Critical Review

Trace Elements and Electrolytes Homeostasis and Their Relation to Antioxidant Enzyme Activity in Brain Hyperexcitability of Epileptic Patients

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Abstract. Epileptogenesis is a big challenge. Various experimental and human studies suggested that the homeostasis of trace elements, electrolytes, membrane lipid peroxidation, and antioxidants is crucial for brain function, and they were directly or indirectly implicated as taking part in the pathophysiology of neuronal excitability, neuronal excitotoxicity, and seizure recurrence and its resistance to treatment with antiepileptic drugs (AEDs). In addition, AEDs can also alter the homeostasis of trace elements, electrolytes, and seriously increase membrane lipid peroxidation at the expense of protective antioxidants, leading to an increase in seizure recurrence and an idiosyncratic drug effect. Differential effects were detected among different AEDs treatments in which carbamazepine (CBZ) was found to be better anticonvulsant for the control of free radical related seizures and the level of trace elements were better regulated with CBZ than with valproate (VPA) and phenytoin (PHT) therapies. It is concluded that adequate trace elements and antioxidants supply is important for brain functions and prevention of neurological diseases and further elucidation of the pathological actions of such substances in the brain should result in new therapeutic approaches. Trace elements and antioxidant might have neuroprotective biological targeted benefits when used in epileptic patients.

Keywords: trace element, lipid peroxidation, antioxidant, neuronal excitotoxicity, antiepileptic drug

Introduction

Trace elements (e.g., zinc, selenium, and copper) are minor building components in tissues including the nervous system. The very complex balance of trace elements is crucial for all areas of maintaining human health, preventing as well as overcoming health problems (1). The brain barrier system, that is, the blood-brain and blood cerebrospinal fluid (CSF) barriers, is important for trace element homeostasis in the brain (2). The concentration of trace elements in cerebral tissue is not equal in all parts of the brain (3).

Trace elements play important functional roles in peripheral and central nervous systems (4–9). Zinc,

selenium, and copper are indispensable components for certain enzymes responsible for various metabolic processes in different tissues including the brain (10, 11). They are important parts of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) as well as transport protein with antioxidant properties, ceruloplasmin (Crl) (a copper-binding protein), providing protection against peroxidative superoxide radicals damage. Through enzymatic or indirect action, they block destructive alteration of lipids, proteins, and nucleic acids by oxygen-derived free radicals, radiation, certain heavy metals, and other toxic substances (12). Vitamin E and GSH-Px have similar and complementary physiological roles in protecting cells from damage caused by endogenous peroxides (13). Furthermore, trace elements are also important for the development of the nervous system, myelination of nerve fibers (11, 14), and neuronal excitability (8).

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Many previous studies demonstrated that the abnormal metabolism of trace elements, electrolytes, and antioxidants might be involved in the pathophysiology of severe mental and neurological disorders including epilepsy (2, 15, 16).

The pathophysiological mechanisms yielding epileptogenic and neuronal excitotoxic effect of trace elements, membrane lipid peroxidation, and anti-oxidant system

Epilepsy is a big challenge for scientists and many workers have been exploring different aspects of this disease. The mechanisms of epileptogenesis are not well established. Research employing experimental models of epilepsy suggested that alteration of homeostasis of some trace elements in the brain may be involved in the susceptibility, development, and termination of seizures in animal models of genetically determined epilepsy (17). Theoretically, trace elements may play a role in the production of seizures and their control in humans. This concept has led to several studies. However, the relation between epilepsy and trace elements has never been convincingly documented in human studies because the data in the literature are conflicting and this association is poorly understood (18–20). Many studies suggested that the body electrolytes, the level of some trace elements, and the membrane lipid peroxidation due to increase in free radicals or decrease in activities of antioxidant defense mechanisms have been causally involved in some forms of epilepsies and seizure recurrence (21–23). Moreover, some antiepileptic drugs (AEDs) may alter trace element metabolism and free radical scavenging enzyme activities in humans and experimental animals (7, 10, 24–30).

Zinc

In the brain, Zinc (Zn^{2+}) is abundant, having, after iron, the highest concentrations among all transition metals. Most of this brain zinc (approximately 90%) is bound up in metal-protein complexes. Many neurons in addition contain a significant amount of reactive ionic zinc. Within the telencephalon, zinc-containing fiber systems form vast association networks that reciprocally interconnects isocortical, allocortical, and limbic structures. Large amounts of chelatable Zn^{2+} are concentrated also in the limbic region, notably in the hippocampus formation (31). Approximately 10% of the total Zn^{2+} in the brain, probably ionic Zn^{2+} , exist in the synaptic vesicles of what is known as zinc-containing neurons (a subclass of a glutaminergic neurons) and is released in a calcium and impulse-dependent manner (9). Because the hippocampal, amygdalar, and perirhinal regions are

prominent nodes in this glutaminergic network, it is presumed that vesicular Zn^{2+} is involved in modulation of neuronal excitability and in the synaptic plasticity of developmental and experiential learning (9). Zn^{2+} is a potent modulator of amino acid receptors (especially the NMDA receptor) and co-release of zinc along with glutamate would provide a mechanism for modulating postsynaptic excitability with little or no effect at physiological firing rates, but selectively depressing excitability (by NMDA-receptor depression) when firing rates reach dangerous, paroxysmal levels (9). The precise mechanism by which zinc interferes with NMDA function is unknown, but appears to act as a non-competitive antagonist whose major site of action is outside the channel pore in contrast to the action of divalent cations, notably magnesium (Mg^{2+}), which binds within the pore to block ion permeation (32). In addition, glutaminergic synapses may have differential sensitivities to zinc inhibition and might even be capable of modulating their sensitivity by altering NMDA subunit composition (33). It has been reported that vesicular zinc enriched regions, for example, the hippocampus, are responsive to dietary zinc deprivation, which also causes brain dysfunction such as learning impairment and olfactory dysfunction (2).

Pathophysiologic mechanisms yielding epileptogenic effect of zinc: Altered zinc metabolism may play a role in the development of epilepsy. Zinc homeostasis in the brain is closely related to neuronal activity. It has been reported that susceptibility to epileptic seizures, which may decrease vesicular zinc, is also enhanced by zinc deficiency (2, 34). Experimental studies revealed that the concentration of zinc was decreased in the piriform cortex and amygdaloid nuclei complex during convulsions. The decrease in activity functioning zinc in the brain may be associated with the increase in the susceptibility to seizures in El mouse (a genetically animal model of epilepsy) (34). In contrast, zinc ions were found to inhibit the activity of $\text{Na}^+\text{-K}^+$ ATPase enzyme, which is known to concentrate in the hippocampus (4, 35). It was claimed that this situation increased the neuronal excitability and led to seizure (10). In addition, Zn^{2+} can inhibit glutamic acid decarboxylase activity, thereby resulting in decreased levels of GABA or an increase in carbonic anhydrase activity (36). Several lines of evidence point to the possible role of zinc in the pathological changes occurring in the hippocampus in temporal lobe epilepsy, death of hippocampal neurons, and aberrant sprouting of hippocampal mossy fibers so that they form recurrent synapses onto the dentate gyrus granule cells. The distribution of zinc in the brain was altered under certain pathological conditions including epilepsy. It is

abundant in areas prone to seizures. Aberrantly sprouted mossy fibers release zinc and this contributes to the ultimate collapse of GABA-mediated inhibitory drive in the dentate gyrus leads to chronic excitability and the spread of seizure activity (37).

Moreover, studies examining the effect of zinc on neurotoxicity have shown that micromolar concentrations of zinc protect cortical neurons from the toxic effects of excessive glutamate exposure (38). However, several lines of evidence support the idea that upon excessive synaptic zinc release, its accumulation in postsynaptic neurons contributes to the selective neuronal loss caused by generation of toxic free radicals and then cause necrotic neuronal degeneration (39). Excitotoxic brain lesions such as epilepsy lead to increasing destruction of neurons hours after the insult. This deadly cascade of events involves detrimental actions by free radicals and the activation of proapoptotic transcription factors, which finally result in neuronal destruction. The Zn^{2+} neurotoxicity was not attenuated by antiapoptotic agents, inhibitors of protein synthesis, caspase or glutamate receptor, or nitric oxide synthase (40).

Selenium

Selenium (Se^{2+}) is known to reduce lipid peroxides. The best known biological activity of Se^{2+} is in the seleno-enzyme GSH-Px. GSH-Px (in the form of selenocystine) with catalase and SOD is part of the cellular antioxidant defense system against free radical peroxides (41, 42). Cellular Se-GPX is ubiquitous in prokaryotes as well as eukaryotes (41).

Pathophysiologic mechanisms yielding epileptogenic effect of selenium: Selenium depletion in the brain amongst patients with epilepsy may constitute an important triggering factor for the origin of intractable seizures and subsequent neuronal damage (43). Savaskan et al. (16) provided evidence that Se^{2+} deficiency in vivo results in a massive increase in susceptibility to kainate-induced seizures and cell loss. Primates and other animals made Se^{2+} -deficient by dietary restriction develop alopecia, weight loss, epilepsy, and degeneration of the liver, pancreas, and kidney. Patients with systemic Se^{2+} deficiency develop liver disease, depigmented hair, osteoarthropathy, neuroimpairments including progressive neuronal degeneration, severe mental retardation, and intractable epilepsy (44, 45).

Many studies provided evidences for the role of Se^{2+} in neuronal susceptibility to excitotoxic lesions. In neuronal cell culture, addition of Se^{2+} in the form of selenite within a physiological range protects against excitotoxic insults and even attenuates primary damage. The neuroprotective effect is not mediated via a direct

antioxidant effect of selenite but requires de novo protein synthesis. Gel shift analysis demonstrates that the effect is connected to the inhibition of glutamate-induced NF-Kappa B and AP-I activation (16).

Copper

Copper (Cu^{2+}) is involved in number of enzymes with catalase and oxidase-type reactions. Some studies reported relationship between the serum levels of Cu^{2+} and Zn^{2+} and CuZn-SOD activity and the serum concentration of Se^{2+} and GSH-Px activity in the group of healthy subjects (46). Crl (a copper-binding protein) appears to have two antioxidant properties: firstly, it binds Cu^{2+} and therefore prevents this transition metal from catalyzing hydroperoxide decomposition to radicals. Secondly, Crl oxidizes ferrous iron to ferric and concomitantly converts O_2 to H_2O , thereby inhibiting iron-dependent lipid peroxidation (47). A moderate linear correlation was estimated between serum values of Cu^{2+} and Crl (46).

Pathophysiologic mechanisms yielding epileptogenic effect of copper: Tsaryuk et al. (48) demonstrated that the copper-rutin complex completely eliminated epileptiform potentials induced by a combination of chlorpromazine and microwave radiation, 1 – 2-min post-injection and suppressed convulsive activity provoked by application of penicillin to the sensorimotor cortex. On the contrary, Cu^{2+} and Zn^{2+} are known to produce seizures in animals at low dosages, which is possibly attributable to the inhibition of Na^+ - K^+ ATPase activity (10).

Relation of lipid peroxidation and antioxidant system to neuronal excitability and excitotoxicity

Many studies suggest that membrane lipid peroxidation may be causally involved in some forms of epilepsies, and the decrease in free radical scavenging enzyme activity is believed to cause the increased risk of seizure recurrence (49 – 51) and idiosyncratic drug reaction encountered in the management of epilepsy (52 – 54). It has been suggested that, an increase in free radicals may cause neuronal degeneration through membrane lipid peroxidation and a decrease in GSH-Px level (21, 55). GSH-Px deficiency is suggested to be a cause of childhood seizures (56). In an experimental study, any increase in lipid peroxide levels in the epileptic focus was found to be prevented by antioxidant treatment in epileptic rats, thereby resulting in a decreased number of epileptic discharges (57, 58). GSH-Px deficiency has been known as a cause of chronic granulomatous disease (59) and idiosyncratic drug reactions (60), particularly acute pancreatitis and increased lipid peroxides after therapy with valproic acid.

Weber et al. (56), described 4 children with intractable seizures, repeated infections, and intolerance to anticonvulsants with evidence of glutathione peroxidase deficiency. Two had low intracellular enzyme activity but normal blood selenium and high plasma GSH-Px concentrations. The other two had low intracellular GSH-Px activity with low circulating GSH-Px activity and selenium concentrations. The clinical state of the children improved after discontinuation of anticonvulsant medication and selenium substitution. The first two children had primary deficiency of the intracellular enzymes, whereas the other two may have a disturbance of selenium resorption or transport.

Effect of AEDs on trace elements, membrane lipid peroxidation, and antioxidant system

Zinc

Many studies attributed altered homeostasis of trace elements in epileptic patients to epilepsy per se and/or the effect of anticonvulsant drugs therapy or due to other reasons (10, 27, 60–66). However, conflicting results were reported by different studies. Zinc levels were reported to be normal or decreased in untreated and treated epileptics (10, 19, 20, 24–26, 50, 54, 66–70). Kuzuya et al. (19) reported lower levels of zinc in patients who were treated with mono-AEDs (carbamazepine (CBZ), phenobarbital (PB), phenytoin (PHT), and valproate (VPA)) when compared to healthy volunteers. No dose-related changes, but the difference from controls remained within the normal range, indicating that there is no need to adjust serum concentrations in order to treat diseases related to them. Yuen et al. (71) reported normal levels of zinc in white blood cells of epileptic patients on VPA or CBZ. They suggested that AEDs might affect the intracellular zinc level concentrations. Steidl et al. (72) reported significant lowering of serum Zn^{2+} in patients treated with AEDs for ≥ 5 years. They suggested that various AEDs and their combination have different effects on the degree of lowering of Zn^{2+} . Lerman-Sagie et al. (18) in their study of evaluating the effect of VPA on Zn^{2+} metabolism in 15 children with absence seizures found that the erythrocyte Zn^{2+} content was significantly lower than that found in controls. However, the plasma and urine values of Zn^{2+} and Cu^{2+} were within the normal limits. Smith et al. (73) reported significant alteration of serum Zn^{2+} with CBZ monotherapy in epileptics.

Hair analysis has been used to evaluate the trace element status in the body (74–76). Hair levels of Zn^{2+} were found to be decreased in patients on AEDs compared to those in controls (77, 78, 80). In the prospective study done by Altunbasak et al. (78), the serum

and hair levels of Zn^{2+} were found to be higher in untreated epileptic patients than those treated with VPA and controls and returned to normal level after VPA treatment. The authors concluded that there is no Zn^{2+} deficiency and replacement with Zn^{2+} therapy may be considered unnecessary. The authors believed that hair is a reliable specimen for retrospective search of trace elements status of the body. Unlike blood, serum, and urine, the hair, in addition to being less traumatic to the patient, provides historical information on concentrations of trace elements in the body as well as nutritional condition over a long period of time (74, 79, 80). Furthermore, trace elements are more concentrated in the hair than in the body fluids. Hair analysis provides information about intracellular accumulation of trace elements. Lastly, serum levels of trace elements in epileptic patients are unstable and may show variability during the day (81).

The real mechanism (s) of the possible effect of AEDs on Zn^{2+} concentrations is not completely understood, although some authors (61) have suggested that AEDs could decrease Zn^{2+} (with a concomitant Cu^{2+} increase) concentrations in epileptic patients, modifying the neurotransmitter regulation. VPA can bind zinc, thus protecting glutamic acid decarboxylase from the inhibitory effect of zinc, which results in an increased level of GABA (82).

In contrast, some studies reported slightly increased Zn^{2+} levels in epileptic patients (29, 54, 83). In the studies of Kürekçi et al. (50) and Hamed et al. (83), the higher levels of zinc in treated groups of patients remained within the normal range. In the study of Kürekçi et al. (50), although weak, the correlation between the serum VPA level and plasma Zn^{2+} was satisfactory significant ($P < 0.05$, $r = 0.554$), which might be explained mainly by the normal physiological variation in serum zinc concentrations (circadian variation) and is unlikely related to the anticonvulsant drugs or epilepsy. It was known that plasma Zn^{2+} level is higher than the mean between 10:00–20:00 (68, 84). In the study of Kürekçi et al. (50) and Hamed et al. (83), the Zn^{2+} levels were measured after an overnight fast before the next morning VPA dose was taken. Other studies attributed the increased serum Zn^{2+} levels to measuring such levels at least 2 h later than VPA intake, that is, at the peak of VPA concentration (85).

In general, as previously discussed, the conflicting results of different studies of different trace elements can be mainly explained by the differences in the Materials and Methods, most studies have not been prospective, the patients receiving different AEDs were classified in the same group, and the information about the patients included was not satisfactory in most of the

studies. In addition, the duration of drug treatment and the prescribed dose are important variables. Age of the patients whether children or adults is not a strong variable as it is believed that the plasma levels of these trace elements (Cu^{2+} , Zn^{2+} , Se^{2+} , Mg^{2+}) and antioxidant enzymes (GSH-Px and SOD) do not change after the age of 3 years (86). Further prospective and experimental studies are necessary for confirmation (7, 23, 87).

Selenium

Selenium levels were reported to be normal, decreased, or increased among epileptics. Pippenger et al. (60, 65), Kuzuya et al. (19), Kürekçi et al. (50), Liu et al. (70), and Verrotti et al. (20) found normal values of Se^{2+} in epileptic patients (treated and untreated). Hurd et al. (88) reported reduced plasma Se^{2+} level in patients and animals treated with VPA. Hepatic levels of Se^{2+} were also reduced in rats (54). It has been believed that deficiency of trace elements (e.g., Se^{2+} and Cu^{2+}) is associated with the increased risk and idiosyncratic drug reaction encountered in the management of epilepsy. VPA has also been associated with pancreatitis, renal tubular abnormalities, and hepatotoxicity (89, 90). VPA may cause hepatotoxicity like that in Reye's syndrome (RS) (52, 53), which occur primarily in prepubertal children. Children who died of RS and mice treated with 4-pentenoic acid (a fatty acid that causes RS-like syndrome) had altered selenium and copper levels in plasma and liver (91, 92). In contrast, Hamed et al. (83) reported significantly increased levels of serum Se^{2+} in a group of patients treated with VPA in comparison to healthy controls and a CBZ-treated group of patients ($P < 0.01$). The authors in this study attributed the higher levels of Se^{2+} in the VPA-treated group of patients to the associated significant increase in GSH-Px levels that were also reported in the same group.

Copper

The plasma Cu^{2+} levels in epileptic patients contain conflicting information (10, 25, 54, 67–69). Smith and Bone (7), Pippenger et al. (60, 65), Kürekçi et al. (50), Sozuer et al. (66), Liu et al. (70, 93), Shah et al. (94), and Verrotti et al. (20) reported normal serum Cu^{2+} levels in different groups of epileptics included in their study (untreated and treated). In contrast, Hamed et al. (83) reported significant higher levels of mean serum copper in untreated epileptics in comparison to controls ($P < 0.05$) that may be attributed to the inverse relationship between Zn^{2+} and Cu^{2+} concentrations (61). Fichsel et al. (95) found low levels of plasma copper and ceruloplasmin in children taking VPA. Tange et al. (77) reported low hair level of Cu^{2+} in epileptic patients on AEDs compared to that in the normal control group. The

authors suggested that AEDs had more effect on hair Zn^{2+} than on Cu^{2+} and the increase in the hair Zn^{2+} and Cu^{2+} , and the $\text{Zn}^{2+}:\text{Cu}^{2+}$ ratio seems to show the efficiency of the drug. Kaji et al. (29) reported low serum Cu^{2+} levels, relative to normal controls, in epileptic patients treated with VPA monotherapy or VPA in addition to other AEDs, while patients treated with other AEDs alone had unaltered Cu^{2+} levels. Hamed et al. (83) reported significantly lower levels of mean serum Cu^{2+} in treated epileptics (particularly those on CBZ monotherapy) in comparison to controls and the untreated group of patients ($P < 0.05$ and $P < 0.001$, respectively). None of the patients reported side effects attributed to Cu^{2+} deficiency.

It has been found that side effects of valproate therapy, including gastrointestinal tract (GIT) disturbance, alopecia, hyperammonemia, stupor lethargy, tremor, and anorexia (89, 96, 97), may also follow trace metal deficiencies, particularly deficiencies in Zn^{2+} , Cu^{2+} , and Se^{2+} (12, 79, 98). Alopecia, achromotrichia, and changes in hair consistency are seen with both VPA treatment and Cu^{2+} deficiency (62, 89, 99, 100). Both Zn^{2+} deficiency and high-dose VPA (Package insert) produce decreased growth, decreased food consumption, and testicular and atrophy of the thymus gland in animals (101).

In contrast, increased plasma levels of Cu^{2+} was reported by some studies in children taking PHT therapy (67, 95, 102). Kuzuya et al. (19) reported significant increased levels of Cu^{2+} in patients who were treated with mono-AEDs (CBZ, PB, PHT, and VPA) when compared to healthy volunteers. No dose-related changes, and the difference from the controls remained within the normal range. Sozuer et al. (66) reported that combination therapy and CBZ increased copper level but not with VPA. Motta et al. (103) reported elevated levels of serum copper and Crl in adult patients on chronic AED treatment. They suggested that the AEDs may influence the serum Cu^{2+} and Crl concentrations by hepatic enzymes induction. The clinical picture of epilepsy and treatment duration does not influence serum Cu^{2+} and Crl concentrations.

Lipid peroxidation and antioxidant enzymes: relation to AEDs

Modification in GSH-Px and/or Cu/Zn-SOD (which are important antioxidants for detoxification of xenobiotics) may be involved in the oxidative injury of AEDs; in fact, inadequate supply of down stream scavengers of hydrogen peroxide might facilitate the transformation of hydrogen peroxide to hydroxyl radicals (51, 104). Maertens and associates (51) reported that long-term use of AEDs may result in an increased

production of free radicals and elevated oxidative damage in neuronal cells. Many conventional AEDs are metabolized to generate reactive metabolites with capability of covalent binding to macromolecules. Therefore, the AEDs may not only suppress the epileptic spikes but also elicits systemic toxicity through covalent binding of their metabolic intermediates to proteins or other vital biomolecules (105).

Although decreased or normal levels of GSH and increased levels of lipid peroxidation were usually noted in many studies, the results have been rather varied. Maertens et al. (51), Liu et al. (93), Niketic et al. (22), Yüksel et al. (23), Sudha et al. (106), Martinez-Ballesteros et al. (107), and Hamed et al. (83) reported significant increased levels of malondialdehyde (MDA) (an index of extracellular lipid peroxidation) in untreated and treated patients in comparison to controls. Also significant increased levels in treated groups (CBZ, VPA, and polytherapy) with higher levels among the VPA-treated group. The higher levels of MDA observed among the VPA-treated group may be due to VPA metabolism generating an increased body burden of free radicals, which is responsible for serious side effects (23, 108). Pippenger et al. (60, 65) reported that GSH-Px and SOD activities in erythrocytes of children with epilepsy receiving VPA were significantly reduced. Graf et al. (108) have demonstrated that GSH-Px can be depressed in VPA-treated patients with clinically defined toxicity of the drug. On the contrary, those patients with good clinical tolerance of VPA showed normal GSH-Px activity. They considered that GSH-Px deficiency is a mediator of risk for toxicity in diverse applications of VPA. In the prospective study done by Yüksel et al. (23), in patients receiving VPA, the level of GSH-Px and SOD was markedly lowered after treatment and normal before treatment, while the level of lipid peroxidation was mildly increased before treatment and markedly increased after treatment. No significant differences of these parameters were found in epileptic children on CBZ therapy compared with the control group and the results before treatment, except that lipid peroxidation level was slightly higher in epileptic patients before treatment.

It has been known that the metabolism of VPA generates an increased body burden of free radicals (109, 110), and failure to remove this highly reactive species before they can inflict damage on various cellular constituents may be expressed by an increased susceptibility to the drug. The potential teratogenicity of changes in the serum trace elements and the fetal abnormalities due to VPA are similar, and the side effects encountered with chronic VPA use are also evident in disturbance of trace metal homeostasis. These

deficits may be inherent or secondary to the nutritional or systemic condition (108). During VPA therapy, a decrease in GSH-Px activity has been reported due to a breakdown in the antioxidant system as well as secondary to total body depletion of Se^{2+} , Cu^{2+} , and Zn^{2+} (cofactors for GSH-Px activity). VPA enhances the clearance of Se^{2+} , Cu^{2+} , and Zn^{2+} , thereby decreasing the synthesis of free radical scavenging enzymes (50). Indeed, young children have been shown to be at a higher risk to develop undesired effects upon treatment with VPA. Graf et al. (108) reported that while the level of GSH-Px in children showing good clinical tolerance to VPA treatment was close to normal, the level in children with serious side effects was decreased. They have demonstrated that GSH-Px can be depressed in VPA-treated patients with clinically defined toxicity of the drug: in particular these patients showed protracted emesis and behavioral changes and/or VPA-associated thrombocytopenia, pancreatitis, and elevated aspartate transaminase (AST). On the contrary, those patients with good clinical tolerance of VPA showed normal GSH-Px activity. The daily doses should be taken into account because high doses of VPA could modify GSH-Px; in fact, Cotariu et al. (111) reported that GSH levels in rat liver was not affected by non-toxic doses of VPA but were decreased by toxic doses. VPA and its unsaturated metabolite 4-en-VPA generated by the microsomal cytochrome P450-dependent mixed function oxidase system (109, 112) undergo further metabolic activation to electrophilic intermediates that bind covalently to liver macromolecules, resulting in hepatotoxicity (110, 113). This type of toxicity already reported for a number of xenobiotics may also involve reduced GSH depletion (114). Yüksel et al. (23) believed that VPA affects the antioxidant system, but did not agree with the comment that a decreased level of GSH-Px is an indicator of the risk of toxicity in some clinical applications. Hamed et al. (83) reported decreased level of GSH-Px in an untreated group of epileptics and increase in their levels in treated patients but they did not reach the level of significance in comparison to controls. However, patients treated with VPA and combined therapy showed significantly increased levels of GSH-Px ($P < 0.001$ and $P < 0.01$, respectively) in comparison to controls and CBZ group. In contrast, Kürekçi et al. (50), reported significantly higher plasma GSH-Px levels in the VPA group than in controls (in well-controlled group under treatment). How VPA increases the level of GSH-Px in plasma is unknown, but it could be attributed to induction of hepatic synthesis of GSH-Px and transport to blood (50).

Liu et al. (93) reported significantly increased levels of MDA and serum CuZn-SOD levels but the

glutathione level was significantly decreased in all epileptic patients with PHT monotherapy compared with those of the controls. However, there was no significant difference of these parameters in the CBZ-treated patients except for a mild elevation of the activity of serum CuZn-SOD. They concluded that CBZ compared to PHT produce less disturbance in trace element metabolism, antioxidants, and lipid peroxidation in the serum of epileptic patients. The results of Liu et al. (70, 93) supported the notion that teratogenicity of PHT may be associated with or caused by disturbance of free radical scavenger systems, which are critical for efficient disposal of the reactive PHT metabolites (50, 51, 70, 105). PHT is metabolized by arene oxide, an electrophilic reactive intermediate, which is highly associated with increased lipid peroxidation and teratogenicity (115). In contrast, the metabolic intermediates of CBZ (e.g., the 10,11-epoxide and trans-diphenyldiol derivative) were found to be stable, and the teratogenicity has rarely been reported in patients with CBZ monotherapy (116). An exacerbation of seizure through the release of neurotransmitters was observed in acute intoxication of patients with PHT (50, 117).

Furthermore, studies carried out on patients receiving CBZ, the antioxidant enzyme activities and lipid peroxidation levels were usually similar to the levels of epileptic patients receiving no AEDs and the control group (50, 93) except the study of Liu et al. (93) who found mild elevation of serum SOD activity and Niketic et al. (22) who found that decrease SOD and GSH-Px. Yüksel et al. (23) reported that in epileptic patients that received CBZ, the level of GSH-Px was found to be normal while the level of serum lipid peroxidation was found to be increased in patients receiving CBZ. They concluded that antioxidant systems in epileptic patients on CBZ therapy are better regulated in comparison with epileptic children on VPA therapy. CBZ may be a better anticonvulsant for the control of free radical-related seizures (post-traumatic epilepsy) and for clinical management and treatment of female epileptic patients during pregnancy (93).

Fichsel et al. (95) and Hurd et al. (54) reported low levels of CrI in patients taking VPA, in contrast to the increased level among those receiving PHT (67, 95). Tutor-Crespo et al. (118) reported increased CrI and attributed this to a drug-induced cholestasis. Hamed et al. (83) reported significant decrease in the serum CrI in patients treated with CBZ and VPA in comparison to controls and untreated group.

Hyperuricemia was reported in all groups of epileptics (untreated and treated) ($P < 0.001$) (83). Hyperuricaemia observed in VPA therapy may be related to alteration of the renal excretion of the drug (119) or cellular

protective mechanism against peroxidative damage. Uric acid is known to be an effective antioxidant that contributes to the effective mechanisms against oxygen radicals. The increased uric acid may be a compensatory mechanism trying to counteract oxidative stress encountered in epilepsy (120).

Effect of epilepsy and antiepileptic drugs on body electrolytes

Many previous studies suggested that routine laboratory estimation of serum sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}), and calcium (Ca^{2+}) are essential for the rational understanding and management of epileptic patients. Several studies suggested that the body electrolytes play a vital role for seizure conditions to prevail (30, 121).

Magnesium

Serum levels of Mg^{2+} were found to be lowered in epileptic patients (94). Papierkowski et al. (122) reported lowered serum Mg^{2+} concentrations in children with febrile convulsions. A variety of pathological conditions are associated with low serum Mg^{2+} levels in humans. Hypocalcemia and hypokalemia frequently accompany magnesium deficiency in humans (121, 123). Mg^{2+} is an essential element with a role in neuronal excitability. It inhibits the facilitating effect of calcium (Ca^{2+}) on synaptic transmission, Mg^{2+} alters Ca^{2+} mobilization and may stabilize excitable membranes and also exerts a voltage-dependent blockage of the NMDA-receptor channel (124). It has been shown previously that low Mg^{2+} level-induced epileptiform activity in rat entorhinal cortex slices changes with time from a pattern of serial seizure-like events (SLEs) to a state of continuously recurring epileptiform activity. Low magnesium levels are observed in patients given anticonvulsant drugs designed for the clinical problem of pharmacoresistant epilepsy (76). In addition, efficacy of magnesium sulfate in the prevention and control of eclamptic convulsions has been validated in randomized controlled trials performed worldwide (125).

Steidl et al. (72) stated that various AEDs result in deficiency of red blood cells (RBCs) Mg^{2+} and serum Zn^{2+} , and therapy of magnesium lactate produces significantly higher RBC Mg^{2+} and serum Zn^{2+} with improved clinical EEG and biochemical findings (72). Iihan et al. (30) reported reduced serum level of Mg^{2+} in epileptic patients whether treated or not and also regardless to the type of AEDs utilized. Shah et al. (94) reported a significant decrease in serum Mg^{2+} levels in all seizure groups in comparison to controls, and they attributed this decrease to be responsible for hyperexcitability

encountered for uncontrolled seizures on AEDs. However, many studies reported unchanged levels of Mg^{2+} in patients receiving AEDs (7, 54, 72). In contrast, Hamed et al. (83) reported normal levels of serum Mg^{2+} among all groups of epileptics (treated or untreated) and irrespective to the degree of control on AEDs.

Calcium

It is well documented that alterations in the levels of serum Ca^{2+} are responsible for initiation of convulsions (126–128). Neonatal hypocalcaemia and hypomagnesaemia have also been reported to be the cause of convulsions (129, 130). Serum Ca^{2+} levels were reported to be unaltered in generalized and unspecified seizures (94). Rutter et al. (131) reported normal serum levels in children with febrile convulsion. Hamed et al. (83) reported no difference in the levels of Ca^{2+} among the untreated group of patients and the normal healthy group, but reported a significant increase in the levels of serum Ca^{2+} in the CBZ- and VPA-treated groups of patients in comparison to controls ($P<0.05$) and untreated group of patients ($P<0.001$). The authors concluded that the increased serum Ca^{2+} levels among the treated group of patients could be a marker for better seizure control by AEDs. Shah et al. (94) reported a rise in Ca^{2+}/Mg^{2+} ratios compared to control mean value, with unaltered Ca^{2+} serum levels and significantly lowering in Mg^{2+} levels, and they concluded that the Ca^{2+}/Mg^{2+} ratio is a new concept and may help to judge enhancement of neuronal excitability. The elevated Ca^{2+}/Mg^{2+} ratio was found to be closely associated with initiation and continuation of seizures. Leaver et al. (132) suggested that decline in both Ca^{2+} and Mg^{2+} concentrations results in frequent seizures.

Potassium

The deficiency in K^+ , is generally correlated to hypomagnesaemia and hypocalcaemia and other disturbance electrolyte homeostasis. Natelson et al. (133) and Shah et al. (93) reported reduced K^+ levels in untreated group of epileptics but normal levels in the treated group. The authors concluded that hypokalemia that is observed in untreated epileptics may be expressed as an increase in the ratio of intracellular to extracellular K^+ concentrations, which may result into serious neurological symptoms. Hamed et al. (83) reported significant reduction in K^+ levels in untreated epileptics (interictal) in comparison to controls ($P<0.01$) and treated patients ($P<0.001$). While no difference was observed in serum K^+ levels in treated group (CBZ, VPA, and polytherapy) in comparison to controls and to each other.

Sodium

In general, no abnormality was noticed in serum Na^+ in epileptic patients during and after seizure activity (94, 133). The observed hyponatraemia leading to grand mal seizures in some cases of epileptic children treated with CBZ was associated with water intoxication, which is a rare side effect of CBZ (134). Shah et al. (94) reported a rise in Na^+/K^+ ratio as compared to control mean value, with unaltered Na^+ level and hypokalemia in patients was suggested to increase the recurrence of seizures (94). Natelson et al. (133) reported unaffected Na^+ and low K^+ levels during seizures. Biochonski (135) reported hypernatremia and hypokalemia in epileptic children. White et al. (136) reported elevation of K^+ levels during periods of intense seizure activity.

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