EVALUATION OF THE EFFECTS OF A DIETARY ANTIOXIDANT IN ANIMAL MODEL OF DEXAMETHASONE-INDUCED NEUROTOXICITY: A PRELIMINARY STUDY

*[1]Zo□a Danilczuk, [2]Hanna Koszel

[1]Department of Experimental and Clinical Pharmacology, Medical University of Lublin, Lublin, Poland
[2]Department of Experimental and Clinical Pharmacology, Medical University of Lublin, Lublin, Poland, Volunteer

*Corresponding author e-mail: zo□a.danilczuk@umlub.pl

Summary. Chronic stress or prolonged exposure to high levels of glucocorticoids induces neuropathological alterations, which are frequently associated with cognitive de□cits, such as the impairment of memory and learning. The results of our study have shown the prolongation of climbing time in "chimney" test, decreased retention time in the memory task, reduction of the body weight, but no change in the lethality in the mice chronically treated with dexamethasone (DEX). Spirulina, administered alone at both doses (0.5 or 1.0 mg/kg/day) changed neither behaviour of mice nor lethality in comparison with the control group. However, Spirulina at the dose of 1.0 g/kg/day reduced the body weight of the tested mice, as compared to the control group. In th mice treated with DEX, Spirulina (at the dose of 1.0 but not 0.5 g/kg/day) signi□cantly reduced the climbing time in 'chimney' test, improved acquisition of memory and did not affect lethality in comparison with DEX alone, however it further reduced the body weight of the mice subjected to dexamethasone for 14 days. Although the preliminary study suggests protective effect of Spirulina in this experimental model, it should be con□rmed in further research.
Keywords: neurotoxicity, neuroprotection, glucocorticoids, antioxidants, memory, mice

INTRODUCTION

Glucocorticoids (GCs) and other preparations, e.g. dexamethasone (DEX - a synthetic GCs receptor agonist) are often used therapeutically for their potent anti-inflammatory and immunosuppressive properties in different diseases such as allergic, rheumatic, neurological, and autoimmune diseases. (Parker et al., 2007, Salvarani et al., 2008, Hermandez-Rodriguez et al., 2009, Bijlsma et al., 2010). Despite many documented side effects, e.g. diabetes, osteoporosis, infections, glaucoma or cataract (Fardet et al., 2007), or cognitive disorders, glucocorticosteroids are still widely used in therapy. Natural endogenous glucocorticosteroids, such as cortisol (in humans) or corticosterone (in animals), secreted in greater amounts, e.g. in stress or in patients with severe depression, as well as glucocorticosteroid therapy can damage the central nervous system (CNS) (Seckl et al., 1995, Kiraly et al., 1997, Brown et al., 1999, Haynes et al., 2001), and lead to ventricular expansion and cortical atrophy, which results in cognitive dysfunctions [Rothschild et al., 1989]. The study has also shown that when administered alone, glucocorticosteroids affect memory. When applied in therapeutic doses, glucocorticosteroids (Keenan et al., 1995), dexamethasone (Wolkowitz et al., 1990, Newcomer et al., 1999) or cortisol (Kirschbaum et al., 1996) induce verbal dysfunction of declarative memory in healthy patients.

Our previous study also indicated that dexamethasone impeded both motoric and cognitive function of animals. (Danilczuk et al., 2006, 2005, 2001).

Various studies con□rm that extreme stress, i.e. a strong psychological stressor leads to an abrupt increase in blood cortisol (Seemann et al., 1997), and is connected with many memory de□cits (Kirschbaum et al., 1996), and that memory function improves with the reduction of cortisol level (Seemann et al., 1997, Wolkowitz et al., 1997). After having experienced an extremely stressful situation that could cause post-traumatic stress disorder (PTSD), patients showed various memory prob-

lems, including de□cits in declarative memory (e.g. recalling and mentioning facts), as well as memory fragmentation (autobiographic or traumatic memory, respectively) (Brewin et al., 1996).

Moreover, brain injury, caused by ischemic or hemorrhagic stroke or trauma, may also lead to the increased production of reactive oxygen species (ROS), and result in tissue damage via several different cellular molecular pathways. Stopping these processes may potentially play a great therapeutic role in safe treatment with glucocorticoids. Researchers have been searching for protective substances against the effects of oxidant stress, such as e.g. Spirulina whose antioxidant properties have already been described (Deng et al., 2010, Hernandez et al., 2009, Wu Q et al., 2010). Hawaiian Spirulina is the only cultured microalgae grown with ultra pure deep ocean water as a source of minerals and trace elements. It contains among others carotenoids, sulfolipids, glycolipids, phyocyanin, enzymes (superoxide dismutase) [Uchihara et al.,2016, Hernandez et al., 2009], which are constantly tested for their protective properties, and possibilities of using them in different diseases.

Therefore, the purpose of this study was to investigate the effect of Spirulina (blue-green algae) in the animal model of dexamethasone-induced neurotoxicity designed by the author.

MATERIALS AND METHODS

All procedures were conducted according to NIH Animal Care and Use Committee guidelines, and approved by the Ethics Committee of the Medical University of Lublin.

Male Albino Swiss mice (weighing initially 25-30 g) were used in the experiments. The animals were housed twelve in a cage. The laboratory temperature was 20^0C, with natural light-dark cycle, food and water provided *ad libitum*. All procedures were performed between 8:00 and 14:00.

Drugs

Spirulina paci□ca (Cyanotech Corporation, Kailua-Kona, Hawaii, USA) was administered at the doses of 0.5 g and 1.0 g/kg/day, *per os*, 4 h before dexamethasone-DEX (Dexaven, Jelfa, Poland) (16 mg/kg/day, *ip*) for 14 days. The long-term memory acquisition (the step-through passive avoidance test), motor performance (‘chimney’ test), as well as body weight and lethality were evaluated 14 days after the drugs administration.

Chimney’ test

The effect of chronic treatment with DEX, alone or with Spirulina, on motor performance was evaluated in the ‘chimney’ test (Boissier et al., 1960).

The mice had to climb backwards up a plastic tube (‘chimney’, 3-cm diameter, 30-cm long). Motor impairment was indicated by the inability of mice to climb backwards up the tube within 60 s. The mice were pretrained 24 h before the treatment, and those unable to perform the test were rejected from the experimental groups.

Long-term memory acquisition test

The step-through passive avoidance test is regarded as a measure of long-term memory acquisition (Venault, et al.,1986).

The mice were placed in an illuminated box (10 x 13 x 15 cm) connected to an electric grid floor. In this test, entry into the dark compartment was punished by an electric footshock (0.6 mA, for 2 s) for facilitation of acquisition. The mice that did not enter the dark compartment within 60 s were excluded from the experiment. On the following day (24 h later), the same animals were again placed in the illuminated box, and the time of entry into the dark compartment was registered. Retention was evaluated as the mean time (in seconds) required for entering the dark compartment.

Body weight and lethality of mice was controlled each day of the experiment.

Statistical analysis

The behavioural data and body weight were analysed by one-way analysis of variance (ANOVA) and Tukey-Kramer post-test, and lethality results by Fisher`s Exact test 2x2.

RESULTS

The effect of prolonged treatment with Spirulina on DEX-induced lethality

DEX given at the dose 16 mg/kg/day did not evoke mortality in mice during 14 days of the experiment. Spirulina, at the doses of 0.5 or 1.0 g/kg/day, administered both alone and with dexamethasone, did not cause mortality in mice, either (data not shown).

The effect of prolonged treatment with Spirulina on DEX-induced reduction of body weight gain in mice

DEX given alone at the dose 16 mg/kg/day for 14 days decreased the body weight in the mice by about 8% of initial body weight, and about 13%

in comparison with the control group. Spirulina (0.5 or 1,0 g/kg/day) given alone did not modify the body weight of mice in comparison with the control group. However, Spirulina at the dose 1.0 g/kg/day potentiated the reduction of body weight during the experiment by about 5% (data not shown).

The effect of prolonged treatment with Spirulina on motor coordination impaired by DEX

As shown in Fig.1, DEX given for 14 days at the dose of 16 mg/kg/day signi□cantly increased the time of climbing in the 'chimney' test by about 140%. When given alone, Spirulina, at both doses did not change the motor coordination in mice.

However, Spirulina at the dose of 1.0 g/kg/day (but not at the dose of 0.5 g/kg/day) signi□cantly improved the DEX-impaired motor coordination in mice.

The effect of prolonged treatment with Spirulina on long-term memory acquisition impaired by DEX

DEX given alone for 14 days signi□cantly decreased the retention time in the memory task by about 40% in comparison with the control group (Tab.1).

Although Spirulina administered alone at any of the two doses did not affect the long-term memory of mice in comparison to the control group, however it signi□cantly improved long-term memory acquisition impaired by DEX at the higher dose.

DISCUSSION

The results of this study indicate that prolonged administration of DEX for 14 days at the dose of 16 mg/kg/day signi□cantly decreased the retention time of mice in the memory task and impaired motor coordination. It also reduced the body weight but did not evoke mortality in mice. Spirulina, administered alone at the dose of 0.5 or 1.0 g/kg/day did not change signi□cant parameters in these tests. In spite of leading to a greater reduction in the body weight, Spirulina applied at the dose of 1.0 g/kg/day prevented impairment of motor coordination, and prolonged the retention time in the long-term memory task in the mice treated with DEX.

Dexamethasone, like other glucocorticosteroid preparations, due to its ef□ciency is still widely used in the treatment of many diseases, despite its undesirable side effects such as e.g. cognitive disorders including dif□culties in focusing attention and concentration, or memory loss and thinking disorders. (Young et al., 1999, Bjelaković et al., 2007).

In stress response, activation of the hypothalamic-pituitary-adrenal axis, and particularly the release of glucocorticoids plays a critical role. Dysregulation of this system and maintained high plasma levels of glucocorticoids can result in depression. Recent studies have suggested the involvement of reactive oxygen species (ROS), such as superoxide anions in depression. However, direct evidence for a role of ROS in the pathogenesis of this disorder is still missing. The results of Uchihara et al. (2016) suggest that overexpression of SOD1 protects mice against glucocorticoid-induced depressive-like behaviours by decreasing cellular ROS levels.

Other authors have found that most of the dietary bioactives display antioxidant and anti-inflammatory activity through the protection of cortisol response (Raciti et al., 2016). All compounds, except for quercetin, reveal antioxidant activity, and also protect cortisol response. This indicates that the antioxidant activity of compounds plays an important role in the protection of GC response. However, in addition to antioxidant activity of bioactives, other mechanisms also seem to be involved in this protective, anti-inflammatory effect. These novel □ndings point to increased ROS concentrations playing a direct role in the heritable alterations in differentiation potential induced by DEX exposure [Ruijters et al., 2016].

Spirulina is now widely used as nutraceutical food supplement worldwide. Recently, great attention and extensive studies have been devoted to evaluate its therapeutic bene□ts in an array of pathological conditions including hypercholesterolemia, hyperglycerolemia, cardiovascular diseases, inflammatory diseases, cancer, and viral infections. The antioxidant and/or anti-inflammatory activity of Spirulina was demonstrated in a large number of preclinical studies (Deng et al., 2010).

Spirulina has been said to have numerous bene□cial effects, such as activation of cellular antioxidant enzymes, inhibition of lipid peroxidation and DNA damage, scavenging free radicals, and increasing the activity of superoxide dismutase and catalase. It will taper off antioxidant activity above a certain threshold level. In clinical trials, Spirulina has been shown to prevent skeletal muscle damage under exercise-induced oxidative stress, and to stimulate the production of antibodies. It is also capable of up- or downregulating the expression of cytokine-encoding genes to induce immunomodulatory and anti-inflammatory responses. Still, it has not yet been found out what molecular mechanism(s) make Spirulina induce these activities, although phycocyanin and β-carotene are certainly important molecules

in this process. Moreover, Spirulina has a regulatory effect on the ERK1/2, JNK, p38, and IκB pathways. These findings may give new insight into the potential therapeutic applications of Spirulina and may bring new ideas for the future (Patil et al., 2016, Wu Q et al., 2016).

The results of our experiment indicate the potential protective properties of Spirulina. When administered at higher dose, Spirulina demonstrated its protective activity against DEX-induced motor and cognitive disorders in mice, which allows us to direct the research accordingly and search for neuroprotective drugs/substances against undesirable effects of glucocorticosteroids on the CNS.

CONCLUSIONS

Although the preliminary study suggests protective effect of Spirulina in this experimental model, it should be confirmed in further research. Neurotoxic activity of glucocorticosteroids, observed both in clinical and animal tests has inspired researchers to look for neuroprotective substances. Finding protective drugs/substances without undesirable side effects may lead to explaining the mechanism of the observed neurotoxic activity of glucocorticosteroids. It may also make the therapy safer, and if these drugs need to be used, it may help prevent neurodegenerative changes resulting from the activity of these hormones in the human brain. Moreover, using substances of plant origin makes the therapy more accessible and less expensive.

Table 1. The effect of spirulina on long-term memory acquisition impaired by dexamethasone (dex).

DRUGS (dose/24h)	**RETENTION TIME (S) Means ± SEM**
Vehicle	161.1± 8.9
SPIRULINA 0.5 g	144.7 ± 7.9
SPIRULINA 1.0 g	167.7± 8.1
DEX 16 mg/kg	119.3 ± 11.6[a]
SPIRULINA 0.5 g + DEX 16 mg/kg	141.0 ± 11.3
SPIRULINA 1.0 g + DEX 16 mg/kg	159.6 ± 7.4[b]

DEX (16 mg/kg/day) was administered for 14 days, the last dose 48 h before the test.
Spirulina was given 4 h before the injection of DEX.
a - vs vehicle, b - vs DEX alone treated mice. $P<0.05$; N=6-8
One-way analysis of variance (Anova) and Tukey-Kramer Multiple Comparisons test.

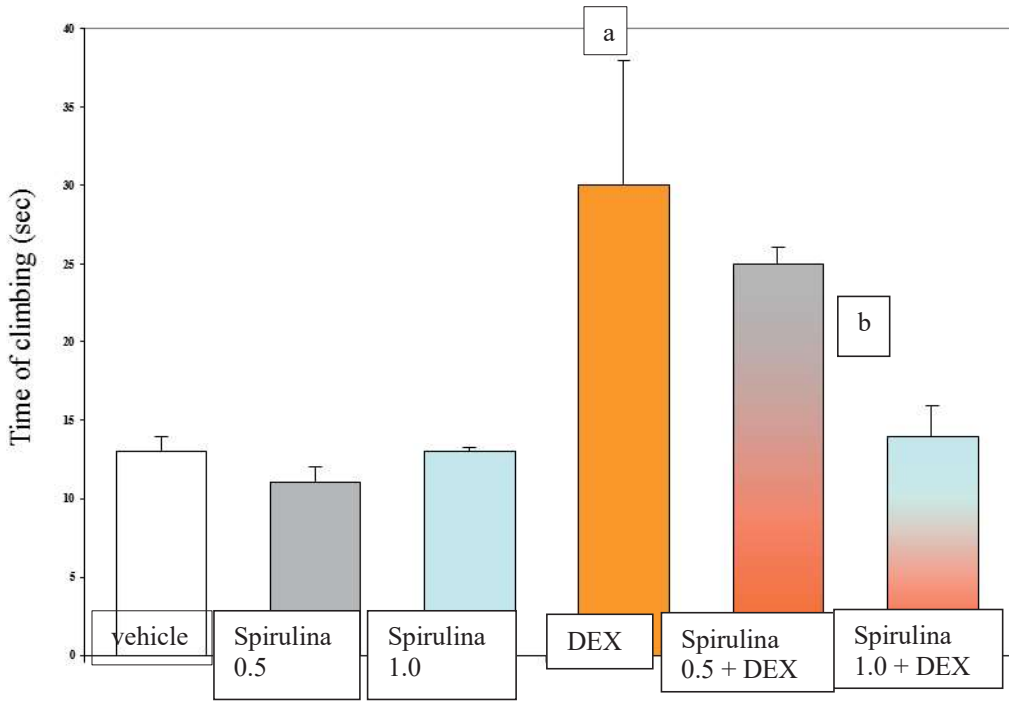

Figure 1. The effect of prolonged treatment with spirulina on the motor impairment in mice treated with dexamethasone (dex) ('chimney' test).

DEX 16 mg/kg/ (24h) was administered for 14 days, the last dose 24 h before the test. Spirulina (0.5 or 1.0 g/kg/day) was injected for 14 days, 4 h before DEX. a-p< 0.001 vs vehicle, b-p<0.001 vs DEX alone treated mice. Parametric Anova test and Tukey-Kramer post-test. N=6-9

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