The effect of penicillin-induced epileptiform activity on proinflammatory cytokines levels in the rat brain

Ahmet Kemal FILİZ 1, Sebahattin KARABULUT 2,*

1 Sivas Cumhuriyet University, School of Medicine, Department of Physiology, Sivas/ TURKEY
2 Sivas Cumhuriyet University, Vocational School of Health Services, Department of Medical Services and Techniques, Sivas/ TURKEY

Abstract
Emerging evidence indicates a pathogenic role of protracted neuroinflammation in the various neurodegenerative diseases, including epilepsy. Neuroinflammation may contribute to neuronal hyperexcitability underlying seizure formation. The current research aims to examine the changes in the levels of proinflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the penicillin epilepsy model. In the present study, 12 male Wistar albino rats were randomly divided into two groups as sham and penicillin epilepsy model. Seizures were induced with the intracortical (i.c.) single microinjection 500 IU of penicillin-G into neocortex. Rats were decapitated after observing the cortical epileptic activity and brains were removed by craniotomy. Proinflammatory cytokines (TNF-α and IL-1β) were measured by using ELISA methods in the cortical and hippocampal brain regions. Penicillin significantly up-regulated the expression of IL-1β and TNF-α in the rat cortex, but did not affect the hippocampal cytokines levels. This study is indicative of the neuroinflammatory potential of cortical penicillin administration.

1. Introduction
Epilepsy is a neurological disease that is characterized by recurrent unprovoked seizures and the associated comorbidities, including cognitive, psychiatric, and social consequences [1]. Patients with epilepsy are generally suffer from behavior such as loss of awareness, jerking, and déjà vu caused by the suddenly synchronous and excessive discharges in the brain. Unfortunately, 30% of all cases of epilepsy patients are unresponsive to pharmacotherapy, most of them are not convenient for surgery and have to continue to suffer from recurring seizures and debilitating side effects of antiepileptic drugs [2]. Furthermore, the available antiepileptic drugs have been developed for antiepileptogenesis (prevention of seizures) and not for antiepileptogenesis (prevention of epilepsy or disease-modifying). Hence, this calls for more research to investigate new and effective therapies for the treatment of epilepsy, by primarily understanding the basis for the initiation and progression of seizures. This need including therapies that target epileptogenesis as well as ictogenesis.

Epileptogenesis, by which a normal brain becomes epileptic, is a dynamic process that increasingly alters neuronal excitability. The evidence to date suggests that seizures can be derived from cortical or limbic lesions, including focal cortical dysplasia and hippocampal sclerosis. Moreover, the hippocampal formation is more vulnerable to epileptic activity in comparison to the cerebral cortex [3]. Hippocampal sclerosis caused by status epilepticus is characterized by pathological structural and functional changes that can affect epileptogenesis such as neuronal damage, gliosis and mossy fiber sprouting. There is strong evidence that inflammatory processes within the brain might constitute an essential mechanism in the etiopathogenesis of epileptogenesis [4]. Also, it is well-accepted that brain inflammation takes part to be an integral part of the diseased hyperexcitable brain tissue from which spontaneous seizures arise. Several lines of evidence from animal and human studies suggest that epileptic seizures can induce the production of proinflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the brain, which in turn may affect the epileptogenesis and the course of epilepsies [4-5]. Therefore, the increase in knowledge about the role of the inflammatory aspects of seizure may encourage the use of anti-inflammatory agents for developing disease-modifying therapy.
Animal models have played a crucial role in epilepsy research, including a better understanding of the disease and the discovery of new antiepileptic drugs. These experimental models of epilepsy have been instrumental in the understanding of the association of inflammation with epileptogenesis, and to resolve the role of inflammation and associated pathways in the formation of a seizure in the brain as well. Findings from the studies performed in rodent epilepsy models suggested the role of inflammation as either the cause or the consequence of epilepsy contributing to its etiopathogenesis [6]. The penicillin-epilepsy model has been commonly used in experimental animal studies to research the neuronal basis of epilepsy. Seizures are induced in a generalized fashion by the intracortical, intracerebroventricular, or intraperitoneal administration of penicillin, a GABA receptor antagonist, on the experimental animals [7-9]. There is no study in the literature on the roles of proinflammatory cytokines in the penicillin rat model of epilepsy. For that reason, this study was planned to assess whether penicillin-induced seizures affect the proinflammatory cytokines such as IL-1β and TNF-α in the cortex and hippocampus.

2. Materials and Methods

2.1. Animals

This study was performed using twelve adult male Wistar rats (230-250 g in weight) were obtained from the Cumhuriyet University Animal Laboratory. All animal care and experimental procedures were performed in accordance with the guidelines of the Local Ethics Committee for the welfare of experimental animals and were approved (approval code 65202830-050.04.04-422). Animals were maintained in an air-conditioned room with controlled temperature (22 ± 2 °C) under a 12-h light/dark cycle with lights on at 07:00 am.

2.2. Drugs

Penicillin G (İ.E. Ulagay, Turkey) was dissolved in physiological saline. All other general agents were used in the studies were of analytical grade.

2.3. Experimental protocol

Adult male Wistar rats (weighing 230-250 g) were used in the experiments. The rats were fully anesthetized with Ketamine at a dose of 90 mg/kg administered intraperitoneally (i.p.). After the onset of anesthesia, rats were placed in a stereotaxic frame and the heads of rats were shaved. The head skin was incised by using a surgical blade at midline rostral-caudal level of the scalp and the periosteum was removed by scraping. With reference to bregma point with the stereotaxic tool, a total of 3 holes having 2 mm diameters each were opened using a micro drill.

Figure 1. Representative illustration of ECoG recordings for Sham (A) and Penicillin groups (B).
500 IU penicillin-G was injected into the neocortex (by a Hamilton microsyringe with an infusion rate of 0.5 μL/min), with the guidance of Paxinos and Watson stereotactic atlas, which is located at coordinates of 1.1 mm anteroposterior, 1.5 mm lateral to bregma, and 3.2 mm vertical from the dura [10]. Saline was given i.c. to the sham group instead of penicillin-G. The recording electrodes were located on the cortex to record cortical electrical activity during electrocorticography (ECoG). The first electrode was placed in a position 3 mm lateral to sagittal suture and 4 mm anterior to bregma, while the second one was mounted in a position 3 mm lateral to sagittal suture and 4 mm posterior to bregma. A third electrode was used as a reference and implanted in the skin [7]. The ECoG activity was monitored online using a PowerLab 4/25 (AD Instruments, Australia) data acquisition system (Figure 1). After observing the epileptic activity of penicillin injection, all rats were decapitated and their brains were removed surgically for biochemical assessment.

2.4. Biochemical analysis

The rat's brains were excised after killed, and the cortex and hippocampus were dissected. Then these tissue samples were homogenized in ice-cold Phosphate-Buffered Saline (PBS) solution (pH 7.4) using a mechanical homogenizer (Analytic Jena speed mill plus, Jena, Germany). The homogenates were centrifuged at 12,000 × g for 10 min at 4°C. Then, the supernatants were collected for determination of total protein levels in samples by a Bradford protein assay kit. (Merck, Germany) [11]. The levels of cortical and hippocampal TNF-α and IL-1β were determined by using rat ELISA commercial kits (Shanghai Sunred Biological Technology, Shanghai, China). In brief, the standard solution and tissue samples were incubated for 60 min at 37°C after they were added to the plate. Following the washing phase, after addition of staining solutions, the tissue samples were incubated for 15 min at 37°C. Then, following the application of stop solution, the wells were read at 450 nm without delay.

2.5. Statistical analysis

The data are expressed as mean ± standard error of mean (SEM). The differences between the mean values for each group of the levels of TNF-α and IL-1β were analyzed using the independent samples t-test. A p-value of less than 5% was accepted as statistically significant.

3. Results and Discussion

Increasing evidence from patient studies and experimental models implicate the presence of neuroinflammation in epilepsy [12]. Proinflammatory cytokines, such as IL-1β and TNF-α, contribute to hyperexcitability and can cause spontaneous seizures [13,14]. These results of which signify the great value of cytokines in the development of new therapeutic strategies against epilepsy. It has been proposed that drugs that block specific inflammatory processes may have therapeutic potential for epilepsy is associated with proinflammatory signals in the brain. Therefore, in the current study, the levels of IL-1β and TNF-α were investigated in the hippocampus and cortex after penicillin-treatment. We found that the levels of IL-1β and TNF-α increased in the cortex after i.c. penicillin administration, but did not change in the hippocampus.

IL-1β belongs to the IL-1 family of proinflammatory cytokines and acts as an amplifier of immune reactions [15]. It is secreted by activated microglia and astrocytes within nearly two hours of central nervous system insult, such as status epilepticus [16]. In the present study, the level of IL-1β in the cortex was significantly up-regulated by penicillin administration (Figure 2A, p<0.001). The regulation of the level of IL-1β in several epileptic animal models is well-known, but there are few studies on the involvement of this cytokine in penicillin model epilepsy. In an early pharmacological study, it has been reported that penicillin-induced electrocorticographic and motor seizures are weakened by using nonsteroidal antiinflammatory drugs in rats [17]. Zhu et al. reported that penicillin leads to the release of inflammatory factors (IL-1β and TNF-α) and activates the MAPK signaling pathway, ultimately cause epilepsy in primary astrocyte cell culture [18]. This is coherent with the results of the present study where we demonstrate that penicillin cause an increase in the level of cortical IL-1β. However, unlike the cortical IL-1β, no significant difference between the groups was found in hippocampal IL-1β levels (Figure 2B, p>0.05). In penicillin model epilepsy, the seizures start from the cortex and then spread to limbic structures such as the hippocampus. In addition, as in our study, there may not be enough time to obtain an inflammatory response in an acute seizure model. Supporting this possibility, in a recent study, Taskiran et al. compared inflammatory markers in the brain in PTZ-induced acute seizure and chronic epilepsy model and reported more significant results in the chronic PTZ model compared to acute seizure model [19].
Similar to IL-1β, pro-inflammatory cytokine TNF-α is also released from activated glial cells [20]. Although TNF-α is rapidly induced after seizures in rodent epilepsy models, its role in epilepsy remains controversial. TNF-α plays a bidirectional role in epilepsy. Baloso et al. showed that transgenic mice with astrocytic overexpression of TNF-α display reduced susceptibility to seizures [21]. Conversely, Shandra et al. reported that i.p. injection of TNF-α elongated the duration of epileptic discharges in amygdala-kindled animals [22]. These apparent contradictions can be reconciled by considering either the activation of its different receptors in the brain or the differences in TNF-α concentrations. According to this, lower concentrations of TNF-α were proconvulsive, whereas higher concentrations of TNF-α have an anticonvulsive effect. In our study, we found that penicillin caused a significant increase in the level of proinflammatory cytokine TNF-α in the rat cortex (Figure 3A, p<0.01).

This finding implies that TNF-α contributes to penicillin-induced epileptic seizures. In animal models, seizures can increase the concentration of TNF-α in the brain while its mechanism remains obscure. Moreover, due to the lack of clinical studies, the role of TNF-α in epilepsy has not yet been elucidated in humans [3]. However, detected no differences across the two groups after penicillin administration in hippocampal TNF-α levels (Figure 3B, p>0.05). As noted above, this result can be attributed to the area of the brain where penicillin was injected and/or the use of an acute seizure model.

In conclusion, we firstly demonstrated that i.c. penicillin administration upregulated proinflammatory cytokines such as IL-1β and TNF-α in the rat cortex. To the best of our knowledge, the presence of proinflammatory cytokines in the penicillin model of epilepsy remains to be elucidated. Clarifying changes in the specific brain region of other proinflammatory...
cytokines involved in epilepsy will be important for future targeted treatments for this disease.

Acknowledgment

The authors thank the institution for using the facilities of the Sivas Cumhuriyet University.

Conflicts of interest

The authors state that did not have conflict of interests.

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