PROTECTIVE ROLE OF CARNITINE AGAINST THE TOXIC EFFECTS OF VALPROIC ACID ON THE SKELETAL MUSCLES IN ALBINO RATS

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ABSTRACT

Objective: To determine the protective role of carnitine against the Valproic Acid (VPA) induced toxicity in the skeletal muscles of Albino Rats.

Material and Methods: It was an experimental study of concurrent parallel design in albino rats, carried out in the Department of Pathology Khyber Medical College, Peshawar from January 2012 to December 2013. Thirty adults and apparently healthy albino rats were selected. The animals were divided into 5 equal groups i.e. group A (Control-kept on the diet of animal house only), group B treated with therapeutic regimen of VPA), group C (treated with therapeutic regimen of VPA + supplement of Carnitine), group D treated with toxic regimen of VPA) & group E (treated with toxic regimen of VPA + supplement of Carnitine). Two sections of each biopsy were taken, thus total 60 sections were taken to see the histological findings. All the animals were fed on the routine diet of animal house and then sacrificed after 03 weeks of treatment. The calf muscle of each animal was properly fixed, sectioned, processed, stained with H & E and then seen under the light microscope.

Results: No significant changes were seen in group A & C. Foci of hyalinization were seen in 2 (16.66%) sections of the muscles of group B & E, and in 4(33.33%) of group D.

Conclusion: The results of the present study shows that carnitine plays a significant protective role against the VPA induced myopathies.

Key Words: Volproic acid;carnitine; Skeletal muscles.

INTRODUCTION

Carnitine (3-hydroxy-4-trimethylamino-butyric acid or β-hydroxy-gamma-N-trimethylamino-butyrate) an amino acid derivative is an important nutrient; 75% comes from the diet, particularly in red meat and dairy products. It is also biosynthesized endogenously from dietary amino acids (lysine .methionin). especially in the liver and in the kidneys[1]. Most body carnitine is stored in skeletal muscles, but it is also stored in other tissues (myocardium, liver, suprarenal glands[2].

The two main metabolic functions of carnitine are to facilitate fatty acyl group transport into mitochondria and to maintain the ratio of acyl-CoA to free CoA in the mitochondria[3]. Carnitine facilitates transport of long-chain fatty acids from the cytosol compartment of the muscle fibre into the mitochondria, where they undergo β-oxidation and produce acetyl-CoA[4].

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Carnitine facilitates prevention of intramitochondrial accumulation of acyl-CoA by transforming acyl-CoA into acylcarnitine. In this way, carnitine protects the cell from the membrane-destabilizing effects of toxic acyl groups. Carnitine thus plays a central role in the metabolism of fatty acids and energy by regulating the mitochondrial ratio of free CoA to acyl-CoA[5].

Primary carnitine deficiency is rare wheres secondary carnitine deficiency is associated with several inborn errors of metabolism and acquired medical conditions[6].

Several drugs, including VPA are associated with decreased carnitine levels and occasionally with true carnitine deficiency[7] by depleting carnitine stores, especially with high-dose or long-term therapy[8] through several mechanisms, particularly the reduction in tubular reabsorption of both free carnitine and acylcarnitine during VPA treatment[9,10]. In addition there is the mitochondrial depletion of CoA-SH which impairs β-oxidation of fatty acids (and VPA) and ATP production[11]. A few studies have shown carnitine supplementation in patients receiving VPA to result in subjective and objective improvements and to prevent valproiac acid induced hepatotoxicity[12]. Carnitine supplementation may be

useful in patients treated with VPA for seizure disorders[13]. Carnitine deficiency may cause muscle symptoms in patients on maintenance hemodialysis. L-carnitine treatment may result in some improvement in muscular symptoms among two-thirds of such patients. Carnitine fractions were normal or slightly above normal, and lipid profiles were seemingly unaffected. A correlation between carnitine deficiency and months on dialysis was indicated. Long-term L-carnitine treatment may alleviate muscle symptoms during dialysis by replenishing the muscle tissue with carnitine and washing out acyl moieties[14].

Valproic acid (VPA), an acidic chemical compound, has found clinical use as an anticonvulsant and mood-stabilizing drug, primarily in the treatment of epilepsy, bipolar disorder, and, less commonly, major depression. Valproic acid (VPA). VPA- a branched chain carboxylic acid, with a very similar structure to that of short chain fatty acids[15]. Mitochondrial β-oxidation of VPA involves its transport within the mitochondrial matrix, using the same pathway as do long-chain fatty acids. VPA has fewer common side effects than do other AEDs, however rare serious complications may occur in some patients receiving VPA chronically. According to some data the hyperammonemia and hepatotoxicity may be promoted either by a pre-existing carnitine deficiency or by deficiency induced by VPA per se. However, serious toxicity and even deaths have been reported[16]. During long-term or high-dose VPA therapy, or after acute VPA overdose, ω-oxidation occurs, potentially increasing the risk for toxicity. Less than 3% of VPA is excreted unchanged in the urine[17], much of which is in the form of valproylcarnitine[18]

MATERIAL AND METHODS

This study was carried out in the Department of Pathology Khyber Medical College, Peshawar from January 2012 to December 2013. We selected 30 adults and apparently healthy albino rats of Sprague – Dwaley strain. The animals were divided into 5 equal groups (A, B, C, D & E each group comprising six animals. The animals of group A were kept just on the routine diet of animal house. No VPA/& carnitine were given. The animals of group B were treated with therapeutic regimen of VPA i.e 35 mg/Kg/day), increased by 5-10 mg /Kg/ week. group C treated with therapeutic regimen of VPA +supplement of Carnitine in adose of 60 mg/kg / day), group D treated with toxic regimen i.e. 85 mg/ kg/day, increased by 10-20 mg/Kg/ week) & group E treated with toxic regimen of VPA +supplement of Carnitine in a dose of 100mg/ Kg/day. Two sections of each biopsy were taken. All the animals were fed on the routine diet of animal house and then sacrificed after 03 weeks of treatment. The calf muscle of each animal was properly fixed, sectioned, processed, stained with H & E and then seen under the light microscope.

Drugs & Nutritions: (1) Valproic Acid (VPA) is available as oral immediate-release, enteric-coated and delayed-release preparations, and as an intravenous formulation. Therapeutic daily doses range from 1 to 2 g in adults, and from 15 to 60 mg/kg in children[18]. The acid, salt, or a mixture of the two (valproate semisodium) are marketed under the various brand names Depakote, Depakote ER, Depakene, Depakene Crono (extended release in Spain), Depacon, Depakine, Valparin and Stavzor. Mitochondrial β-oxidation of VPA involves its transport within the mitochondrial matrix, using the same pathway as do long-chain fatty acids. (2) Carnitine. L-Carnitine is available in some countries as an oral preparation (1 g/10 ml solution, 330 mg tablets) or as an injectable drug (intramuscular or intravenous, 1 g/5 ml solution; e.g. Levocarnil®[Sigma-Tau, Ivry-sur-Seine, France] and Carnitor® [Sigma-Tau, Gaithersburg, MD, USA]). L-Carnitine is available in some countries as an oral preparation (1 g/10 ml solution, 330 mg tablets) or as an injectable drug (intramuscular or intravenous, 1 g/5 ml solution; e.g. Levocarnil®[Sigma-Tau, Ivry-sur-Seine, France] and Carnitor® [Sigma-Tau, Gaithersburg, MD, USA]). It has been administered in senile dementia, metabolic nerve diseases, HIV infection, tuberculosis, myopathies, cardiomyopathies, renal failure and anaemia, and has been included in baby foods and milk[19].

RESULTS

The histopathological changes were seen as below, No significant changes were seen in group A (control) and C (when therapeutic dose of VPA was supplemented with carnitine). Foci of hyalinization were seen in 2 out of 12(16.66%) sections of the muscles of group B (when therapeutic dose of VPA alone given) and E (when toxic dose of VPA was supplemented with carnitine) and in 4(33.33%) of group D (when toxic dose of VPA alone given).

DISCUSSION

We observed that VPA has induced 16.66% of hyalinization in the therapeutic (B) group and 33.33% in the toxic (B) group. When supplemented the carnitine to the therapeutic regimen (i.e. C) group, no significant changes were observed in any section of muscles whereas only 16.66% changes were seen in group E (toxic+ carnitine) cf group of toxic regimen (D) alone i.e. 33.33%.

Our observations are comparable with some studies in which the authors have observed systemic carnitine deficiency and ragged red fibers (abnormal mitochondria in muscle) on Electroan Microsopy (EM).[20] and evidence of depletionof carnitine stores, especially during long-term or high-dose therapy, through various mechanisms[21]. Muscle carnitine concentration was similar to that of control roup of animals in the VPA+ carnitine treated ratsin asome other study[22], which supports our observations.

Our findins as VPA induced toxicity in the toxic regimen is more than the therapeutic one is in almost complete aggrement with the view[23]. that the effect of

VPA is directly proportional to to the concentration of VPA & duration of exposure.

CONCLUSION

Carnitine plays a very important and protective role in preventing skeletal muscles myopathies caused by Valproic Acid. Further studies on humans is recommended.

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