Journal of Addiction Research & Therapy

Shushpanova et al., J Addict Res Ther 2016, 7:2
http://dx.doi.org/10.4172/2155-6105.1000271

Research article Open Access

Treatment of Alcoholic Patients Using Anticonvulsant Urea Derivative Influences the Metabolism of Neuro-active Steroid Hormones - The System of Stress Markers

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Received date: Feb 24, 2016; **Accepted date:** Mar 16, 2016; **Published date:** Mar 23, 2016

Abstract

Objective: Disturbed homeostasis of neuroactive steroid hormones (NAS) may be a risk factor for the development of mental illness and alcohol addiction; psychopharmacological drugs that modulate the activity of NAS can cause clinical effects through their impact on the balance of hormones. We investigated the levels of NAS: cortisol, adrenocorticotropin (ACTH), dehydroepiandrosterone (DHEA) and dehydroepiandrosterone - sulfate (DHEA-S) in the blood serum of male alcoholics and healthy volunteers at baseline and at the background anticonvulsant therapy galodif, showed a positive trend in the reduction of craving for ethanol.

Materials and Method: The study included 68 patients with alcoholism only men and 23 healthy volunteers, standardized to the main group in age. Patients were treated at the Department of addictive states Mental Health Research Institute with the diagnosis according to ICD - 10: F10.232 and F10.302. The studies of the effect of therapy with galodif on the levels of NAS were performed by assigning galodif at dose of 300 mg/day during the ongoing course of treatment 21 days. ACTH, cortisol, DHEA and DHEA-S were determined with use kits for enzyme-linked immunosorbent assay (ELISA).

Results: Our research has shown that increased levels of hormones index ratio cortisol/DHEA in patients with alcoholism during abstinence significantly reduced on the background of the course of therapy with galodif. Reduced levels of cortisol and increasing DHEA levels during therapy with galodif indicates the stimulation of synthesis of DHEA, providing anti-glucocorticoid effect on the background of high levels of cortisol. DHEA and DHEA–S can protect neurons from glucocorticoid-induced neurotoxicity.

Conclusion: The ratio of cortisol/DHEA is of particular importance because the anti-glucocorticoid effect of DHEA underlies the reduction of anxiety and depression in humans. Galodif reduces the ratio of cortisol/DHEA that can enhance GABAergic neurotransmission and provide a positive effect on the therapy.

Graphical Abstract: Anticonvulsant galodif having anti-glucocorticoid effect and reduces the ratio of cortisol/DHEA that can enhance GABAergic neurotransmission in the brain and provide a positive effect of treatment of patients with alcoholism.

Keywords: Alcohol; Alcoholism; Neuroactive steroids; Adrenocorticotropin (ACTH); Cortisol; Dehydroepiandrosterone (DHEA); Dehydroepiandrosterone - Sulfate (DHEA-S); Anticonvulsant; Galodif

Abbreviations

AWS: Alcohol withdrawal syndrome; BzD: Benzodiazepine; BzDR: Benzodiazepine receptor (binding site for benzodiazepine); CNS: Central nervous system; Cl^-: Chloride ion; GABA: Gamma-aminobutyric acid; GABAA receptor: Receptor for gamma-aminobutyric acid type A; GABAA/BzDR: Gamma-aminobutyric acid type A receptor, coupled with allosteric binding site for Benzodiazepine (receptor complex); m-ch-BHU: meta-chloro-Benzhydryl urea; NAS: Neuroactive steroid hormones; CRF: Corticotropin-releasing factor; ACTH: Adrenocorticotropin; HPA: Hypothalamic-pituitary-adrenal axis; HPG: Hypothalamic-pituitary-gonadal axis; DHEA: Dehydroepiandrosterone; DHEA-S: Dehydroepiandrosterone sulphate; ELISA: Enzyme-linked immunosorbent assay

Introduction

One of the problems in the treatment of alcoholism is the need to find new approaches to prevention and treatment of this disease, the search for effective pharmacological correction aimed at molecular targets alcohol, eliminating the effects of alcohol intoxication and dependence, as well as prevention of this disorder. The main criteria

for alcohol dependence are: a strong or irresistible urge to consume alcohol and physiological withdrawal state when discontinuation or dose reduction of alcohol - alcohol withdrawal syndrome (AWS).

Alcoholic behaviour is determined by positive and negative reinforcement, and the contribution of each of these processes is various in different people and can vary according to the stage of development of alcohol dependence. There are two main reasons to continue to consume alcohol - positive and negative reinforcement: drink to feel good and drink not to feel bad [1-5]. Changes in brain stress system; increased sensitivity to stress influences is an important mechanism in the development of addiction, and the formation of alcohol relapse during abstinence in alcohol-dependent patients. The behavioural effects of ethanol can be secured through the positive (causing pleasure, activating) or negative (relieving anxiety, stress reducers) support mechanisms [6-9].

One of the alcoholism development theories suggests a shift in the general excitability of the brain as a result of reduction of the braking process. Violation of balance of excitation and inhibition in the brain may underlie the formation of high-risk alcohol abuse. Patients with alcohol dependence, there is increased irritability, impulsivity, extravagance and other disorders associated with these processes. Violation of braking processes and behavioural responses associated with increased sensitivity to stress in patients with alcoholism can provoke craving - "craving" for alcohol and to manage it [3,10-12]. Alcohol has pronounced anxiolytic properties; in connection with its use of this ability are a form of self-medication when an alarm occurs and the development of anxiety [7,13,14].

There is an "epileptogenic concept" emergence unmotivated paroxysmal - compulsive craving for alcohol. GABA-stimulating effect of drugs that increases the inhibitory processes in the brain is effective for the relief of symptoms of anxiety and depression, reducing irritability and aggression, reduction of withdrawal symptoms and craving for alcohol [15,16]. Questions the effectiveness of prevention and treatment of alcoholism are closely connected with the study of neurochemistry and neuropharmacology of brain changes that underlie the mechanisms of preference for alcohol, development of tolerance and withdrawal syndrome. Alcohol abuse causes neuroadaptive changes of inhibitory GABAA receptor system in the brain and metabolism of neuroactive steroids (NAS) that modulate the function GABAA/BzDR [14,16-18].

Disturbed homeostasis of NAS can be a risk factor for the development of mental illness and alcoholism [9,12,19]. Cortisol and, on the contrary, psychopharmacological drugs that modulate the activity of the NAS, may cause clinical effects through their impact on the balance of the NAS. Studying the effects of drugs have modulatory effects on brain metabolism, including neurosteroidogenesis can give a new understanding of the basic laws of formation of motivation and alcohol addiction, to develop new approaches to the treatment of this disease.

The aim of this work was to study the effect of therapy with an anticonvulsant galodif urea derivative (meta-chloro-benzhydryl urea) on metabolism of NAS in patients with alcoholism.

Materials and Method

In a study of 68 patients suffering from alcoholism have been included, only men aged 24 to 53 years old (mean age - 38.3 ± 8.9 years) living in Tomsk. The comparison group consisted of 23 healthy volunteers, standardized to the main group in age (36,50 ± 9,51 years), without having to present at the time of the study no complaints and found to be healthy on a standard set of clinical and laboratory tests. Patients were treated at the department of addictive states Mental Health Research Institute with the diagnosis according to ICD - 10: "Mental and behavioural disorders due to use of alcohol, dependence syndrome» (F10.232) and "Mental and behavioural disorders due to use of alcohol, withdrawal state" (F10.302). Alcoholism flow type in the surveyed patients wore secondary progression of the character. Patients with other psychiatric and endocrine disorders, or who use drugs that could alter the levels of steroid hormones in the study were not included. In all patients there was no serious liver disease. Patients included in the study, were characterized by a pronounced compulsive (paroxysmal) attraction to alcohol, affective psychopathology with dysthymic and dysphoric manifestations.

The study of the effect of therapy with an anticonvulsant galodif on the levels of NAS was performed by assigning galodif at the recommended dose of 300 mg/day (100 mg x 3 times a day) during the on-going course of treatment 21 days. ACTH, cortisol, DHEA and DHEA-S were determined with use kits for enzyme-linked immune-sorbent assay (ELISA).

To determine the concentration of NAS in serum from patients and healthy volunteers venous blood samples were collected from individual subjects in the morning on an empty stomach. Patients were informed about the planned study and gave their consent.

Collected serum samples were frozen and kept until analysis at -70°C freezer. To determine the levels of ACTH, cortisol, DHEA and DHEA-S we used kits for enzyme-linked immunosorbent assay (ELISA) Bio-Rad company (Germany) with the use of multi-analyzer «Victor» (Pribory Oy, Finland). The principle of the method is universal. Hormone levels in all groups were measured simultaneously. Statistical data processing was carried out using «Statistika 10.0» standard software for the «Windows». The study was approved by the Ethics Committee of Mental Health Research Institute.

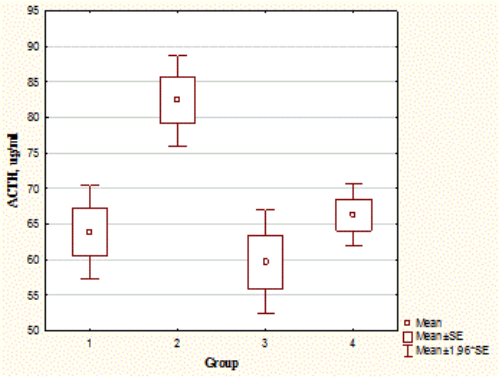

Figure 1: Statistical analysis of the level of ACTH in the blood serum of patients with alcoholism and volunteers before (Gr.1 - volunteers; Gr.2 - patients) and after treatment (Gr.3 - volunteers; Gr.4 - patients) with an anticonvulsant galodif.

Results

The study of cortisol and ACTH levels showed elevated levels in patients with alcoholism, compared with a control group of healthy volunteers (Table 1, Figures 1 and 2). Elevated cortisol is typical for the state of distress and depressive disorders peculiar to alcohol withdrawal syndrome (AWS) associated with a deficiency of GABA.

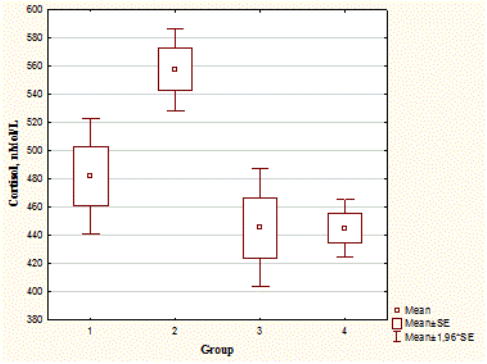

Figure 2: Statistical analysis of the level of cortisol in the blood serum of patients with alcoholism and volunteers before (Gr.1 - volunteers; Gr.2 - patients) and after treatment (Gr.3 - volunteers; Gr.4 - patients) with an anticonvulsant galodif.

In affective disorders and alcoholism is a violation of a negative feedback mechanism by which released from the adrenal cortisol inhibits the production of CRF, causing the contents of CRF, ACTH and cortisol increases abnormally.

We have identified a significant reduction in the level of DHEA and DHEA-S (Table 1, Figure 3 and 4) in blood serum from patients with alcoholism, likely a result of chronic exposure to alcohol and abstinent status in these patients.

DHEA has greater sensitivity to increased levels of ACTH, compared with cortisol. DHEA has expressed psychotropic effects - improved memory, antidepressant, anxiolytic and anti-aggressive action.

We have also found that the ratio of cortisol/DHEA in alcoholic patients twice the index of healthy volunteers in the control group (Table 2), it is indicating a severe stressor activity in alcoholic patients in a state of withdrawal.

The increased value of the coefficient cortisol/DHEA in patients with alcoholism during abstinence changes against the background of the course of therapy with an anticonvulsant galodif: ratio cortisol/DHEA decreases, but does not reach the value of this index in the control group of healthy volunteers. Increasing DHEA levels against the background of the therapy (Table 1, Figure 3) indicates the stimulation of synthesis of DHEA, providing anti-glucocorticoid effect on the background of high levels of cortisol.

Reducing elevated levels of cortisol and the ratio of cortisol/DHEA, also associated with an increase in DHEA levels in patients with alcoholism on the background of the therapy is a positive response to treatment with an anticonvulsant galodif.

The ratio of cortisol/DHEA is of particular importance, since the anti-glucocorticoid effects of DHEA led to a decrease in anxiety and depression in humans. Raising DHEA allosteric modulator GABAAR can improve the overall GABAergic neurotransmission in the CNS.

Type of Hormone	Volunteers		Alcoholic Patients	
	Prior to Therapy	After Treatment	Prior to Therapy	After Treatment
	(n = 23)	(n = 19)	(n = 68)	(n = 63)
ACTH (u/ml) {M ± SE}	63.82 ± 8.23	59.70 ± 9.16	82.41 ± 18.12*	66.26 ± 12.39
Cortisol (nmol/l) {M ± SE}	481.85 ± 41.31	445.30 ± 32.07	557.52 ± 2.84*	445.13 ± 7.52*
DHEA (u/ml) {M ± SE}	33.18 ± 10.54	31.92 ± 10.13	19.85 ± 5.00*	24.02 ± 6.48*
DHEA-S (ng/ml) {M ± SE}	2.48 ± 0.51	2.38 ± 0.56	1.75 ± 0.42*	1.14 ± 0.37*

Table 1: Levels of hormones in the blood serum of alcoholic patients and healthy volunteers before and after treatment, *: The level of significant difference $P < 0.005$.

DHEA exerts anxiolytic effects of chronic stress. In addition to the anti-glucocorticoid effects of DHEA and its ability to modulate the release of other modulatory GABA neurosteroids may also be involved in the implementation of its anxiolytic effect [20].

Reducing the level of DHEA-S in alcoholic patients during therapy with galodif (Table 1, Figure 4) can indicate a decrease in the pool of DHEA-S in connection with its transition into the more active non-sulfated form having greater lipophilicity and the permeability of the blood-brain barrier.

Group	Cortisol/DHEA	DHEA/DHEA-S
Volunteers prior to therapy	14.52	13.38

Volunteers after treatment	13.95	13.41
Patients prior to therapy	28.07*	11.34*
Patients after treatment	18.53*	21.07*

Table 2: The ratio cortisol/DHEA and DHEA/DHEA-S in alcoholic patients and healthy volunteers before and after treatment, *: The level of significant difference $P < 0.005$.

The decline in DHEA-S (Table 1, Figure 4) under the action of anticonvulsant therapy with galodif can testify to its transition into a more active form of DHEA, the level of which increased significantly during therapy. DHEA and DHEA-S have a potent neuroprotective and anti-glucocorticoid to the brain and can protect neurons from neurotoxicity induced by glucocorticoids.

A course of treatment with anticonvulsant galodif (21 days at a dose of 300 mg/day) of abstinence in alcoholics after withdrawal caused reduction state AWS symptoms and led to normalize balance of steroid hormones that modulate the function of GABAA/BzDR.

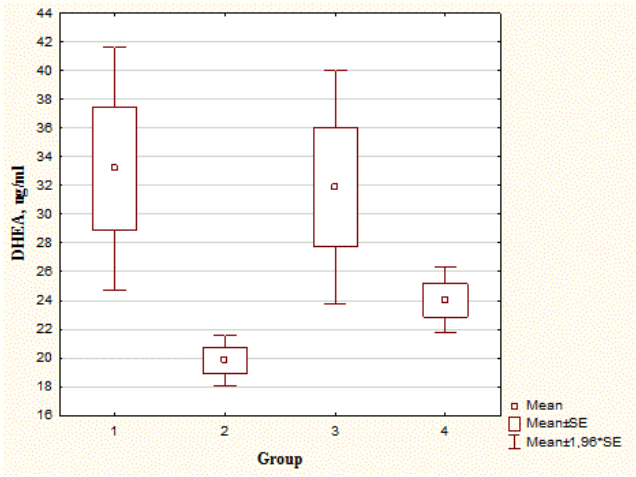

Figure 3: Statistical analysis of the levels of DHEA in blood serum in patients with alcoholism and volunteers before (Gr.1 - volunteers; Gr.2 - patients) and after treatment (Gr.3 - volunteers; Gr.4 - patients) with an anticonvulsant galodif.

Discussion

Alcohol and stress hormones cause neuroadaptive changes in the brain that can contribute to functional changes of the neuroendocrine system, and increased tendency to relapse, which ultimately can lead to the development of dependence [8,21-23]. Mechanisms of reward in the brain may be impaired as a result of chronic stress and alcohol abuse [3]. These processes are modulated by the hypothalamic - pituitary-adrenal (HPA) axis, the end product of which is cortisol [24-26].

The increase in the base level of cortisol could be due to anomalies in the functioning of the HPA - axis in patients with alcoholism. These disorders may be associated with abnormalities in neurotransmitter systems (dopaminergic, glutamatergic, GABA) and benzodiazepine receptor modulatory system involved in the regulation of the HPA – axis [27].

Role of cortisol can be caused by indirect influence its 3α, 5α- and 3α, 5β- reduced metabolites on GABAA receptors [27,28]. Cortisol metabolites have antagonistic effect at the GABAAR in a physiological concentration [28] of GABAergic interneurons.

GABAergic interneurons are the main component of the cortico-limbic system. They provide a regulation as the inhibitory and excitatory modulation of cortical and hippocampal circuits and contribute to the regulation of oscillatory rhythms, discriminatory treatment of information and sensory information in the cortico - limbic system [29].

Stress reaction within a dysfunctional system can lead to an imbalance of steroid hormones. This in turn may lead to excessive exposure to glucocorticoid corresponding neuroadaptive changes in various brain regions. Alcohol and neuroadaptive stress hormones induce changes in the brain, which may contribute to functional changes of the neuroendocrine system and increased tendency to relapse, which ultimately can lead to addiction [17,23-25,30].

Current data in neuropharmacology studies emphasize the NAS value in the regulation of inhibitory neurotransmission in the brain [31]. It suggests that NAS will have a significant role in the development of new approaches to the treatment of intractable patients with neurological and psychiatric disorders including depression, schizophrenia, alcoholism, multiple sclerosis, and other neurodegenerative disorders. This may be due to their ability to regulate the inhibitory neurotransmission in the brain, as well as the function of the HPA - axis and the HPG - axis of myelin formation, and other inflammatory processes. NAS are endogenous neuromodulators, which can be synthesized de novo in the brain as well as in the adrenal gland and gonads [29,31].

Disturbed homeostasis of NAS may be a risk factor for the development of mental illness and alcoholism; psychopharmacological drugs that modulate the activity of NAS may exert clinical effects by their impact on the balance of the NAS. The ability of NAS to reduce the activation of the HPA - axis may play an important role in the return to normal homeostasis. This physiological effect is critical for mental health in violation of its regulation associated with various psychiatric disorders, including depression, post-traumatic stress, dysphoric condition in women associated with ovulatory cycles, as well as alcohol addiction and AWS [32].

CRF - ACTH - cortisol axis has a high sensitivity to acute and chronic effects of alcohol is an important system in the implementation of anxiety and stress, which provide a balance between anxiolytic and anxiogenic effect of having an impact on GABAA - receptor system of the brain and regulated in its turn NAS, it represents an important target for the search for new psychopharmacological agents with the

anti-alcohol focus [19,21,24,25]. Activation of NAS to allosterically modulate the binding of GABA and BzD binding sites, can enhance the conductivity of GABA-mediated Cl^- ion conductivity, and directly stimulate [$^{36}Cl^-$] current in neuronal membranes [33].

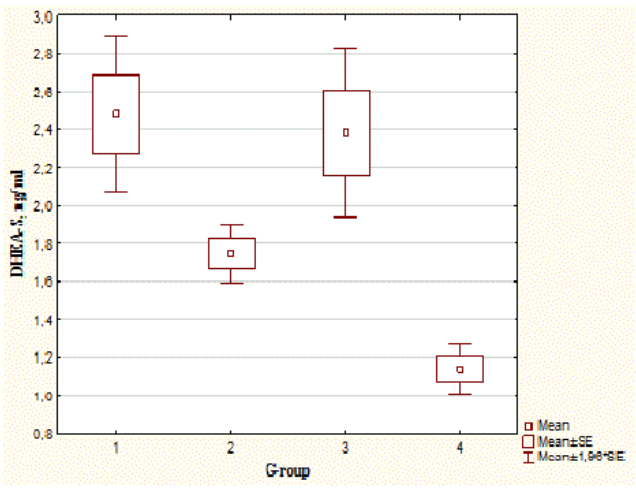

Figure 4: Statistical analysis of the levels of DHEA-S in blood serum in patients with alcoholism and volunteers before (Gr.1 - volunteers; Gr.2 - patients) and after treatment (Gr.3 - volunteers; Gr.4 - patients) with an anticonvulsant galodif.

Treatment with drugs that modulate the concentrations of these compounds can promote neurogenesis processes, improve neuronal survival, myelination processes, improve memory, and reduce neurotoxicity, which is especially important for toxic effects associated with alcohol abuse [9,22,34-36]. Galodif affects the NAS level in alcoholic patients that have a modulating effect on the BzDR and the GABAA - receptor complex, which as a result reduces the compulsive craving for alcohol [37].

NAS are targets for anticonvulsant galodif in the treatment of compulsive craving for alcohol and alcohol dependency, and this may be due to the influence of alcohol on emotiogenic areas of the brain - limbic structure, which is especially important, because they often localized focus of epileptiform activity, which can serve as the basis craving for alcohol in patients with alcohol addiction.

Conflict of Interest

Authors declare no conflict of interest.

Acknowledgement

The authors would like to thank the anonymous reviewers for their valuable comments and suggestions on an earlier version of this paper. The authors wish to express their appreciation to Mr. Vladimir N. Khudoley, general manager of Company LLC "Science Technology Medicine" for their assistance in carrying out the work and presentations and to Scientific Program «Nauka» No.2387, Scientific Program «Nauka» No. № 4.1991. 2014/K.

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