Seizure 19 (2010) 514–516

Contents lists available at ScienceDirect

Seizure

journal homepage: www.elsevier.com/locate/yseiz

The effects of levetiracetam on urinary 15f-2t-isoprostane levels in epileptic patients

Hilmi Ozden [a], Sibel Canbaz Kabay [b,*], Aysun Toker [c], Mehmet Cengiz Ustüner [d], Cansu Ozbayer [d], Derya Ustüner [e], Hasan Veysi Günes [d]

[a] Eskişehir Osmangazi University, Medical Faculty, Department of Anatomy, Eskisehir, Turkey
[b] Dumlupınar University, Medical Faculty, Department of Neurology, Kutahya, Turkey
[c] Yoncalı State Hospital, Clinics of Biochemistry, Kutahya, Turkey
[d] Eskişehir Osmangazi University, Medical Faculty, Department of Medical Biology, Eskisehir, Turkey
[e] Eskişehir Osmangazi University, Medical Faculty, Department of Genetic, Eskisehir, Turkey

ARTICLE INFO

Article history:
Received 24 April 2010
Received in revised form 28 June 2010
Accepted 9 July 2010

Keywords:
Levetiracetam
15F-2t-isoprostane
Oxidative stress
Epilepsy
Antiepileptic drugs

ABSTRACT

Purpose: We aimed to investigate the effects of levetiracetam on oxidative stress which is one of the new antiepileptic drugs in epileptic patients.
Methods: The study consisted of 21 patients with cryptogenic partial epilepsy. We determined the urinary 15F-2t-isoprostane levels of the 30 patients which is a marker of oxidative stress. Morning urine samples were collected from the patients before beginning LEV and after 3 months treatment. Of these patients 9 were excluded from the study that had seizure history in the last 1 month. Urinary levels of 15-F2t-isoprostane determined by ELISA initially and after 3 months treatment for each patient.
Results: Mean age of the 21 patients was 29.6, of these 11 were females and 10 males. Mean urinary 15F-2t-isoprostane level of the patients was 876 ± 447 ng/mg Cr before the treatment of LEV. After 3 months treatment the mean 15F-2t-isoprostane level of the patients was 1560 ± 630. The patients had significantly higher levels of urinary 15F-2t-isoprostane when compared with initial levels ($p = 0.025$).
Conclusion: Our results showed the increase of urinary 15F-2t-isoprostane levels in epileptic patients whom were treated with LEV which may indicate that LEV induces the oxidative stress in epileptic patients.

1. Introduction

Reactive oxygen species (ROS) are atoms or molecules with one or more uncoupled electrons. ROS that occur during physiological and pathological processes may damage cell compounds such as lipid, protein, carbohydrate and DNA.[1,2] Oxidative stress appears when the production of ROS exceeds the removal capacity. The ROS reactivity towards various molecular targets leads to oxidative damage contributing to different human pathologies. Despite the presence of the cell's antioxidant defense system to counteract oxidative damage from ROS, radical-related damage may play a key role in the development of age-dependent diseases such as cancer, arteriosclerosis, arthritis, neurodegenerative disorders and other conditions.[3] Some studies reported that free radicals may play role in epilepsy.[4,5] Generalized epilepsy may increase the content of ROS and superoxide generation in the brain.[6,7] Seizure-induced lipid peroxidation has been reported by measuring products such as thiobarbituric acid reactive substances[8] and F2-isoprostanes (F2-IsoPs) in rat brain tissue.[9] In vivo detection of free radicals is difficult because of their very short lifetimes; other parameters are used to measure oxidative stress. Considering that lipid peroxidation is one of the most biological important free radicals reactions,[10] changes in lipid oxidation markers are commonly used as indicators of oxidative stress in clinical laboratory settings.[11] Lipid peroxidation often appears in response to oxidative stress, and a great variety of aldehydes are formed when lipid hydroperoxides break down in biological systems. The methods used detection of conjugated dienes, lipoperoxides, and aldehydes are poorly reproducible and unreliable when carried out in plasma because of the extreme reactivity and instability of the species.[12] Some researchers reported the production of a series of prostaglandin F2-like compounds (F2-isoprostanes, F2-IsoPs), which are formed in vitro and in vivo by free radical-catalyzed peroxidation of phospholipid-bound arachidonic acid, a pathway which is independent of the cyclooxygenase pathway.[13,14] F2-isoprostanes are chemically stable prostaglandin isomers consisted by free radical peroxidation of polyunsaturated fatty acids, which are stored in tissues, circulate in plasma, and are

* Corresponding author. Tel.: +90 2742271555.
E-mail address: scanbazkabay@yahoo.com (S.C. Kabay).

1059-1311/$ – see front matter © 2010 British Epilepsy Association. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.seizure.2010.07.010

excreted in urine.[15] F2-IsoPs may be considered as the most reliable markers of oxidative stress[16] and can be used to evaluate the oxidative stress in several of human pathologies. At the same time, this is also the conclusion of a multilaboratory validation study of the National Institute of Environmental Health Sciences.[17] In some studies it was shown that valproic acid treatment in epileptic children increased the urinary levels of 15-F2t-IsoP which is a marker of oxidative stres.[18] But there is not any study about the effect of levetiracetam on levels of the urinary15-F2t-IsoP.

The aim of our study was to evaluate levels of urinary isoprostane levels of epileptic patients, and then to determine whether the treatment of levetiracetam might influence the urinary isoprostane levels before and 3 months after therapy.

2. Materials and methods

All research procedures were approved by the University of Dumlupinar Institutional Review Board and Eskisehir Osmangazi University, Medical Faculty. Initially, the study consisted of 30 patients with cryptogenic partial epilepsy. Of these patients 9 were excluded from the study that had seizure history in the last 1 month. All patients were subjected to detailed clinical examination for evaluation of neurological status. Electroencephalography (EEG) was conducted on all patients. MRI scan was done for more detailed examination. All patients were suffering from cryptogenic partial epilepsy. The patients who received levetiracetam (1000–3000 mg/day) for 3 months and who did not have any clinical epileptic seizure during for the last 1 month were included. Our exclusion criteria were abnormal neurological examination, abnormal cerebral MRI scan, endocrinopathies, liver, heart, kidney diseases, cancer, and any disease likely to affect lipid metabolism, any inflammatory disease, and a body mass index (BMI) > 25. Patients with past or concurrent diseases which might affect the activity of antioxidant enzymes were excluded from the study. Mean age of the 21 patients whom lasted the study were 29.6, of these 11 were females and 10 males. Blood samples were taken first day of therapy. Urine samples were collected from the participants beginning LEV and after 3 months treatment. Blood samples were collected in serum separator tubes, allowed to clot for 30 min, centrifuged at room temperature for 15 min at 2000 × *g*. The biochemical measurements were done immediately, by using commercial available kits. The urine samples were stored at −80 °C until analyzed. Routine blood count and chemistry analysis and urinalysis were performed for each patient, and those with abnormal results were excluded. Urinary levels of 15-F2t-isoprostane determined by ELISA first and last day for each patient.

2.1. Urinary 15-F2t-IsoP immunoassay

Urinary 15-F2t-IsoP levels were analyzed using immunoassay kits from Oxford Biomedical Research (Product number; EA 85). Urine samples without additives added were thawed to room temperature and diluted 1:2 using the dilution buffer provided with the kit. Analysis was done according to the recommendations of the manufacturer according to Fig. 1. Urinary levels are dependent on the hydration status of the subject. To normalize 15-F2t-IsoP levels, urinary creatinine was assayed using a creatinine kit from Oxford Biomedical Research (Product number; CR 01) in Fig. 2. 15-F2t-IsoP concentration was expressed as nmol 15-F2t-IsoP/mmol creatinine.

2.2. Statistical analysis

All statistical analysis was performed with the computer program "SPSS for Windows" (SPSS Inc; Release 11.5; September 6, 2002). All of the data were expressed as means ± SD. Differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The significance was tested at n.s. $p > 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.001$.

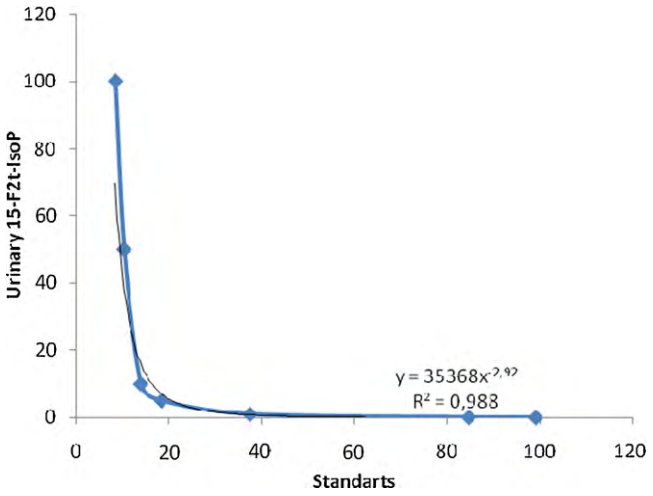

Fig. 1. Calculation of 15-F2t-IsoP levels according to standard in urinary levels of 15-F2t-isoprostane kit.

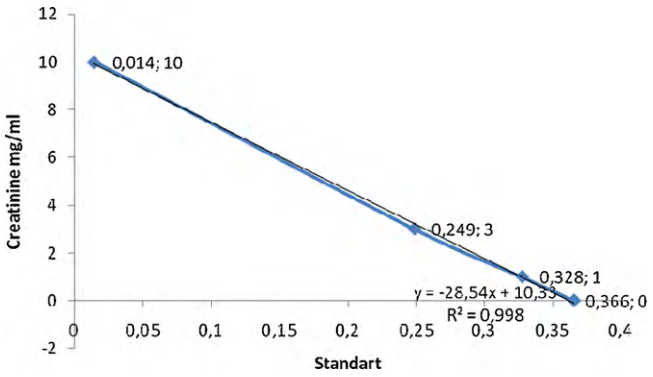

Fig. 2. Calculation of creatinine according to standard in creatinine kit.

Urinary 15-F2t-Isop Levels	N	Mean	Multiple Comparisons Tukey HSD and *p* values
Before LEV Treatment	21	876 ± 447	-
After LEV Treatment	21	1560 ± 630	0,025*

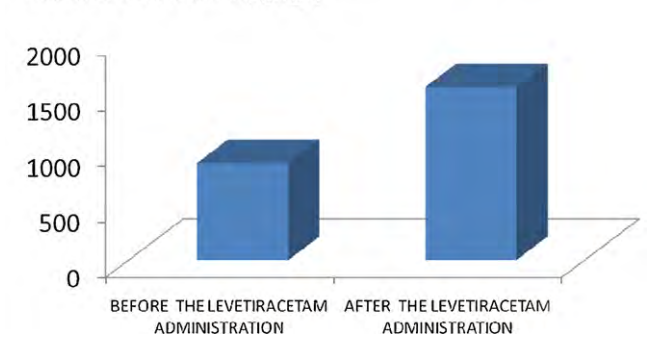

Fig. 3. Urinary 15-F2t-IsoP levels (ng/mg creatinine) before and after LEV of the treatment. The mean difference is significant at the .05 level.

3. Results

Initially, mean urinary 15F-2t-isoprostane levels of the patients were 876 ± 447 ng/mg Cr. After 3 months treatment of LEV, the mean 15F-2t-isoprostane levels of the patients were 1560 ± 630. The patients had significantly higher levels of urinary 15F-2t-isoprostane when compared with initial levels ($p = 0.025$) in Fig. 3.

4. Discussion

In this study, the oxidative status in epileptic patients before and 3 months after levetiracetam therapy was evaluated. The seizures mediated brain injury is a dynamic process that includes multiple factors such as genetic factors, the extent of glutamate-mediated excitoxicity leading to disturbances in intracellular electrolyte metabolism, mitochondrial dysfunction, oxidative stress and changes in cytokines.[19] Some studies reported an increase in oxidative status in the brain of animals probably caused by recurrent seizures.[6,7] Free radical generation may induce seizure activity by inactivation of glutamine synthase. Thus, oxidative stress could exacerbate the etiology of epilepsy.[6] There are some conflict results in oxidative stress in epilepsy. Some authors reported that increased lipid peroxidation of brain tissue was in ferric chloride and kainate-induced epilepsy in experimental studies.[8,20] Patel et al. demonstrated that seizures induced the increase in isofurans (novel products of lipid peroxidation) formation and its correlation with changes in hippocampal pO_2 and mitochondrial dysfunction.[21] The conflicting results in oxidative stress in epilepsy have not been exactly explained in the literature. The variation of reported results may result from patients included the study different forms of medication, type of seizure, obesity. F2-Isops are desirable index of lipid peroxidation in vivo because they are specific products of lipid peroxidation, detectable in normal biological fluids, modulated by antioxidant status; and unaffected by dietary lipids and they increase dramatically in models of oxidant injury.[22] F2-IsoPs are not only most reliable markers of oxidative stress[16] but also have some biological effects such as vasoconstriction with ischemia of the retina which account for retinopathy of prematurity.[23] Patel et al., showed that status epilepticus results in the formation of lipid peroxidation end products. Status epilepticus transiently decreases hippocampal oxygen tension and F2-IsoP occurs during these hypoxic phase whereas the formation of isofurans occurs when the tissue oxygen levels return to normal. These data suggest that seizure-induced changes in tissue oxygen levels and mitochondrial dysfunction may differentially influence the formation of F2-IsoPs and IsoFs.[21] Free radicals and ROS may play role in epilepsy.[4,5,24] Long-term use of anticonvulsants has been shown to increase free radicals formation and cause oxidative damage within neuronal cells.[25] There are several studies about the effect of valproic acid treatment on 15-F2t-isoprostane levels.[26,27,18] Tong et al. reported that the plasma and liver levels of 15-F2t-isoprostane was increased in rats after their treatment with VPA.[26] Moreover, they reported VPA-induced formation of 15-F2t-IsoP is associated with VPA glucronidation.[27] Michoulas et al., demonstrate that treatment of children with VPA is associated with higher urinary levels of 15-F2t-IsoP, a marker of oxidative stress.[18] But there is no knowledge about effect of levetiracetam treatment on urinary 15-F2t-isoprostane levels. The report of the subcommittees of American Academy of Neurology and American Epilepsy Society suggested that levetiracetam have no serious adverse events and no known interactions with oral contraceptives, warfarin, enzyme inducer and other antiepileptic drugs.[28] Our results showed the significant higher levels of urinary 15F-2t-isoprostane levels in epileptic patients under LEV treatment when compared with the initial levels. Considering the present findings, it can be suggested that patients on LEV therapy may be at risk of developing oxidative stress as shown by the remarkably high urinary 15-F2t-isoprostane levels.

References

1. Knight JA. Diseases related to oxygen-derived free radicals. *Annals of Clinical and Laboratory Science* 1995;**25**:111–21.
2. Halliwell B, Gutteridge JMC, Cross CE. Free radicals, antioxidants, and human diseases: where are we now? *The Journal of Laboratory and Clinical Medicine* 1992;**119**:598–620.
3. Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. third ed. Oxford University Press; 1999. pp 140–163, 393–430.
4. Kovacs R, Schuchmann S, Gabriel S, Kann O, Kardos J, Heinemann U. Free radical-mediated cell damage after experimental status epilepticus in hippocampal slice cultures. *Journal of Neurophysiology* 2002;**88**:2909–18.
5. Patel M. Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures. *Free Radical Biology & Medicine* 2004;**37**:1951–62.
6. Oliver CN, Starke-Reed PE, Stadtman ER, Lin GJ, Correy JM, Floyd RA. Oxidative damage to brain proteins, loss of glutamine synthetase activity and production of free radicals during ischemia/reperfusion induced injury to gerbil brain. *Proceedings of the National Academy of Sciences of the United States of America* 1990;**87**:5144–7.
7. Armstead WM, Mirro R, Leffer CW, Busija DW. Cerebral superoxide anion generation during seizures in newborn pigs. *Journal of Cerebral Blood Flow and Metabolism* 1989;**9**:175–9.
8. Bruce AJ, Baudry M. Oxygen free radicals in rat limbic structures after kainate-induced seizures. *Free Radical Biology & Medicine* 1995;**18**:993–1002.
9. Patel M, Liang LP, Roberts 2nd LJ. Enhanced hippocampal F2-isoprostane formation following kainate-induced seizures. *Journal of Neurochemistry* 2001;**79**:1065–9.
10. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature* 2000;**408**:239–47.
11. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clinical Chemistry* 1995;**41**:1819–28.
12. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology & Medicine* 1991;**11**:81–128.
13. Morrow JD, Harris TM, Roberts LJ. Noncyclooxygenase oxidative formation of a series of novel prostaglandins: analytical ramifications for measurement of eicosanoids. *Analytical Biochemistry* 1990;**184**:1–10.
14. Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts LJ. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. *Proceedings of the National Academy of Sciences of the United States of America* 1990;**87**:9383–7.
15. Pratico D. F (2)-isoprostanes: sensitive and specific non-invasive indices of lipid peroxidation in vivo. *Atherosclerosis* 1999;**147**:1–10.
16. Morrow JD, Roberts LJ. The isoprostanes: current knowledge and directions for future research. *Biochemical Pharmacology* 1996;**51**:1–9.
17. Kadiiska MB, Gladen BC, Baird DD, Germolec D, Graham LB, Parker CE, et al. Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? *Free Radical Biology & Medicine* 2005;**38**:698–710.
18. Michoulas A, Tong V, Teng XW, Chang TK, Abbott FS, Farrell K. Oxidative stress in children receiving valproic acid. *The Journal of Pediatrics* 2006;**149**:692–6.
19. Ferriero DM. Protecting neurons. *Epilepsia* 2005;**46**:45–51.
20. Singh R, Pathak DN. Lipid peroxidation and glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase, and glucose-6-phosphate dehydrogenase activities in $FeCl_3$-induced epileptogenic foci in the rat brain. *Epilepsia* 1990;**31**:15–26.
21. Patel M, Liang LP, Hou H, Williams BB, Kmiec M, Swartz HM, et al. Seizure-induced formation of isofurans: novel products of lipid peroxidation whose formation is positively modulated by oxygen tension. *Journal of Neurochemistry* 2008;**104**:264–70.
22. Roberts LJ, Morrow JD. Measurement of F (2)-isoprostanes as an index of oxidative stress in vivo. *Free Radical Biology & Medicine* 2000;**28**:505–13.
23. Reynaud X, Dorey CK. Extraretinal neovascularization induced by hypoxic episodes in the neonatal rat. *Investigative Ophthalmology & Visual Science* 1994;**35**:3169–77.
24. Torbati D, Church DF, Keller JM, Pryor W. Free radical generation in the brain precedes hyperbaric oxygen induced convulsions. *Free Radical Biology & Medicine* 1992;**13**:101–6.
25. Maertens P, Dyken P, Graf W, Pippenger C, Chronister R, Shah A. Free radicals, anticonvulsants, and the neuronal ceroid lipofuscinoses. *American Journal of Medical Genetics* 1995;**57**:225–8.
26. Tong V, Chang TK, Chen J, Abbot FS. The effect of valproic acid on hepatic and plasma levels of 15-F2t-isoprostane in rats. *Free Radical Biology & Medicine* 2002;**34**:1435–46.
27. Tong V, Teng XW, Karagiozov S, Chang TK, Abbott FS. Valproic acid glucuronidation is associated with increases in 15-F2t-isoprostane in rats. *Free Radical Biology & Medicine* 2005;**38**:1471–83.
28. French JA, Kanner AM, Bautista J, Abou-Khalil B, Browne T, Harden CL, et al. Efficacy and tolerability of the new antiepileptic drugs, I: treatment of new-onset epilepsy: report of the TTA and QSS Subcommittees of the American Academy of Neurology and the American Epilepsy Society. *Epilepsia* 2004;**45**:401–9.