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Original Communication

Valproic acid-associated hypoalbuminemia in medically fragile pediatric and young adult patients in a long term care facility: Potential mechanisms for decreased albumin synthesis

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ABSTRACT

Valproic acid (VPA) has been associated with numerous side effects including hypoalbuminemia though the mechanism for this side effect has not been established. The purpose of this study was to investigate a potential mechanism of VPA-associated hypoalbuminemia in medically fragile pediatric and young adult epileptic patients of a long-term care facility. Medical charts were reviewed for residents of a pediatric long-term care facility, admitted from January 1, 2001 to December 31, 2009, for the case-control study if they had either received VPA ≥3 months at the start of the study or were matched as a control patient. Demographic and laboratory data was obtained from patient charts. Prospectively, blood samples were obtained from 11 residents on VPA to assess for serum concentrations of albumin, VPA, and amino acids. For the primary outcome, ANOVA revealed a significant difference in serum albumin concentrations between the three groups, p=0.002. The correlation analysis for free VPA serum concentrations and serum amino acid concentrations, controlled for patient weight and daily protein intake, found the serum arginine concentration had the best correlation although not statistically significant, p=0.064. Patients on long-term VPA therapy were found to have lower serum albumin concentrations than our control groups, which correspond to reports in the literature. There was a correlation, although not statistically significant, with free serum VPA concentrations and serum arginine concentrations when assessed prospectively. For medically fragile patients on VPA, it may be reasonable to try amino acid supplementation prior to an extensive work-up for hypoalbuminemia.

KEYWORDS: valproic acid, albumin, hypoalbuminemia, medically fragile, pediatric

ABBREVIATIONS

VPA - valproic acid; HLCCD - Hattie Larlham Center for Children with Disabilities; ACH - Akron Children's Hospital; AED - antiepileptic drug; BUN - blood, urea, nitrogen; sCr - serum creatinine; AST - aspartate aminotransferase; ALT - alanine aminotransferase

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INTRODUCTION

Valproic acid (VPA) is a highly protein bound long-chain fatty acid frequently used as an antiepileptic agent in pediatric and adult patients. Unfortunately this drug has been associated with a number of side effects/toxicity including hepatotoxicity, pancreatitis, altered mitochondrial dysfunction, hyperammonemia, and decreased serum protein concentrations [1-3]. Alterations in the percentage of free drug available, primarily due to decreased serum protein, increases the risk for toxicity.

Decreased serum albumin concentrations in patients receiving chronic VPA therapy have been identified in multiple studies [2-5]. The exact mechanism or mechanisms by which VPA affects albumin have not been fully elucidated though multiple theories have been postulated including impaired vesicle transport within the hepatocyte, inhibition of hepatic synthetic metabolic pathways and renal protein loss [1-3]. It is unfortunate that investigation into the mechanism(s) of VPA-associated hypoalbuminemia has been minimal because despite absolute differences in serum albumin concentration appearing to be of limited clinical consequence, increased medical costs may be incurred by unnecessary testing in attempts to determine the etiology of hypoalbuminemia. The purpose of this study was to investigate a potential mechanism of VPA-associated hypoalbuminemia in medically fragile pediatric and young adult epileptic patients of a long-term care facility.

METHODS

Subjects & study design

Hattie Larlham Center for Children with Disabilities (HLCCD) is a specialized long-term care facility for pediatric and young adult residents with cognitive and neuromuscular impairments in the severe to profound range. The majority has frequent medical problems such as seizures, frequent pneumonia, gastroesphageal reflux, urinary tract infections, constipation, spasticity, scoliosis, and osteoporosis and is considered medically fragile, most being fed via a gastrostomy tube. In order to study the effects of VPA on serum albumin concentrations, a retrospective case-control study was designed along with a prospective study arm for patients receiving VPA. Informed consent was obtained from the parents/legal guardians of residents eligible for participation in the VPA prospective study arm. This study design was reviewed and approved by the HLCCD Human Rights Committee and the Akron Children's Hospital (ACH) Institutional Review Board; the study was registered at www.clinicaltrials.gov (NCT00723762).

Retrospective study arm

Residents were eligible for the case-control study if they were admitted to HLCCD between January 1, 2001 through December 31, 2009 and had received VPA ≥3 months at the start of the study. Control patients were identified if they were not receiving VPA and were matched based on age and gender. Patients were excluded from the study if they received albumin products in the past month, had a medical need for a specific protein supplementation, or had been diagnosed with protein-losing nephropathy or enteropathy.

Residents receiving VPA were case-control matched by age and gender to two different control groups; control group one: patients were matched to VPA patients based on concomitant antiepileptic (AED; non-VPA), age, and gender; control group two: patients not receiving any AED therapy. Twenty-two HLCCD residents receiving VPA were identified and included in the retrospective review; 12 male and 10 female, see Table 1 for demographics. Twenty-one HLCCD residents were matched as control group one patients; seventeen HLCCD residents were matched as control group two patients. Serum VPA concentrations, if available, serum albumin, blood urea nitrogen (BUN), serum creatinine (sCr), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein concentrations and urine protein concentrations were obtained from the charts of all eligible patients. Daily patient protein and caloric intake were determined by the nutritional and dietary records documented for each patient.

Prospective study arm

Among the 22 residents receiving VPA included in our retrospective review, 17 were eligible for possible prospective evaluation; 3 residents were deceased and 2 residents had VPA discontinued after the start of our retrospective review.

Table 1. Summary of the demographic and clinical characteristics of the study population.

Characteristic	VPA group n=22	AED control group n=21	No AED control group n=17	p-value
Age[+]	19.2 (5.81)	19.9 (5.69)	19.8 (6.04)	0.925
Male (n)	12 (54.5%)	12 (57.1%)	9 (52.9%)	0.966
Weight (kg) [+]	41.4 (10.59)	43.1 (7.56)	33.7 (11.80)	0.014
Race*(n)				0.454
Black	1 (4.5%)	5 (23.8%)	2 (11.8%)	
Caucasian	19 (86.4%)	15 (71.4%)	14 (82.4%)	
Other/Unknown	2 (9.1%)	1 (4.8%)	1 (5.9%)	
Daily Caloric Intake[+] (calories/day)	1257.1 (199.03)	1263.2 (207.22)	1248.4 (279.15)	0.981
Daily Protein Intake (g/day)[+]	47.9 (11.30)	49.9 (11.23)	41.3 (15.01)	0.112
Antiepileptic Drugs (n)				
Lamotrigine	5 (22.7%)	5 (23.8%)		
Carbamazepine	1 (4.5%)	2 (9.5%)		
Topiramate	3 (13.6%)	2 (9.5%)		
Phenobarbital	3 (13.6%)	5 (23.8%)		
Phenytoin	1 (4.5%)			
Levetiracetam	5 (22.7%)	4 (19.0%)		
Zonisamide	2 (9.1%)			
Primidone	1 (4.5%)			
Felbamate	1 (4.5%)			
Clonazepam	2 (9.1%)	3 (14.3%)		
Oxcarbazepine	1 (4.5%)	4 (19.0%)		

[+]Data presented as mean (±SD).
*For race categorical comparisons were performed on Caucasian/Non-Caucasian collapsed categories.
Continuous variables mean (SD) are reported and comparison performed using single factor ANOVA.
Categorical variables frequencies (%) are reported and compared using Pearson chi-square test.
Antiepileptic drug (AED).

Parental consent was obtained for 12 of the 17 eligible residents on VPA for the prospective evaluation; one consented patient was being weaned from VPA and not eligible for this part of the study and 5 declined to participate (n=11 for prospective study arm).

Blood samples were obtained from these 11 residents and urine samples were available from 5; all samples were obtained in conjunction with routinely scheduled laboratory tests. All study specific blood and urine samples were collected by a phlebotomist and transported by study members to ACH in a temperature-controlled container. Specimen blood samples for serum amino acids and free VPA were frozen and stored at ACH until all samples were available for testing. Blood samples were tested for serum concentrations of albumin, VPA, free VPA, total protein, arginine, citrulline, ornithine, BUN, serum creatinine, ammonia, AST, ALT, and alkaline phosphatase. Spot urine samples were tested for concentrations of protein and urea.

Statistical analysis

The population was described overall and by groups, including demographic variables and relevant clinical values. Nominal values were described with frequencies and percents while continuous variables were described by using the appropriate measures of central tendency and dispersion. For the primary outcome variable, comparison of mean concentrations of albumin in residents on VPA and each control group, with other potentially confounding factors statistically controlled, an ANOVA was utilized; the Tukey HSD test was used for post-hoc analysis. A backwards stepwise regression analysis was performed to identify the linear model predicting average serum albumin concentrations. The prospective data from patients receiving VPA was assessed for a correlation between serum VPA concentrations and the amino acids citrulline, arginine, and ornithine. All statistical testing was performed using SPSSv17.0 software.

RESULTS

Relevant demographic results for the 3 patient study groups are shown in Table 1. For the primary outcome, ANOVA revealed a significant difference in serum albumin concentrations between the three groups, p=0.002, see Table 2. The Tukey HSD post-hoc test indicated that the VPA group had the lowest serum albumin (mean albumin 3.18g/dL). A weak inverse relationship was identified between VPA dose and serum albumin concentration, r^2=0.08. A backwards stepwise regression analysis identified patient weight, daily protein intake, and use of VPA as predictors of serum albumin concentration, r^2=0.334. The correlation analysis for free VPA serum concentrations and serum amino acid concentrations, controlled for patient weight and daily protein intake, found serum arginine concentration had the best correlation, followed by serum citrulline although neither were statistically significant, p=0.064 and 0.364 respectively; see Table 3 for serum amino acid concentrations.

DISCUSSION

Our retrospective analysis revealed a significant difference in serum albumin concentrations between the three groups, p=0.002 (see Table 2), which corresponds to previous reports of lower albumin in VPA-treated patients [2-4]. The post-hoc Tukey analysis indicated that the patients in the VPA group had the lowest serum albumin while predictive factors included patient weight, daily protein intake, and use of VPA based on regression analysis. Adequate dietary protein intake has been associated with albumin synthesis [6, 7] however body weight as a predictive factor is an interesting finding, especially considering that patients on VPA tend to gain weight [8].

Decreased serum albumin concentrations have been observed in patients taking VPA, with transient decreases described in ambulatory pediatric patients [4] or persistent decreases despite increased protein intake in medically fragile patients [3]. Rugino *et al.* reported hypoalbuminemia during

Table 2. Serum laboratory test results by group.

Lab Parameter	VPA group n=22	AED control group n=21	No AED control group n=17	p-value*
Albumin (g/dL)	3.18 (0.40)	3.57 (0.32)	3.49 (0.38)	0.002
BUN (mg/dL)	15.10 (4.43)	14.0 (5.53)	14.69 (4.50)	0.758
sCr (mg/dL)	0.47 (0.15)	0.46 (0.15)	0.63 (0.98)	0.561
AST (units/L)	30.54 (12.51)	28.10 (12.51)	30.10 (15.61)	0.858
ALT (units/L)	21.71 (12.59)	31.46 (15.05)	29.26 (22.50)	0.143

Data presented as mean (±SD).
*p-value from single factor ANOVA with effect for treatment group.
Valproic acid (VPA), Antiepileptic drug (AED), Blood urea nitrogen (BUN), serum creatinine (sCr), aspartate aminotransferase (AST), alanine aminotransferase (ALT).

Table 3. Amino acid serum concentrations from patients on valproic acid.

Pt #	Weight kg	Protein g/day	[VPA] µg/mL	[Free VPA] µg/mL	[albumin] g/dL	[arginine] µmol/L	[ornithine] µmol/L	[citrulline] µmol/L
Normal Range			50-100	4-50	3.5-5	10-140	10-163	1-46
1	27	33	54.9	10.7	3.3	78	82	29
2	45	46	92	23.2	3.2	66	38	30
3	41.1	53	65	14.5	3	85	82	32
4	35	43	78	12.4	3.9	78	46	34
5	26.8	36	18	4	2.5	79	60	22
6	60.5	NA	91	34.7	2.9	63	61	64
7	47.5	57	105	33.9	3	83	49	24
8	44.2	44	85	21.3	3.7	80	54	30
9	41.3	53	59	10.6	3.8	46	42	19
10	41	41	57	10.1	3.3	55	49	19
11	61.6	75	96	34.9	2.5	58	70	25

Valproic acid (VPA), not available (NA).

VPA use in 5 pediatric/adolescent residents of a long-term care facility for severely disabled, neurologically injured children [3]. Three of these patients had been receiving VPA for 4 to 12 months with a high protein diet however low serum albumin concentrations persisted until after VPA therapy was discontinued; the two other patients had normal serum albumin concentrations until VPA was initiated. This response, or lack thereof, in medically fragile patients raises numerous questions about the mechanism of VPA-associated hypoalbuminemia. It is also interesting to note that while our patients received increased amounts of daily protein their albumin concentrations did not change appreciably though our statistical analysis found protein intake to be a predictive factor. The influence of a patient's body weight should be explored more as well to determine its potential role in predicting serum albumin, especially in patients on VPA.

Considering the numerous published reports of low serum albumin in conjunction with VPA [2-5], there has been minimal research exploring potential mechanisms of VPA's effect on albumin synthesis. We attempted to investigate whether patients receiving VPA had changes in serum concentrations of different amino acids in the urea cycle, thus affecting albumin synthesis and might provide some insight into the mechanism of VPA-associated hypoalbuminemia. In our study, there was a correlation, although not statistically significant, with free serum VPA concentrations and serum arginine concentrations along with total serum VPA and serum citrulline concentrations; see Table 3 for serum amino acid concentrations.

Albumin synthesis occurs in hepatocytes via unbound or endoplasmic reticulum-bound polysomes. The bound polysomes export albumin out of the liver whereas unbound polysomes produce albumin for intracellular requirements [7]. Albumin synthesis is sensitive to tryptophan concentrations although other amino acids are also able to stimulate albumin synthesis whereas oncotic pressure near the synthetic site and energy supply while albumin release is sensitive to intrahepatocellular potassium concentrations [6, 7]. The potential correlation between the urea cycle and albumin synthesis (see Figure 1) was identified after the administration of various amino acids increased both albumin and urea synthesis [9].

Ornithine, one of these amino acids, is an intermediate amino acid within the urea cycle and

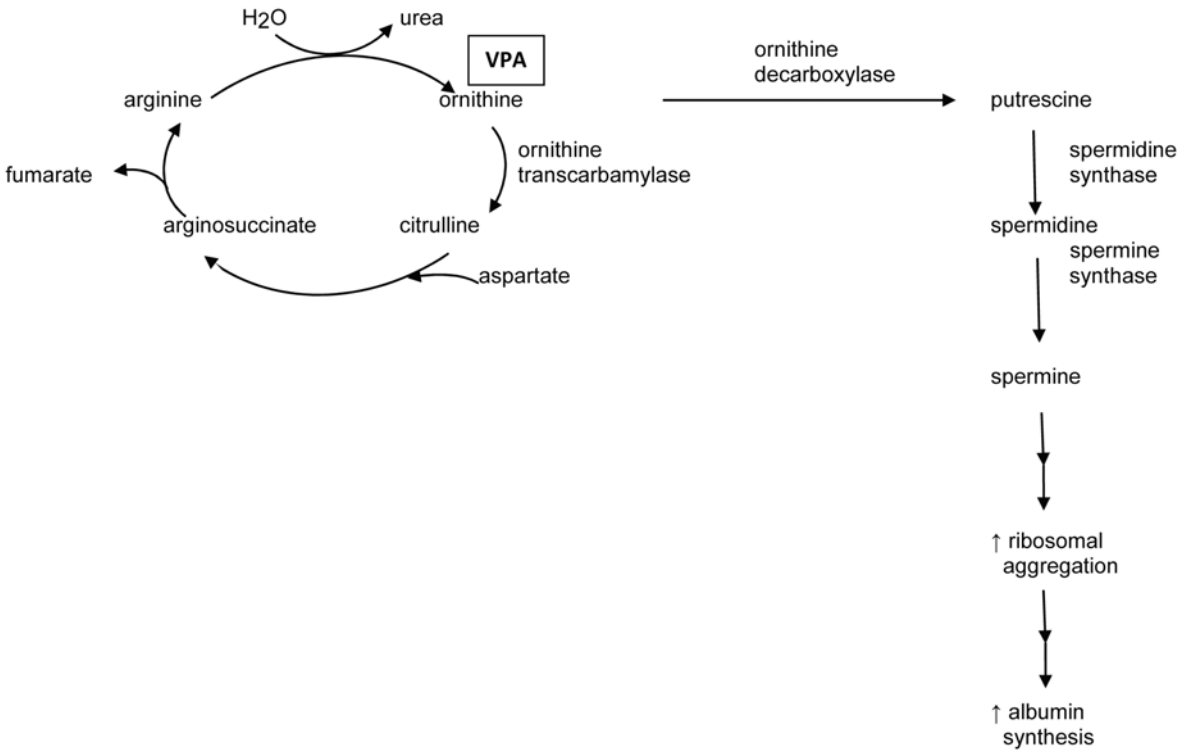

Figure 1. The urea cycle synthesizes the amino acid ornithine which is also necessary for albumin production. VPA may indirectly decrease albumin synthesis by inhibiting the urea cycle

also a precursor to polyamines (see Figure 1) which have been shown to increase the degree of aggregation of polysomes bound to the endoplasmic reticulum and responsible for protein synthesis [7, 9]. Thus, VPA may indirectly inhibit protein synthesis by interfering with the urea cycle [10, 11] leading to decreased ornithine concentrations and subsequently a decrease in polyamine concentrations and the number of bound polysomes resulting in alterations in albumin synthesis and release. Citrulline is converted to arginine via argininosuccinate and arginine is converted to ornithine when urea is released (see Figure 1), which may explain the correlation of these amino acids to VPA concentrations observed in our study. Based on these correlations, it appears possible that VPA affects the urea cycle either before or after the conversion of arginine to ornithine. If this is the part of the urea cycle affected, than amino acid supplements, either arginine or ornithine, may overcome the inhibition, increasing serum albumin concentrations via an easily employed and economical approach.

LIMITATIONS

There are several limitations to this study. First, the case-control part of the study was performed retrospectively with all the usual limitations of a retrospective study, e.g. missing data, thoroughness of documentation. Secondly, serum amino acid concentrations were not analyzed across all three study groups since these were not routine tests. Therefore, any differences found between actual serum concentrations and accepted normal concentrations cannot be fully attributed to the use of VPA since it is not known if medically fragile patients have altered serum amino acid concentrations at baseline. However, differences in amino acid concentrations have been reported in the literature with non-medically fragile patients thus this is likely due to VPA. Lastly, serum ammonia concentrations may be falsely elevated due to sample breakdown as these samples needed to be transported for analysis. Trained medical assistants were responsible for proper sample storage and transportation, reducing this risk. The affects of VPA on ammonia was not the primary objective of this study.

CONCLUSION

Our data supports previous reports [2-5] that patients on long-term VPA therapy have lower serum albumin concentrations than patients receiving non-VPA anticonvulsant drugs (see Table 2). In our small group of patients available for prospective evaluation a non-statistically significant correlation between free serum VPA concentrations and serum arginine concentrations was observed. Thus, for medically fragile patients on VPA, it may be reasonable to try amino acid supplementation prior to an extensive diagnostic work-up for hypoalbuminemia. Other predictive factors, besides VPA exposure, included daily protein intake and patient weight. Further investigation into this link with a larger sample size may help to emphasize any differences between those on VPA and control groups and possible interventions or supplementations.

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REFERENCES

1. Bellringer, M., Rahman, K. and Coleman, R. 1988, Biochem. J., 249, 513.
2. Hauser, E., Seidl, R., Freilinger, M., Male, C. and Herkner, K. 1996, Brain Dev., 18, 105.
3. Rugino, T., Janvier, Y., Baunach, J. and Bilat, C. 2003, Pediatr. Neurol., 29, 440.
4. Attilakos, A., Voudris, K., Katsarou, E., Prassouli, A., Mastroyianni, S. and Garoufi, A. 2007,Clin. Neuropharmacol., 30, 145.
5. Jaffe, S. and Sanford, M. 2005, Epilepsia, 46, 599.
6. Rothschild, M. A., Oratz, M., Mongelli, J., Fishman, L. and Schreiber, S. S. 1969, J. Nutr., 98, 395.
7. Doweiko, J. and Nompleggi, D. 1991, J. Parenter Enteral Nutr., 15, 476.
8. Martin, C. K., Han, H., Anton, S. D., Greenway, F. L. and Smith, S. R. 2009, J. Psychopharmacol., 23, 814.
9. Oratz, M., Rothschild, M., Schreiber, S., Burks, A., Mongelli, J. and Matarese, B. 1983, Hepatology, 3, 567.
10. Cotariu, D. and Zaidman, J. 1988, Clin. Chem., 34, 890.
11. Castro-Gago, M., Rodrigo-Saez, E., Novo-Rodriguez, I., Camiña, M. and Rodriguez-Segade, S. 1990, Childs Nerv. Syst., 6, 434.