on the brain levels of GSH (A) and the activities of catalase (CAT) (B) and SOD (C) in untreated kindled mice (PTZ) and treated kindled mice at termination of the experiment. All values are presented as mean ± SEM (n = 4). *, $p < 0.05$; ***, $p < 0.001$ as compared with untreated kindled group.

duced repeated seizures [4]. Likewise, the total activity of SOD was considerably decreased following acute seizures in kindled rats [40]. Accordingly, improving the antioxidant capacity of the brain might provide a beneficial effect against seizures-induced neuronal and oxidative damages. Based on our results, fenofibrate administration improved the antioxidant capacity of the brain in kindled mice through increasing the antioxidant enzymes, including SOD and catalase activities. SOD is a key antioxidant enzyme that neutralizes superoxide [22]. Similarly, catalase is another key enzyme of the brain antioxidant system that is responsible for the neutralization of H_2O_2 derived from SOD-mediated dismutation of superoxide [27, 41]. According to our results, fenofibrate was able to potentiate the brain enzymatic antioxidant capacity by enhancing the activities of catalase and SOD in the kindling seizures. Therefore, fenofibrate administration might improve the seizures-induced oxidative and neuronal damages by enhancing both the enzymatic and non-enzymatic antioxidant capacity of the brain.

ACKNOWLEDGMENTS

This study was performed in the Department of Physiology and Biophysics, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran. The authors are cordially appreciating Student Research Committee of Baqiyatallah University of Medical Sciences. The authors declare no conflicts of interest.

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