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Effect of antiepileptic drugs on antioxidant status in epilepsy

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Abstract

The present study comprised of 60 patients of epilepsy grouped as 30 patients with regular antiepileptic drugs (phenytoin and carbamazepine) treatment and 30 patients with irregular treatment with antiepileptic drugs (phenytoin and carbamazepine) and compared with normal 30 controls. Lipid peroxidation in patients with regular and irregular treatment was significantly high compared with controls. Erythrocyte superoxide dismutase (SOD) and plasma vitamin E levels were significantly lower in both the groups of patients, while the levels were also found significantly lower in irregular treatment patients as compared with regular treatment patients. This study suggests that though epilepsy is lowering antioxidant status of patients, regular treatment with antiepileptic drugs is beneficial to restore antioxidant levels near normal.

Keywords- Epilepsy, antiepileptic drugs, lipid peroxidation, vitamin E, superoxide dismutase

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Introduction

Epilepsy or more correctly a seizure, is most easily defined in physiological terms. It is the occasional, sudden, excessive, rapid and local discharges in grey matter.

Generalized seizures involve diffuse regions of the brain simultaneously in a bilaterally symmetric fashion which may result from cellular, biochemical or structural abnormalities that have a widespread distribution. The inhibitory transmitter gamma amino butyric acid(GABA) is thought to be particularly important in a role of keeping the interconnected neurons of the cerebral cortex in a state of relative quiescence. It is likely that both reduction in inhibitory systems and excessive excitation of excitatory neurotransmitters (acetyl choline, glutamate, aspartate) play a part in the genesis of the seizure activity. [1]

Disrupted tissues undergo lipid peroxidation more quickly than healthy ones. The potential toxicity of reactive oxygen species is counteracted by a large number of cytoprotective enzymes and antioxidants which limit the damage caused by such species.[2]

Excessive activation of excitatory amino acid neurotransmitter receptors during seizures is known to generate reactive oxygen species (ROS),including accelerated production of neurotoxic guanidino compounds in the pattern of vicious cycle.[3] High levels of guanidino compounds accumulating near or within cells such as neurons(in an epileptogenic focus) may cause free radical damage. The convulsions are thought to be due to depressed functions of serotonergic neurons and accumulated free radicals. [4]

Fewer studies have reported on the effect of antiepileptic drugs on antioxidant system in epilepsy. Treatment with antiepileptic drugs may be able to exert pro-oxidant and/or antioxidant effect and increasing the antioxidant activity in patients with treatment, nearly up to the normal range. [5] Hence, this study was aimed to show that regular treatment with antiepileptics may reduce the oxidative stress and normalize the antioxidant status.

Patients and Materials

The study included 60(44 males and 16 females) epileptic patients age and sex matched with control group (30) aged between 20-50 years from B. J. Medical college and Sassoon hospitals, Pune.

The patients were diagnosed by clinical examination, Electroencephalograph, and CT brain. These patients were on antiepileptic drugs (phenytoin and carbamazepine). The treatment status whether on regular or irregular treatment was taken into consideration irrespective of classification of antiepileptic drugs and drug therapy. The patients were divided into two groups each with

30 patients as patients with regular treatment (Group A) and patients with irregular treatment.(Group B).

About 5 ml of blood sample was collected (from an antecubital vein of the patients and the controls.) in plain bulb with all aseptic precautions and serum was separated by centrifugation after one hour. This serum was used for measurement of malondialdehyde (MDA), an index of lipid per oxidation by method of Buege and Aust [6] using thiobarbuturic acid. This serum was also used for measurement of vitamin E, as an antioxidant by method of Baker and Frank.[7]

About 2 ml of blood was also collected from an antecubital vein in EDTA bulb and centrifuged for 10 minutes. Plasma was separated, buffy coat was removed and the separated erythrocytes were washed thrice with normal saline. The upper separated layer was removed each time and afterwards the erythrocytes at the bottom were lysed with equal volume of distilled water. This hemolysate was used for measurement of superoxide dismutase by Marklund and Marklund method. [8] In this method pyrogallol auto oxidation is inhibited by superoxide dismutase (SOD).

Data was analyzed statistically. For unpaired T test $p<0.01$ were significant while $p<0.001$ were highly significant.

Results

The patients of epilepsy with regular (Group A) and irregular (Group B) treatment were compared with normal controls as well as with each other.

Serum malondialdehyde (MDA) levels were found to be increased in both the groups compared to normal controls. This increase was highly significant in both the patients groups. While the levels were significantly high in Group B patients compared to Group A patients.

Vitamin E levels were also highly significantly low in both the patients groups compared with the control group. While the decrease was highly significant in group B compared to Group A.

Erythrocyte superoxide dismutase levels were low in both the patients groups. This decrease was highly significant. In Group B, the erythrocyte SOD levels were also highly significantly low compared with Group A.

Table I. *Comparison of serum Malondialdehyde (MDA), Vitamin E, Superoxide dismutase (SOD) levels between normal and patients groups.*

	Normal	**Group A**	**Group B**
Sample size(n)	30	30	30
Serum MDA(nm/ml)	2.58 ± 0.62	4.70+1.27**	6.10+1.39**
Serum Vit.E(mg%)	1..11+0.15	0.89+0.14**	0.74+0.12**
Erythrocyte SOD (Untits/gm of Hb)	650.42+61.93	449.30+37.46**	318.06+74.13**

**p‹0.01-significant *p‹0.001- highly significant*

Table 2. *Comparison of serum Malondialdehyde (MDA), Vitamin E, Superoxide dismutase(SOD) levels between Group A and Group B.*

	Group A	**Group B**
Sample size (n)	n=30	N=30
Serum MDA (nm/ml)	4.70+1.27	6.10+1.39*
Serum Vit.E (mg%)	0.89+0.14	0.74+0.12**
Erythrocyte SOD (Units /gm of Hb)	449.30+37.46	318.06+74.13**

**p‹0.01-significant *p‹0.001- highly significant*

Discussion

Epilepsy is known to accelerate oxidative stress, leading to neurotransmitter changes which in turn accelerate the production of reactive oxygen species. [3]

Oxidative stress is defined as oxygen radical mediated damage to biological material (proteins, lipids, carbohydrates and DNA) caused by either increased generation and build up of the oxygen radicals or due to diminished removal or inadequate protection against these oxygen radicals. Powerful antioxidant enzymes or radical scavengers are present to remove the oxygen radicals. [9]

In the present study, serum MDA levels were increased significantly in both the groups, suggesting the generation

of free radicals in epilepsy. This increase in serum MDA is also significant in Group B patients compared to Group A patients. This suggests that the regular treatment with antiepileptic drugs may have antioxidant effect, thereby decreasing the lipid per oxidation.

This result is also reported by Liu et al [10] as well as by Solowiej E et al [11] where the effect of antiepileptic drug therapy on antioxidant enzymes was studied. The study done by K. Sudha et al [5] also suggests the role of neuroleptic drugs as pro-oxidant and/or antioxidants.

Vitamin E also called as alpha tocopherol is a chain breaking antioxidant. When peroxyl and alkoxyl radicals are generated during lipid per oxidation they will combine with alpha tocopherol converting it into alpha tocoperoxyl radical and thereby inhibit further lipid per oxidation[2].Vitamin E is reported to delay the onset of seizures induced by intracerebral ferrous chloride injection as well as its addition to the drug treatment improves the electroencephalograph.[3][12]

In present study, vitamin E levels were significantly low in both the patients group compared to normal. While this decrease was highly significant in group B compared to group A, suggesting that antioxidant effect may be exerted by the antiepileptic drugs. Furthermore, it gives the scope of addition of vitamin E in the treatment modality along with antiepileptics.

Erythrocyte superoxide dismutase is a cytoprotective antioxidant enzyme. Various studies have reported the decrease in levels of SOD in epileptic patients. For instance, study by Nikushkin EV et al.[13] and Tupeev IR et al [14] reported 30% and 20-25% decrease in SOD levels in epileptic patients.

In present study, SOD activity was about 31% decreased in group A while it was 52% decreased in group B compared to controls. This difference suggests the role of antiepileptic drugs as antioxidants.
Recent studies also show the effectiveness of regular use of antiepileptic drug like benzodiazepines decrease the frequency of seizures by its acute administration.[15] while Bolayir E et al[16] agrees with antioxidant effect of antiepileptic drugs.

Janszky J et al also showed the efficacy of antiepileptic drugs in reducing the convulsion activity by its regular use [17].

Thus, from this study it is evident that epilepsy is linked to oxidative stress that results due to increased free radicals production and defective antioxidant defense. Treatment with antiepileptic drugs may exert the antioxidant effect and addition of antioxidants to the conventional drug therapy may enhance the anticonvulsive effect of the drugs.

References

1. Harrison; Diseases of the central nervous system; Principles of internal medicine; 14th edition; 1998; 2307-25
2. Roberta J.W. Timothy J. P.; Free radicals; Clinical biochemistry metabolic and clinical aspects, edited by Williams J. Marshall; 1995;765-77
3. Akitane Mori, Isao Yokoi, Yasuka Noda ;Natural antioxidants may prevent post traumatic epilepsy; A Proposal Based on Experimental animal studies; Acta. Med. Okayama; 2004;58(3);111-18
4. Hiramatsu M.; A Role for guanidino compounds in the brain; Molecular Cell Biochem;2003; 244(1-2); 57-62
5. K. Sudha , Ashalatha V Rao, Anjali Rao ;Oxidative stress and antioxidants in epilepsy; Clinica Chimica Acta;2001;303;19-24
6. Buege and Aust; Microsomal lipid per oxidation; Methods in enzymology; 1978; 105; 302-10
7. Baker H and Frank O; Determination of serum alpha tocopherol; In: Gowenlock AH, Mcmurry JR, Mchauchian DM; Varlay's practical clinical Biochemistry:6th edition:London:1968;902-
8. Marklund SL and Marklund G ;Involvement of Superoxide Anion Radical in the Auto-oxidation of Pyrogallol and a convenient assay for superoxide dismutase; European J.Biochem;1974;47;469-74
9. V Th Ramaekers ,B Bosman, G.A. Jansen; Increased plasma malondialdehyde associated with cerebellar structural defects; Arch Dis. Child; 1997;77;231-34
10. Liu CS, Wu HM, Kao SH; Serum trace elements, glutathione, copper/zinc ,superoxide dismutase and lipid per oxidation in epileptic patients with phenytoin or carbamazepine monotherapy; Clin Nuropharmacol; 1998,21(1); 62-4
11. Soloweij E, Sobaniec W, The effect of antiepileptic drug therapy on antioxidant enzymes activity and lipid per oxidation in young patients with epilepsy; Neruol Neurochir Pol; 2003;37(5); 991-03
12. Higashi A, Tamari H, Ikeda T, Serum vitamin E concentration in patients with severe multiple handicaps treated with anticonvulsants; Pediatr. Pharmacol (New York);1980;1(2);129-34
13. Nikushkin EV; Kryzhanovskii GN; Tupeev IR; Blood anti-oxidative enzymes during epileptic activity; Biull. Eksp Biol. Med;1987;103(3);297-9
14. Tupeev IR; Kryzhanovskii GN; Nikushkin EV; The antioxidant system in the dynamic combined treatment in epilepsy patients with traditional anticonvulsant preparation of antioxidant-alpha tocopherol; Biull. Eksp. Biol Med.;1993;116(10);362-4
15. Wolf P; Acute administration of Benzodiazepines as part of treatment strategies for Epilepsy; CNS Neurosci. Ther.2010 Apr 8. [Epub ahead of print]PMID: 20406251 [PubMed - as suppliedbypublisher

16. Bolayir E; Celik K; Tas A; The effects of oxacarbazepine on oxidative stress in epileptic patients; Methods find Exp Clin Phamacol; 2004,26(5);345
17. Janzky J.; Kovacs N; Gyimesi C Fogarasi A, Doczi T, Wiebe S. Epilepsy surgery, antiepileptic drug trials and the role of evidence; Epilepsia; 2010;Apr 2 [Epub aheadofprint]

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Acute Administration of Benzodiazepines as Part of Treatment Strategies for Epilepsy.
Wolf P.
CNS Neurosci Ther. 2010 Apr 8. [Epub ahead of print]PMID: 20406251 [PubMed - as supplied by publisher

Abstract

Ad-hoc administration of benzodiazepines (BZD) is well established in status epilepticus, but intermittent BZD use in the treatment of chronic epilepsy is little known beyond catamenial epilepsy. We aim to assess the use of acute drug administration (ADA) in the treatment of 24 patients with epilepsy (9 idiopathic generalized, 14 focal symptomatic/cryptogenic, 1 migraine-epilepsy) receiving ADA for (1) prevention of generalized tonic-clonic seizures (GTCS) after minor seizures, (2) prevention of seizures at perceived risk, (3) prevention of seizure clusters. Standard ADA was 10 mg oral clobazam (CLB); one patient received 10 mg rectal diazepam. Concomitant antiepileptic drugs (AED) remained unchanged whenever possible. Ten patients used ADA always correctly, 7 mostly, 7 sporadically or not. Outcome considering seizure control was positive in 44% of all patients (59% of those who actually used ADA): 5 patients seizure free, 1 free of disabling seizures, 4 with >50% reduction in seizure frequency. Eleven had minor or no improvement, 3 patients could not be rated. Thirteen (of 19 possible) patients attempted prevention of seizures or clusters, 10 with full or >50% success (52.6 resp. 76.9%). Prevention of clusters sometimes required higher or repetitive CLB dosing. Self rating of patients who did use ADA was positive or very positive in 88.2%. Retention rate was 66.7% of all patients, and 88.2% of those using ADA. The best results were obtained in idiopathic generalized epilepsy (IGE) patients with seizures habitually triggered by typical factors (sleep deprival, alcohol) but also some others were successful. The only adverse effect was gait ataxia in a multiple-handicapped patient. ADA is an elegant and often successful but underused treatment option for selected patient groups where it can make the difference between becoming seizure free or not. Depending on the individual case it can be applied as monotherapy or in combination with a basis AED. A controlled investigation should follow.

Adjunctive antiepileptic drugs in adult epilepsy: how the first add-on could be the last.
Cretin B, Hirsch E.
Expert Opin Pharmacother. 2010 May;11(7):1053-67.PMID: 20402552 [PubMed - in process]

Abstract

IMPORTANCE OF THE FIELD: In adult epilepsies, incomplete seizure control under monotherapy affects approximately 20-25% of patients with idiopathic generalized epilepsies (IGE) and approximately 20-40% of patients with epilepsies with focal seizures (FE). The choice of an adjunctive therapy is therefore a common event. AREAS COVERED IN THIS REVIEW: Efficacy studies of add-on anti-epileptic drugs for adult epilepsies--approved since the early 1990s until 2008--were reviewed. An exception was made for valproate. WHAT THE READER WILL GAIN: Efficacy studies give important clues for add-on drug choice but--beyond this--we encourage physicians to consider other parameters, especially co-morbidity(ies) and special situation(s). According to clinical and pharmacological data, an original, practical approach is proposed, by which decisions are based on three main criteria, which aim to optimize patients' seizure control and quality of life. The need for drugs that act not only on 'ictogenesis' but also on 'epileptogenesis' is also discussed briefly. TAKE HOME MESSAGE: Given the increasing disposal of anti-epileptic drugs, the choice of an add-on therapy appears to be partly based on subjective criteria (physician opinions and preferences). In fact, the selection criteria can be clarified as: treatment decisions rely not only on seizure type, efficacy and tolerability profiles but also on patient-related factors.

Epilepsy surgery, antiepileptic drug trials, and the role of evidence.
Janszky J, Kovacs N, Gyimesi C, Fogarasi A, Doczi T, Wiebe S.
Epilepsia. 2010 Apr 2. [Epub ahead of print]

Abstract

Summary Objective: We assessed whether recent randomized controlled trials (RCTs) of antiepileptic drugs (AEDs) are informed by evidence about surgical effectiveness. We explored whether RCTs of AEDs consider the patients' candidacy for surgery in their eligibility criteria, and whether the necessary investigations are requested in participating patients to determine their potential eligibility for surgery. Methods: We systematically analyzed RCTs published in the last 2 years investigating the efficacy of new AEDs in localization-related epilepsy. Results from a surgical RCT and recommendations from an epilepsy surgery practice parameter were used to assess the degree to which surgical evidence informed the drug study design. Results: Eleven RCTs were analyzed. All were conducted in countries with access to epilepsy surgery. None of the studies required magnetic resonance imaging (MRI) with an epilepsy protocol or explicit statement of the epilepsy syndrome, which could lead to the identification of surgical candidates. Having temporal lobe epilepsy or being a potential surgical candidate were not exclusion criteria in any of the trials. The primary efficacy end point was the reduction in seizure frequency or responder rate. Seizure freedom was never the primary outcome, and it was reported in only seven studies. The pooled data analysis of these trials revealed that 1.9% of patients became seizure-free on placebo and 4.4% on the study drug ($p < 0.01$). Conclusions: Important aspects of patient selection for new AED trials are not informed by the evidence about surgical effectiveness. Investigations that could lead to identification of patients for presurgical evaluation were not required in any of the studies.

Re paper: EFFECT OF ANTIEPILEPTIC DRUGS ON ANTIOXIDANT STATUS IN EPILEPSY Surekha T. Nemade and R. R .Melinkeri

I have made corrections directly onto manuscript – these are in red font. I suggest the authors revise accordingly and also add citations of the newest publications in the field of epilepsy and drugs. In fact I have copied from Medline 3 abstracts of such papers (abstracts I copied are at the bottom of the MS). I suggest authors incorporate related information from them or at least cite these papers in their own MS.

It is absolutely important that authors provide the names of drugs the patients were treated with. I understand the list of antiepileptic drugs can be long but this is vital information for the clinicians. The names of drugs can be introduced either in the Abstract or even better in the section **2. Material and methods** (more appropriate heading for this section would be the **"Patients and material")**

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