ORIGINAL ARTICLE

FAVOURABLE RESULTS FOR L-CARNITINE USE IN VALPROIC ACID ACUTE POISONING

RADU CIPRIAN ŢINCU[1,2#]*, CRISTIAN COBILINSCHI[3#], DANA TOMESCU[4,5#], LAURENŢIU COMAN[6#], IULIA ŢINCU[7#], CAMELIA DIACONU[8#], RADU ALEXANDRU MACOVEI[1,2#]

[1]*Clinical Emergency Hospital Bucharest, Critical Care Toxicology Unit, 8 Floreasca Way, 014451, Bucharest, Romania*
[2]*"Carol Davila" University of Medicine and Pharmacy, Faculty of Medicine, Department of Orthopaedics – Anaesthesiology and Intensive Care, Discipline of Pharmacology, Toxicology and Clinical Psychopharmacology, 8 Floreasca Way, 014451, Bucharest, Romania*
[3] *Clinical Emergency Hospital Bucharest, Anaesthesiology Intensive Care Unit, 8 Floreasca Way, 014451, Bucharest, Romania*
[4] *"Fundeni" Clinical Institute, Anaesthesiology Intensive Care Unit, 258 Fundeni Street, 022328, Bucharest, Romania*
[5]*"Carol Davila" University of Medicine and Pharmacy, Faculty of Medicine, Department of Orthopaedics – Anaesthesiology and Intensive Care, Discipline Anaesthesiology and Intensive Care, 258 Fundeni Street, 022328, Bucharest, Romania*
[6]*"Carol Davila" University of Medicine and Pharmacy, Faculty of Pharmacy, Physiology Department, 6 Traian Vuia Street, 020956, Bucharest, Romania*
[7]*"Grigore Alexandrescu" Emergency Children Hospital, Emergency Department, 30-32 Iancu de Hunedoara Avenue, 11743, Bucharest, Romania*
8"Carol Davila" University of Medicine and Pharmacy, Clinical Emergency Hospital Bucharest, Internal Medicine Unit, Bucharest, Romania

**corresponding author: r_tincu@yahoo.com*
[#]*All authors contributed equally to this work and thus share first authorship.*

Manuscript received: December 2016

Abstract

Valproic acid (VPA) is a fatty acid with anticonvulsant properties. The aim of our present study was to investigate the effect of levo-carnitine supplementation on serum NH_3 and clinical recovery in patients with VPA intoxication. This study included all patients admitted for acute VPA poisoning (VPA > 100 μg/mL), in 2014, in our clinic. Blood samples were obtained in order to analyse NH_3, VPA concentrations and biochemical status. The patients were allocated to receive standard therapy (Group 1) or 1800 mg of L-carnitine/day together with standard therapy (Group 2) for 3 days. A total of 62 patients were finally enrolled in the study. The median (IQR) ingested dose of VPA was 1000 mg (800 mg, 1200 mg [range; 800 - 6000 mg]). L-Carnitine supplementation resulted in significant reductions in ammonemia (47.9 ± 6 *vs*. 61.9 ± 11.39 μmol/L), determined after 24 hours, levels compared with baseline ($p < 0.001$). The trend was similar for plasma VPA levels. The use of L-carnitine accelerates the elimination of VPA and facilitates the decrease in ammonia plasma levels.

Rezumat

Acidul valproic (VPA) este un acid gras cu proprietăți anticonvulsivante. Scopul studiului a fost investigarea efectului suplimentării cu levo-carnitină asupra amoniacului seric, în cazul pacienților cu intoxicație cu VPA. Acest studiu a inclus toți pacienții admiși pentru intoxicație acută cu VPA (VPA > 100 μg/mL), în 2014. Probele de sânge au fost prelevate pentru determinarea concentrațiilor de NH_3, VPA și a statusului biochimic. Pacienții au fost randomizați pentru terapie standard (Grupul 1) sau 1.800 mg L-carnitină/zi împreună cu terapia standard (Grupul 2) timp de 3 zile, totalizând 62 de pacienți. Doza medie de VPA a fost de 1.000 mg (800 mg, 1.200 mg [domeniul 800 - 6.000 mg]). Suplimentarea cu L-carnitină a determinat o reducere semnificativă a amonemiei (47,9 ± 6 față de 61,9 ± 11,39 μmol/L), determinată după 24 ore, nivelurile acesteia fiind diferite de valorile inițiale ($p < 0,001$). Nivelurile plasmatice de VPA au fost similare. Utilizarea L-carnitinei accelerează eliminarea VPA și facilitează scăderea concentrațiilor plasmatice ale amoniacului.

Keywords: acid valproic, L-carnitine, poisoning

Introduction

Valproic acid (VPA) is a fatty acid with anticonvulsant properties widely used in various neurological and psychiatric disorders. It is usually well tolerated in chronic treatment, but it may produce several side effects due to long term administration: pancreatitis, hepatotoxicity, hyperammonaemia encephalopathy, bone marrow suppression [1]. VPA proper metabolic pathway uses carnitine as an essential co-factor in order to eliminate NH_3 [2]. VPA acts for increasing γ-aminobutyric acid (GABA) functions in specific parts of the brain [3, 15] and attenuates the neural excitatory system through *N*-methyl-D-aspartate (NMDA)-type glutamate receptors [4]. Glucuronic acid conjugation, mitochondrial β – and cytosolic (endoplasmic reticulum) ω-oxidation are the pathways involved in VPA metabolism [5] and some previous

data consider that ω-oxidation metabolites promote hyperammonaemia [6].

Becker and Harris demonstrated increased levels of medium chain acyl-CoA fraction after valproate administration [7]. They concluded that a rapid accumulation of valproyl-CoA and its metabolites (CoA-ester) leads to an inhibition of fatty acid oxidation. Coenzyme CoA, acetyl-CoA and long chain acyl-CoA were decreased.

VPA crosses the inner and outer mitochondrial membranes using carnitine-independent mechanisms, as a simple branched medium-chain fatty acid. The mitochondrial β-oxidation of VPA involves several metabolic steps, called "carnitine shuttle"; once activated, VPA is linked with reduced acetyl co-enzyme A (CoA-SH) and results valproyl-CoA which cannot cross the inner mitochondrial membrane and needs conjugation with palmityl carnitine transferase 1 (PCT1) in order to form valproylcarnitine. Carnitine translocase will change valproylcarnitine for free carnitine [8].

Among the metabolic adverse events, hyper-ammonaemia encephalopathy (HE) has been described in patients using VPA as chronic treatment or acute overdose [9, 10]. The mechanisms resulting in HE are considered to be either carnitine depletion induced by VPA or pre-existing carnitine deficiency. Acute VPA intoxication induced by suicide intention also produces a severe depletion of carnitine, leading to serious neurologic impairment. Carnitine biochemical main properties results in fatty acyl group transportation into mitochondria and maintenance of the ratio of acyl-CoA to free CoA in the mitochondria (Figure 1) [11].

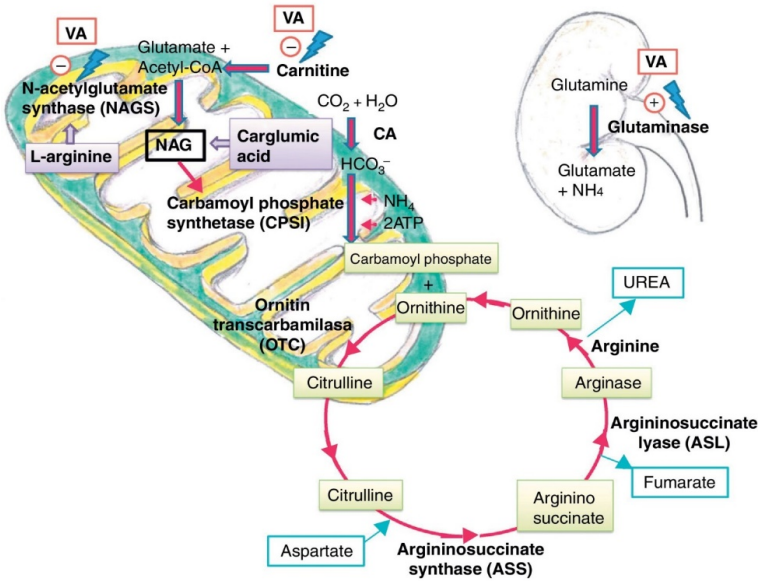

Figure 1.
Urea cycle (after Fernández Colomer *et al.*) [12]

Previous studies have shown that serum ammonia levels are directly correlated with the serum concentrations of VPA, and inversely with the serum concentrations of carnitine [13]. A study on 14 VPA-treated patients having hyperammonaemia and carnitine deficiency was reversible using carnitine supplementation (50 mg/kg per day) for 4 weeks [14]. Exogenous carnitine binds VPA and decreases ammonia levels, enables β-oxidation process and production of acetyl-CoA and relieves the inhibition of urea synthesis, in the meantime.

As acute VPA intoxication also rises in incidence, taking into account intentional and accidental overdose, we deal with serious toxicity that demands new therapy interventions.

The aim of our present study was to investigate the potential effect of levo-carnitine (active isoform of carnitine, L-Carnitine) supplementation on serum NH_3 and clinical recovery in patients admitted for VPA intoxication.

Materials and Methods

Subjects

This was a randomized controlled trial conducted in the Intensive Care-Toxicology Unit of the Clinical Emergency Hospital in Bucharest, that included all patients admitted for acute VPA poisoning (VPA > 100 μg/mL), in 2014. We excluded all individuals suffering of chronic illnesses that may affect NH_3 serum levels, such as liver or kidney conditions, and patients using valproic acid in their current medication for neurologic or mental disorders. Also, patients with multidrug ingestions were excluded.

The ethics committee of Clinical Emergency Hospital approved this study and written informed consent was obtained from all participants.

Clinical design

Recorded data included demographic characterization, physical examination on arrival, laboratory data and outcomes for every patient. We also marked the time elapsed between ingestion and arrival at the hospital, and the amount of medication ingested by the patient, based on his/her or family declaration. After admission in the Toxicology - Intensive Care Unit, all patients underwent standard procedures for poisoning management, including gastrointestinal lavage and activated charcoal and sorbitol, airway management, intravenous fluid resuscitation.

Blood samples were obtained during the admission procedure, collected following standard guidelines, plasma separated and stored at -20°C until use to analyse NH_3 and VPA concentrations together with aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALK) plasma values. For NH_3 levels we used EDTA K3 vacutainers and the laboratory determined the concentration using an enzymatic method (with glutamate dehydrogenases), having normal ranges between 11 - 60 μmol/L. VPA plasma concentration was calculated using fluorescence polarimetry assay principle of Cobas Integra 400 and values over 100 μg/mL were considered abnormal. The kit is a ready-to-use liquid reagent presented in cartridges for 100 tests, which does not require sample pre-treatment. The assay is performed at 37°C. A minimum volume of 70 μL of plasma is required.

Plasma levels of valproic acid were determined every six hours for a period of 72 hours and ammonemia was assessed daily. Cut-off values for aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALK) were considered to be 5 - 40 IU/L, 7 - 60 IU/L and 44 - 150 IU/L, respectively. Patients with severe toxicity were compared to the others in order to identify the potential risk factors for a bad prognostic.

The patients were enrolled consecutively and allocated in 2 groups to receive standard therapy (Group 1) or 1800 mg of L-carnitine/day together with standard therapy (Group 2) for 3 days. Standard therapy was applied according to the ongoing protocol using gastrointestinal decontamination with a single dose of activated charcoal for patients presented early; other interventions were largely supportive such as: blood pressure support with intravenous fluids and vasopressors, correction of electrolyte or acid-base disorders (commonly an anion gap metabolic acidosis) with volemic repletion, mechanical ventilation in patients who required airway protection, mannitol for those who developed cerebral oedema or respiratory depression.

Statistics

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 18.0 for Shapiro-Wilk, Chi-square, Mann-Whitney U-test and binomial logistic regression test. Fisher's exact test was used to compare treatment groups in the analysis of categorical variables (e.g., gender). Some other baseline characteristics were analysed as continuous variables in the groups using a t-test. The differences in serum profile were determined by an analysis of covariance (ANCOVA). For the normally distributed data, Wilcoxon rank-sum test was applied. Statistical significance was set for a p value less than 0.05.

Results and Discussion

A total of 84 patients were initially enrolled in the study, but the final study population consisted on 62 patients, due to incomplete data or patient withdrawn from the study; out of them, 41 (66.1%) were women and the remaining 21 (33.8%) were men. The ages of subjects ranged between 19 and 47 years, with a mean of 29.35 ± 6.85 years.

The median (IQR) ingested dose of VPA was 1000 mg (800 mg, 1200 mg [range; 800 - 6000 mg]). Anyway, for a percentage of 18.6% of all subjects, the exact ingested amount couldn't be evaluated. There was a median (IQR) time elapsed between drug self-administration and hospital presentation of 3 (2, 8 h [range; 1 - 48 h]), as reported by themselves or by their relatives, in 95% of cases.

The most common form of presentation included signs or symptoms like drowsiness (16 patients, 25.5%), nausea and vomiting (11 patients, 18.1%), vertigo (10 patients, 15.6%), and headache (8 patients, 13.7%). Seizures were registered in 4 cases (6.45%). All patients included in the study experienced different degree of encephalopathy symptoms with impaired cognition, sensorium and behaviour alterations. Onset of encephalopathy symptoms ranged after a mean time of 6.8 ± 4.4 hours following the ingestion. Valproate is a well-known anti-epileptic drug, widely used in neurologic and mental disorders, with a potential toxicity affecting the well-being [16, 17]. Its main side effects include pancreatitis, hepatotoxicity, bone marrow suppression and encephalopathy. In this research, we do not report any case of bone marrow suppression, while liver function was affected; anyway this marker was not correlated with patients' outcome. Vital signs at admission were proofed not to indicate the prognosis, while the only negative prediction factors were age, high ingested doses and high plasma VPA. This data are similar with some other findings publish before [18].

In 50 cases (80.6%) there were no abnormalities on electrocardiograms (ECGs) registrations. The others suffered of tachycardia (6, 9.67%), ventricular extra systoles (2, 3.22%) or even bradycardia (4, 6.45%).

The median (IQR) duration of hospitalization in hours was 18.

Table I shows the comparison of potential risk factor for patients having good or bad prognosis. AST and ALT alteration was assessed in 8.7% cases, but these variations did not influence the outcome. ALK was abnormal in 62% of the patients on admission and it was associated with poor patient outcomes.

Table I

Independent risk factors for patients with good or bad prognosis

Independent factor	Total (n = 62)	Good prognosis (n = 52)	Bad prognosis (n = 10)	p value
Age*	29.35 ± 6.85	25.35 ± 4.85	31.45 ± 3.84	0.005[a]
Gender *n (%)*				
Male	21 (33.8)	29	4	NS[b]
Female	41 (66.1)	23	6	
Self-poisoning dose (mg)†	1000 (800 - 1200)	1000 (800 - 1200)	4000 (3000 - 8000)	0.002[a]
Time to admission (hours)†	3 (2 - 8)	3 (2 - 8)	4 (3 - 6)	NS[a]
VPA plasma (µmol/L)*	264 ± 15.3	176 ± 25.3	274 ± 15.3	0.005[a]
Systolic blood pressure (SBP) (mmHg)†	120 (110 - 120)	120 (110 - 120)	130 (110 - 140)	NS[a]
Diastolic blood pressure (DBP) (mmHg)†	75 (70 - 80)	75 (70 - 80)	80 (75 - 85)	NS[a]
ALT	55 (30 - 80)	53 (33 - 80)	60 (55 - 82)	NS[a]
AST	45 (25 - 78)	50 (35 - 75)	55 (45 - 75)	NS[a]
ALK	122 (62 - 178)	101 (73 - 198)	167 (123 - 210)	0.005[a]
HR (beats/min)†	86 (83 - 91)	86 (83 - 91)	90 (85 - 100)	NS[a]

* mean value; † median value; [a] Mann-Whitney U-test; [b] Pearson ; HR: heart rate; NS: not significant

At admission, there was no difference in NH_3 and VPA plasma level between Group 1 and Group 2. Group 1 consisted in 34 subjects and Group 2 enrolled 28 patients. The changes of the values over treatment period are presented in Table II.

Table II

NH_3 and VPA levels over the study period

Outcome measure	Time point (hours)	Total	Group 1[a]		Group 2[b]		*p*
			Levels	*p*	Levels	*p*	
NH_3, µmol/dL	Admission	264 ± 15.3	267.1 ± 15.7	n/a	271.3 ± 16.6	n/a	NS
	6	251 ± 14.1	250.54 ± 15.7	NS	254 ± 14.7	NS	NS
	12	231 ± 16.3	238 ± 11.1	NS	228 ± 12.3	NS	NS
	18	218 ± 14.6	221 ± 10.3	NS	205 ± 15.4	NS	NS
	24	202 ± 6.7	215.3 ± 6.3	NS	183. ± 13.7	NS	NS
	30	189 ± 6.3	198 ± 9.7	NS	162 ± 12.5	NS	NS
	36	178 ± 7.9	188 ± 8.3	NS	139 ± 15.8	NS	*
	42	152.6 ± 8.1	165 ± 12.4	NS	130 ± 11.7	NS	*
	48	148.9 ± 6.8	145 ± 15.5	NS	105 ± 10.7	NS	*
	54	116.1 ± 9.9	119 ± 11.7	*	103 ± 9.8	*	*
	60	90.6 ± 8.1	92 ± 12.5	*	81 ± 8.7	*	*
	66	88.9 ± 6.9	75 ± 8.7	*	68 ± 9.5	*	*
	72	64.1 ± 6.2	65.1 ± 3.2	*	48.1 ± 3.2	*	*
VPA, µg/mL	Admission	264 ± 15.3	255.2 ± 10.7	n/a	279 ± 6.7	n/a	NS
	24	155.1 ± 5.6	165.2 ± 5.7	NS	124.8 ± 10.2	NS	*
	48	93.7 ± 4.3	98.2 ± 11.8	NS	92.8 ± 5.0	NS	*
	72	56.0 ± 7.4	61.3 ± 11.4	NS	38.3 ± 6.3	NS	*

Values are mean ± SE; [a] Standard group; [b] Carnitine group; * $p < 0.05$, comparisons for each time point; n/a: not applicable; NS: not significant; NH_3: ammonia; VPA: valproic acid.

L-Carnitine supplementation resulted in significant reductions in ammonemia and VPA levels determined after 24 hours, levels compared with baseline whereas those parameters remained still high in standard group.

VPA inhibits the biosynthesis of carnitine by decreasing the concentration of alpha-ketoglutarate. We have shown that carnitine supplementation may increase the beta-oxidation of VPA and limit cytosolic omega-oxydation and release of NH_3. Levocarnitine dietary supplements are thought to correct or attenuate metabolic damages, such as hyperammonaemia, induced by VPA because carnitine deficiency mediates these processes [19]. There is a lack of data in this direction, and only a limited number of case reports or minor surveys have been published with this particular concern. Authors like Minville *et al.* reported in 2004, the case of a severe VPA poisoning in a 36-year-old man who was put on haemodialysis to

decrease high VPA concentration together with L-carnitine therapy (50 mg/kg b.w. per day for 4 days) and eventually recovered after 4 days [20].

A great deal of interest is provided for patients treated with VPA for some neurological and mental disorders; such a study revealed normalization in plasma NH_3 concentrations and marked increase in carnitine concentration in all patients to whom were given L-carnitine supplementation [21]. Nevertheless, analyses such as Raskind's demonstrated poor evidence for subjective and objective improvements in paediatrics patients treated with VPA [22]. Despite this, some scientific committees and guide-lines recommend carnitine supplementation in various risk population during VPA therapy [22]. The results of our study could indicate that levo-carnitine add-on therapy in VPA poisoning patients was able to decrease NH_3 plasma levels, improving clinical outcome and recovery.

The limitation of this research resembles in sample size which was relatively small and the fact that the dose of carnitine was independent than the VPA plasma levels, when we considered additional therapy; also, we weren't able to assess carnitine plasma levels on admission and after treatment.

Conclusions

The use of L-carnitine in the treatment of VPA poisoning accelerates the elimination of VPA and facilitates the decrease in ammonia plasma levels, therefore reducing systemic toxicity and also the risk of encephalopathy.

References

1. Gomceli Y.B., Kutlu G., Cavdar L., Sanivar F., Inan L.E., Different clinical manifestations of hyper-ammonemic encephalopathy. *Epilepsy Behav.*, 2007; 10(4): 583-587.
2. Verrotti A., Trotta D., Morgese G., Valproate-induced hyperammonemic encephalopathy. *Metab. Brain Dis.*, 2002; 17: 367-373.
3. Bolaños J.P., Medina J.M., Effect of valproate on the metabolism of the central nervous system. *Life Sci.*, 1997; 60(22): 1933-1942.
4. Löscher W.C.N.S., Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy. *Drugs*, 2002; 16(10): 669-694.
5. Loscher W., Valproate: a reappraisal of its pharmaco-dynamic properties and mechanisms of action. *Prog. Neurobiol.*, 1999; 58: 31-59.
6. Ungureanu A., Rus L.L., Gligor F.G., Lăzăroae A., Prodan L., Roman-Filip C., Intravenous levetiracetam as second line option for status epilepticus. *Farmacia*, 2016; 64(4): 507-511.
7. Becker C.M., Harris R.A., Influence of valproic acid on hepatic carbohydrate and lipidic metabolism. *Arch. Biochem. Biophys.*, 1983; 223: 381-392.
8. Li J., Norwood D.L., Mao L.F., Schulz H., Mitochondrial metabolism of valproic acid. *Biochem.*, 1991; 30(2): 388-394.
9. Kugoh T., Yamamoto M., Hosokawa K., Blood ammonia level during valproic acid therapy. *J. Psychiatry Neurol.*, 1986; 40: 663-668.
10. Nicolai J., Carr R.B., The measurement of ammonia blood levels in patients taking valproic acid: looking for problems where they do not exist? *Epilepsy Behav.*, 2008; 12: 494-496.
11. Roe C.R.M., Kahler S.G., Kodo N., Norwood D.L., Carnitine homeostatis in the organic acidurias. In: Tanaka KC, editor. Fatty Acid Oxidation: Clinical, Biochemical, and Molecular Aspects. New York: Alan R Liss; 1990, 382-402.
12. Fernández Colomer B., Rekarte García S., García López J.E., Pérez González C., Montes Granda M., Valproate-induced hyper-ammonaemic encephalopathy in a neonate: Treatment with carglumic acid. *An. Pediatr.*, 2014; 81(4): 251-255.
13. Ishikura H., Matsuo N., Matsubara M., Ishihara T., Takeyama N., Valproic acid overdose and L-carnitine therapy. *Anal. Toxicol.*, 1996; 20(1): 55-58.
14. Ohtani Y., Endo F., Matsuda I., Carnitine deficiency and Hyperammonemia associated with valproic acid therapy. *J. Pediatr.*, 1982; 101(5): 782-785.
15. Nicolae A.C., Arsene A.L., Vuţă V., Popa D.E., Sîrbu C.A., Burcea Dragomiroiu G.T.A., Dumitrescu I.B., Velescu B.S., Gofiță E., Drăgoi C.M., *In vitro* P-gp expression after administration of CNS active drugs. *Farmacia*, 2016; 64(6), 844-850.
16. Isbister G.K., Balit C.R., Whyte I.M., Dawson A. Valproate overdose: a comparative cohort study of self-poisonings. *Br. J. Clin. Pharmacol.*, 2003; 55(4): 398-404.
17. Sztajnkrycer M.D., Valproic acid toxicity: over-view and management. *J. Toxicol. Clin. Toxicol.*, 2002; 40(6): 789-801.
18. Thanacoody R.H., Extracorporeal elimination in acute valproic acid poisoning. *Clin. Toxicol.*, 2009; 47(7): 609-616.
19. Aires C.C., van Cruchten A., Ijlst L., de Almeida I.T., Duran M., Wanders R.J., Silva M.F., New insights on the mechanisms of valproate-induced hyperammonemia: inhibition of hepatic N-acetylglutamate synthase activity by valproyl-CoA. *J. Hepatol.*, 2011; 55(2): 426-434.
20. Minville V., Roche Tissot C., Samii K., Haemo-dialysis, L-carnitine therapy and valproic acid overdose. *Ann. Fr. Anesth. Reanim.*, 2004; 23(4): 357-360.
21. Böhles H., Sewell A.C., Wenzel D., The effect of carnitine supplementation in valproate-induced hyper-ammonaemia. *Acta Paediatr.*, 1996; 85(4): 446-449.
22. Raskind J.Y., El-Chaar G.M., The role of carnitine supplementation during valproic acid therapy. *Ann. Pharmacother.*, 2000; 34(5): 630-638.
23. Mechanick J.I., Brett E.M., Chausmer A.B., Dickey R.A., Wallach S., American Association of Clinical Endocrinologists medical guidelines for the clinical use of dietary supplements and nutraceuticals. *Endocr. Pract.*, 2003; 9(5): 417-470.