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Original scientific paper

The Use of a Food Supplementation with D-Phenylalanine, L-Glutamine and L-5-Hydroxytriptophan in the Alleviation of Alcohol Withdrawal Symptoms

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A B S T R A C T

We described the use of a food supplementation with D-phenylalanine, L-glutamine and L-5-hydroxytriptophan in the alleviation of alcohol withdrawal symptoms in pateints starting a detoxification terapy. Since abstinence from ethanol causes a hypodopaminergic and a hypoopioidergic environment in the reword system circuits, manifesting with withdrawal symptoms, food supplements that contains D-phenylalanine a peptidase inhibitor (of opioide inactivation) and L-amino-acids (for dopamine synthesis) were used to replenish a lack in neurotransmitters and alleviate the symptoms of alcohol withdrawal. 20 patienets suffering from alcohol addictions starting a detoxification therapy have been included in a prospective, randomized, double blind study. The patients have been randomly devided in two groups. One group recieved for a period of 40 days a food supplement containing D-phenylalanine, L-glutamine and L-5-hydroxytriptophan (investigation group), and the control (placebo) group. On the first day of hospitalization the patients performed a SCL-90-R test, and blood samples were taken for measuring liver enzymes, total bilirubin, unbound cortisol and lymphocyte populations. The same was done on the 40th day of hospitalization. During the therapy a significant decrease in SCL-90-R psychiatric symptoms scores and a significant increase in CD4 lymphocyte count was observed in the investigation group. The cortisol values were significantly, but equally decreased in both groups, the same was with the liver enzymes and the total bilirubin values. We conclude that abstinence causes a major stress for the patients. The use of food supplement containing D-phenylalanine, L-glutamine and L-5-hydroxytriptophan alleviates the withdrawal symptoms and causes a rise in CD4 lymphocyte population, but it dose not affect the serum cortisol levels, which are probably more affected by liver inflammation and the liver restitution.

Key words: *alcohol withdrawal, D-phenylalanine, cortisol, stress*

Introduction

Alcohol dependence is a complex psychiatric disorder with high heritability and high lifetime prevalence[1]. Alcoholism complexly harms human organism. Heavy drinking for an extended period of time causes many toxic injuries to the organs, and at the same time it afflicts the psyche and the social environment[2,3]. In the past years many studies have proved the existence of connections between the nervous, endocrine and immune system[4]. The alcoholism afflicts all three systems. Chronic alcohol consumption induce a decreased expreson of inhibitory GABA A alpha 1 (Gama aminobutyric acid A) receptor and an increased expression of NMDA (N-methyl-D-aspartate) receptor, a major excitatory receptor, responsible for neuronal hyperexcitability. Both receptors constitute an important part of the reward system neuronal circuits[5,6].

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During chronic alcohol consuption the system is relatively stabile, however the abstinence causes instability causing neuronal hyperexcitability due to increased NMDA activity and a »rewording deficit« due to decreased expression of GABA A alpha 1 receptor. The latter causes a hypodopaminergic and a hypoopioidergic environment in the reword system circuits, manifesting with withdrawal symptoms. At the same time HPA axis is stimulated via CRF released from the extended amygdala, causing a stress response[7].

During this period, the probability of a relapse of alcoholism reaches the maximum[7]. Abstinence is accompanied by many conditions such as anxiety, dysphoria, restlessness, etc.. that we attribute to stress axis activation. Abstinence from ethanol in alcoholics produces a rise in the concentration of CRF in the central amygdala[8]. Anxiety in abstinence may be resolved by the administration of CRF receptor antagonist – α-helical CRF9-41 – in the central amygdala. In mice with defective CRFR1 receptor the level of anxiety during abstinence is lower compared to the wild-mice control. At the same time, this same failure results in increased post-stress ethanol consumption in mice exposed to various stressors[8].

Since abstinence from ethanol causes a hypodopaminergic and a hypoopioidergic environment in the reword system circuits, manifesting with withdrawal symptoms, we have tested the effects of food supplements that contains D-phenylalanine a peptidase inhibitor of opioide inactivation and L-amino-acids as nutritional source for dopamine synthesis – in order to alleviate the symptoms of alcohol withdrawal[9]. In a prospective, randomized, double blind study we have followed the effect of 40-day treatment wilth food supplements in 20 patients suffering from alcohol addictions and starting a detoxification therapy.

Material and Methods

The research was conducted at the Department of addictive diseases, Psychiatric Hospital Vojnik. The study was approved by the Commission for Medical Ethics at the Ministry of Health. A prospective, randomized, double-blind clinical study included 20 patients who were treated for alcohol dependence syndrome in psychiatric hospital Vojnik. The volunteers were randomly divided into investigation (IG) and control (CG) group (10 individuals in each group). The IG consisted of seven men and three women, the average age was 41 years (SD 7.2). The CG consisted of eight men and two women, the average age was 42 years (SD 10.4).

The IG received during the regular course of hospitalization (40 days) nutritional supplements in capsule form (each capsule contained 300 mg d/l-phenylalanine, 150 mg l-glutamine, 5 mg l-5-hidroksitriptofane, 1 mg of vitamin B6, 50 mg calcium gluconate, 25 mg of magnesium oxide, 0.01 mg of folic acid), while the control group received the same-size capsules loaded with 1 mg of vitamin B6, 50 mg calcium gluconate, 25 mg of magnesium oxide and 0.01 mg of folic acid.

At day 1 and 40 of hospitalization somatic and psychological status was monitored. Patients were taken blood for haematological and biochemical tests including concentration of free cortisol, the activity of hepatic enzymes (AST, ALT, γ-GT), bilirubin level, and lymphocyte populations analysis by flow cytometry. Patients also passed a standardized psychological test SCL-90-R. Standardized psychological testing of SCL-90-R (Leonard R. Derogatis, PhD).

Psychiatric symptoms were measured using the SCL-90R (Derogatis 1983) and the Diagnostic Interview Schedule (DIS) (Robins et al. 1988). The DIS is a structured interview that was used to obtain psychiatric diagnoses according to the DSM-III-R. The psychological assessments included in the SCL-90R scales were as follows: somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid, and psychoticism. Global index of disturbance give the assessment of the overall psychological distress.

Immunological Assessment

Peripheral human blood leucocytes were collected by the venepuncture procedure and collected into EDTA Vacutainers (Becton Dickinson, Mountain View, CA). 100 μL of blood cells were labeled with 20 μL of monoclonal antibodies for 20 minutes at room temperature. Two-parameter analysis was performed to determine the proportion of T cells (CD3+), B cells (CD19+), T helper cells (CD3+CD4+), cytotoxic T cells (CD3+CD8+) and NK cells (CD16/56+). Isotype controls (Becton Dickinson, Mountain View, CA) and a control of viable cells (LIVE/DEAD kit, Molecular Probes, OR) were included. At least 2000 gated cells were analyzed for each test, and signals from two light scatters and four fluorescence parameters were analyzed with the Becton Dickinson Lysis II software.

Statistical analysis

The data obtained in this study have been analyzed in two steps. First, the internal structure of the SCL-90-R CRI questionnaire was assessed using factor analysis. Afterwards, an analysis of variance (ANOVA) was used to test for differences in biochemical/immunological parameter levels. Statistically significant differences between groups and between the first and second measurements within groups were tested with the Student t-test (assuming equal variances). The result is statistically significant at a value of $p<0.05$.

Results

Psychological status – SCL-90-R test results

At the first day of hospitalization, test results demonstrated high levels of psychiatric symptoms in both groups, resulting in high GID (global index of disturbance), most probably associated with high overall psychological distress at the start of hospitalisation. At the first day of

TABLE 1
MEAN SCL-90-R TEST VALUES ($\overline{X}$) AND STANDARD DEVIATION (SD) OF INDIVIDUAL SYMPTOMS AND INDICES FOR BOTH GROUPS AT ADMISSION (1.DAY) AND ON THE FORTIETH DAY OF HOSPITALIZATION (40. DAY)

Symptoms	Control group				Investigation group			
	1. day		40. day		1. day		40. day	
	$\overline{X}$	±SD	$\overline{X}$	±SD	$\overline{X}$	±SD	$\overline{X}$	±SD
Som	1.64	0.90	1.04	0.66	1.38	1.02	0.43	0.42
O-K	1.50	0.92	1.34	0.62	1.47	1.09	0.58	0.53
I-S	1.58	0.77	1.40	0.70	1.50	0.91	0.54	0.44
Dep	1.90	1.20	1.25	0.68	1.68	1.19	0.49	0.63
Ank	1.58	1.11	1.16	0.70	1.39	1.16	0.51	0.71
Hos	1.33	1.21	0.88	0.60	1.13	1.07	0.30	0.39
Fob	1.03	1.16	0.66	0.70	0.87	0.83	0.16	0.18
Par	1.23	1.02	1.25	0.64	1.66	0.85	0.42	0.27
Psi	1.15	0.85	0.89	0.74	1.17	0.99	0.32	0.32
GIM	1.56	0.99	1.16	0.60	1.45	0.95	0.45	0.42

Legend: Som – somatization, O-K – obsessive-compulsive, I-S – interpersonal sensitivity, Dep – depression, Ank – Anxiety, Hos – hostility, Fob – phobic anxiety, Par – paranoid ideation, Psi – psychoticsim, GIM – Global index of disturbance

hospitalization no significant differences between the groups regarding psychiatric symptoms or GID was found (Table 1).

At the fortieth day of hospitalization a statistically significant decline in all symptoms (except for symptoms of anxiety ($p=0.056$)) and GID was found in IG ($p<0.05$), compared with the first day results. In the CG there we did not find significant changes in any of the observed symptoms or indices. Comparison of IG and CG on the fortieth day of hospitalization showed significant group differences in all measured parameters ($p<0.05$), except in anxiety symptoms ($p=0.07$).

Cortisol levels

At the first day of hospitalization, test results demonstrated high levels of serum free cortisol in both groups, the average of the control group was 644.63 nmol/ L and the average of the investigated group of 689 nmol/L. Between the two groups at admission no statistically significant differences were calculated (Table 2).

TABLE 2
MEAN VALUES OF CORTISOL IN GROUPS ACCORDING TO THE TIME OF HOSPITALIZATION. THE RABLE REPRESENT THE RESULTS OF MEAN VALUES ($\overline{X}$) AND STANDARD DEVIATION (SD) OF SERUM FREE CORTISOL FOR BOTH GROUPS

Norm. range: 138–690 nmol/L	Control group		Investigating group	
	SV (nmol/L)	±SD	SV (nmol/L)	± SD
1. day	644.63	155.52	689.00	117.48
40. day	362.71	63.74	386.44	139.92

At the fortieth day of hospitalization a statistically significant decline in cortisol levels were measured in

TABLE 3
MEAN VALUES OF BLOOD LYMPHOCYTE CONCENTRATIONS (CD3, CD4, CD8, CD19 AND CD16CD56) ON THE DAY OF ADMISSION (1.DAY) AND ON THE FORTIETH DAY OF HOSPITALIZATION (40. DAY) IN CG AND IG. VALUES IN THE TABLE REPRESENT THE PERIPHERIAL BLOOD CONCENTRATION (CELLS/MM3) OD DISTINCT LYMPHOCYTE POPULATIONS AS MEASURED BY FLOW CYTOMETRY

Cells	Control group				Investigation group			
	1. day		40. dan		1. day		40. dan	
	SV	±SD	SV	±SD	SV	±SD	SV	±SD
CD3 /mm^3	1813.1	360.6	1885.5	321.7	1625.5	593.9	2344.5	975.9
CD4 /mm^3	1123.3	275.2	1225.1	272.2	975.4	328.4	1579.0	740.0
CD8/mm^3	712.7	130.3	655.2	137.7	649.5	338.8	724.4	320.5
CD16CD56/mm^3	367.3	104.6	273.6	101.8	314.0	140.8	331.7	180.0
CD19/mm^3	156.2	39.1	179.5	22.1	101.6	16.5	129.4	19.5
HLA DR %	25.2	14.6	179.5	22.1	18.2	10.2	9.5	3.5

Legend: CD3 – T cells; CD4 – T helper cells; CD8 – T cytotoxic cells; CD19 – B cells; CD16CD56 – NK cells; HLA DR – activated T cells

both groups ($p<0.05$). The average of the control group was 362.71 nmol / l, the average of the investigated group and 386.44 nmol / l. Between the two groups at the fortieth day no statistically significant differences were calculated.

Lymphocyte populations

At the first day of hospitalization, all mean values of individual groups of lymphocyte popultions were within the normal boundaries for the population. Between the two groups at admission no statistically significant differences were calculated (Table 3).

At the fortieth day of hospitalization a statistically significant increase in CD4 (T helper) and CD19 (B lymphocytes) lymphocytes were found in the IG. In the CG there was no statistically significant change in any of the observed lymphocyte population counts. Comparison of IG and CG on the fortieth day of hospitalization did not show any significant group differences in all measured parameters ($p<0.05$).

Liver enzymes and total bilirubin

At the first day of hospitalization, test results demonstrated high serum levels of liver enzymes and bilirubin, except for γ-GT in both groups. Among the various measured parameters between the groups was not statistically significant differences (Table 4).

At the fortieth day of hospitalization a statistically significant decline to normal values in serum levels of liver enzymes and bilirubin were demonstrated in both groups. Among the various measured parameters between the groups was not statistically significant differences.

Discussion

In our work we have confirmed that abstinence causes a major stress for the patients. At the first day of hospitalization, test results demonstrated high levels of psychiatric symptoms in both groups, resulting in high GID (global index of disturbance), most probably associated with high overall psychological distress at the start of hospitalisation. Patients also had high levels of serum free cortisol, liver enzymes and bilirubin. The latter may reflect well known data that regular consumption of alcohol (alcoholic beverages) affect directly or indirectly, the whole organism, and lead to increasingly serious psycho-physical degradation including serious health problems[7]. Additionally, hospitalization and start of abstinence caused a major stress (withdrawal syndrom), which is reflected as a strong activation of stress axis, as shown in our results.

Many discoveries demonstrated that the brain has receptor sites for naturally occurring opiatelike substances (endorphins, enkephalins, and dynor-phins) which are produced by the nervous system. Opiates such as morphine and some of the metabolic products of alcohol (tetrahydroisoquinolines) can also attach themselves to these receptors. It has been further demonstrated that the physiological craving for alcohol may be the result of a deficiency of the naturally occurring opiatelike substances as well as other neurochemical deficits (i.e., dopaminergic, GABAergic, and serotonergic). These neurochemical deficits can occur genetically or as a result of adaptive procesess in long-term heavy drinking[10].

Chronic alcohol consumption induce a decreased neuronal expression of naurotransmiter receptors, e.g. GABA A alpha 1 (Gama aminobutyric acid A) receptor. With cessation of alcohol consumption, decreased receptor levels causes a hypodopaminergic and a hypoopioidergic environment in the reword system circuits, manifesting with withdrawal symptoms. At the same time HPA axis is stimulated via CRF released from the extended amygdala, causing a stress response[11].

Ethanol and opiates have a common biochemical mechanism of action; the metabolic condensation by-products of ethanol, tetrahydroisoquinolines, link ethanol and opiate actions by virtue of interaction at multiple opioid receptor sites in the brain; Stress induces significant brain reductions of endogenous opioid peptides and induces copious alcohol consumption. Long-term ethanol ingestion remarkably reduces cerebrospinal fluid endorphin and plasma enkefalin levels in alcoholics[12].

Since abstinence from ethanol causes a hypodopaminergic and a hypoopioidergic environment in the reword system circuits, manifesting with withdrawal symptoms, we have tested the effects of food supplements that contains D-phenylalanine a peptidase inhibitor of opi-

TABLE 4
MEAN VALUES (SV) AND STANDARD DEVIATION (SD) OF SERUM LIVER ENZYMES (AST, ALT, Γ-GT) AND TOTAL BILIRUBIN (BILIRUBIN) ON THE DAY OF ADMISSION (1. DAY) AND ON THE FORTIETH DAY OF HOSPITALIZATION (40. DAY) IN CG AND IG

	Control group				Investigation group			
	spray		40. day		spray		40. day	
	SV	±SD	SV	±SD	SV	±SD	SV	±SD
bilirubin	14.63	7.27	7.83	4.39	18.20	11.59	10.06	2.84
AST	0.54	0.46	0.30	0.15	0.90	1.33	0.37	0.16
ALT	0.55	0.40	0.29	0.13	0.46	0.25	0.32	0.09
γ-GT	2.10	2.77	0.60	0.41	4.26	6.46	0.80	0.79

oide inactivation and L-amino-acids as nutritional source for dopamine synthesis – in order to alleviate the symptoms of alcohol withdrawal. In a prospective, randomized, double blind study we have followed the effects of 40-day treatment wilth food supplements in 20 patients suffering from alcohol addictions and starting a detoxification therapy[9].

At the first day of hospitalization no significant differences between the groups regarding psychiatric symptoms, serum free cortisol, lymphocyte populations, liver enzymes and bilirubin was found. During the therapy a significant decrease in SCL-90-R scores and a significant increase in CD4 and CD19 lymphocyte count was observed in the test group. The cortisol levels were significantly, but equally decreased during hospitalization in both groups, the same was with the liver enzymes and the total bilirubin values. The use of food supplement containing D-phenylalanine, L-glutamine and L-5-hydroxytriptophan alleviated the withdrawal symptoms and caused a rise in CD4 and CD19 lymphocyte population, but it does not affected the serum cortisol levels, which were probably mostly affected by the liver restitution.

Our results showed that abstinence was a severe stress burden for our patients. The test results SCL-90-R demonstrated severe psychological symptoms, in both groups at baseline. This is evidence of severe mental distress in patients at the beginning of the treatment. The difference between the two groups after 40 days of treatment is likely a result of dietary supplement containing D-phenylalanine, L-glutamine and L-5-hidroksitriptofan. This may confirm our hypothesis that administration of such dietary supplements reduces symptoms of withdrawal from ethanol. The success of a mitigation of symptoms of abstinence by using D-phenylalanine and neurotransmitter precursors in the form of L-amino acids (e.g. L-glutamine) have been also described in other studies[13,14].

D-phenylalanine primarily prevent the degradation of endogenous opioid peptides in the body, thereby reducing drop caused by abstinence and thus alleviating the symptoms of withdrawal from ethanol[13]. Much research confirmed that central effects of alcohol are at least partly mediated by the endogenous opioid peptides[12,15] and that the lack of them is associated with signs of withdrawal from ethanol[16]. Our results confirmed this hypothesis. Broad knowledge of this area in future will not affect only the treatment of substance abuse, but the application of this knowledge is also possible for diseases accompanied by chronic pain, such as reumatoid arthritis and the like.

High serum free cortisol, measured the first day of hospitalization, is a confirmation of the stress burden at the beginning of the treatment. In our patients, there are at least two strong factors that activate the stress axis. The first is the abstinence from ethanol itself and its actions on the brain, important for emotional responses[7,8]. The second factor is a chronic liver disorder that may increase the concentration of pro-inflammatory cytokines IL-1, IL-6 and TNF-alpha[17]. As IL-1 directly activates stress-axis reesponse by passing from blood to hipotalamic nuclei, liver inflammation strongly affect plasma cortisol levels. This is confirmed by data in our study, since the elevated free cortisol and elevated liver enzymes dropped simultaneously during therapy.

The concentration of free serum cortisol dropped to normal at the fortieth day of hospitalization in both groups equally. We can conclude that the addition of D-phenylalanine and L-amino acids do not have a measurable effect on free cortisol decrease during abstinence from alcohol. In alcoholics starting abstinence a liver inflammation may be the most important factor determining serum cortisol level since both, levels of cortisol and liver enzymes dropped within normal limits at the fortieth day of hospitalization. A decreased toxic effects of ethanol during abstinence reduce the rate of liver inflammation and consequently the secretion of pro-inflammatory cytokines from liver Kupfer cells[17]. That led to reduced secretion of cortisol from the adrenal[4]. Further research will better define the mechanisms of liver damage and will be particularly important in terms of opportunities for the immunomodulatory treatment of liver failure.

Information on the fortieth day of hospitalization, support our hypothesis that the administration of nutritional supplement containing D-phenylalanine, L-glutamine and L-5-hidroksitriptofan had a statistically significant impact in raising the concentration of T helper lymphocytes (CD4). The increase is in harmony with mental status results; since nutritional supplements did not influence the reduction of cortisol levels, the elevated CD4 counts may be explained by enhancement of endogenous opioids due to improved mental status. Some studies have shown that the lack of endogenous opioids in chronic ethanol abuse is one of the main factors responsible for the decrease in CD4 lymphocytes[18]. Furthermore, some studies showed that endogenous opioids effectively stimulate CD3 and CD4 cell proliferation and IL-2, IL-4 and INF γ synthesis[19,20].

On this basis, one might conclude that the main mechanism responsible for the increase in CD4 lymphocyte populations is the rise of endogenous opioids levels, caused by inhibition of their degradation. Indirectly, we can conclude also that the levels of endogenous opioids returned within the normal range, because high concentrations of opioids (eg heroin) have an immunosuppressive effects. The effects of inhibitors of peptidases (D-phenylalanine) on the concentration of CD4 cells is particularly interesting in terms of chronic alcohol abuse, since the number of T lymphocytes (CD4 and CD8) is usually decreased an chronic alcoholics. This is one of the reasons for increased morbidity due to infections in this population. Since CD4 cells are central cells in the immune response, the control of their concentration is especially important.

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UPOTREBA SUPLEMENTACIJE HRANE SA D-FENILALANINOM, L-GLUTAMINOM I L-5-HIDROKSITRIPTOFANOM U OLAKŠAVANJU SIMPTOMA SUSPREZANJA OD ALKOHOLA

SAŽETAK

Opisali smo upotrebu suplementacije hrane sa D-fenilalaninom, L-glutaminom i L-5-hidroksitriptofanom u olakšavanju simptoma susprezanja od alkohola kod pacijenata koji su započeli detoksikacijsku terapiju. Budući da apstinencija od etanola uzrokuje hipodopaminergičke i hipoopioidergičke uvjete u putevima centra za ugodu, koji se manifestiraju simptomima susprezanja, suplementi hrani koji sadrže D-fenilalanin, peptidazni inhibitor (od opioidne inaktivacije) i L-aminokiseline (za sintezu dopamina) su korišteni za obnovu manjka neurotransmitera i tako ublažuju simptome susprezanja od alkohola. 20 pacijenata koji su ovisnici o alkoholu i koji su započeli detoksikacijsku terapiju, uključeni su u prospektivnu, randomiziranu, dvostruko slijepu studiju. Pacijenti su nasumično podijeljeni u dvije grupe. Jedna je grupa u periodu od 40 dana primala suplemente D-fenilalanin, L-glutamin i L-5-hidroksitriptofan (istraživana skupina), a druga (kontrolna skupina) placebo. Prvog dana hospitalizacije pacijentima je napravljen SCL-90-R test i uzeti su uzorci krvi za mjerenje jetrenih enzima, ukupnog bilirubina, nevezanog kortizola i limfocita. Isto je napravljeno 40-og dana hospitalizacije. Za vrijeme terapije uočeno je značajno sniženje rezultata SCL-90-R kod psihijatrijskih simptoma i značajno povećanje CD4 limfocita u istraživanoj skupini. Vrijednosti kortizola su značajno porasle, ali podjednako u obje grupe. Isto je bilo sa jetrenim enzimima i ukupnim bilirubinom. Zaključili smo da je apstinencija veliki stres za pacijenta. Upotreba suplemenata u hrani koji sadrže D-fenilalanin, L-glutamin i L-5-hidroksitriptofan olakšava simptome susprezanja i uzrokuje povećanje CD4 limfocita, ali ne utječe na nivo serumskom kortizola, koji je vjerojatno više zahvaćen upalnim promjenama jetre i jetrenim oporavkom.