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EFFECT OF THEMULTIPROBIOTIC THERAPY ON THE HYPOTHALAMIC-PITUITARY-ADRENAL SYSTEM AND CYTOKINE PROFILE UNDER CONDITIONS OF STRESS

It was studied the influence of multistrain probiotic (MP) on the stress hormones content (adrenocorticotropic hormone (ACTH) and cortisol) and the content of proinflammatory (interleukin (IL) 1β and IL-12Bp40) and antiinflammatory (IL-4 and IL-10) cytokines in conditions of erosive and ulcerative lesions caused water immersion restraint stress (WIRS). Established that MP significantly accelerated recovery of functioning of the hypothalamic-pituitary-adrenal system in terms of the stress action, that was confirmed by a more rapid return of ACTH and cortisol concentrations to values of intact rats. Also MP decrease proinflammatory (IL-1β and IL-12B p40) and increase antiinflammatory (IL-4 and IL-10) cytokines content in the rats serum after WIRS. These data suggest that one of the mechanism of the therapeutic effect of MP on lesions in the gastric mucosa caused by stress is the impact on the stress system and cytokine profile.

Keywords: adrenocorticotropic hormone, cortisol, cytokines, stress, multistrain probiotic.

Physiological response to stress is a compensatory reaction that eliminates or reduces the degree of homeostasis alteration [1]. A mobilization of all systems is the basis of this response. Due to the activation of the hypothalamic-pituitary-adrenal system (stress system) levels of glucocorticoids grows, which enables increased blood glucose as the main energy source for the adaptation to stress [2]. Under conditions of excessive exposure to stressful factors processes of immune reactivity trigger to protect against possible infections [3, 4]. The result is anexcretion of proinflammatory cytokines (interleukin-1 (IL-1), IL-6, IL-12, tumor necrosis factor α). Today it is known that inflammatory mediators engaged in a stimulating impact on the stress systemthat is detected by the increased synthesis of corticotropin-releasing hormone and glucocorticoids, which in turn suppress the immune response by negative feedback [5-8]. When adaptation to excessive or prolonged stress exposure is insufficient, pathological changes in organism emerge. And first of all, significant release of stress hormones damages the gastric mucosa (GM).

Under prolonged stress action endocrine glands (pituitary and hypothalamus) are depleted, resulting in fall of glucocorticoids levelbelow the physiological. To maintain the integrity of the GM, homeostatic cortisol level is essential, as both too high and too low content of this hormone damages the GM [9]. It is observed disbalance in self-regulation of the "stress system – immune system" loop under the depletion of the hypothalamic-pituitary-adrenal system. Significant release of proinflammatory cytokines in these conditions aggravates the lesions of the GM [10].

Today, more and more data suggest the relationship between symbiotic microflora of the intestine, nervous system and stress system [11]. An immune system plays not the least role in this interaction. In our previous study, we found that the therapeutic administration of multistrain probiotic (MP) "Symbiter ® acidophilic concentrated" reduced erosive and ulcerative lesions under conditions of stress action [12]. To reveal the mechanisms of such influence the aim of current work was to determine the effects of MP on the content of stress hormones (cortisol and adrenocorticotropic hormone(ACTH)) and the content of proinflammatory (interleukin (IL) 1β and IL-12Bp40) and antiinflammatory (IL-4 and IL-10) cytokines under conditions of erosive and ulcerative lesions in GM induced by water immersion restraint stress (WIRS).

Methods

The study was carried on 70 male rats in accordance with the standards of the Convention on Bioethics of the Council of Europe's, 1997, European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and the Law of Ukraine from 21.02.2006 № 3447-IV "On Protection of Animals from Abuse".

Animals were divided into 10 groups of 7 rats each (Table 1).

***Table* 1. The deWIRSion of rats on the research groups**

Group number	The number of therapeutical injections	The substance that was injected	Duration (hours) between stress exposure and measuring of hormones and cytokines in serum
1	–	–	intact rats (not subjected to stress)
2	–	–	stress control (hormones and cytokines level was measured immediately after stress)
3	2	water (control)	24
4	2	multistrain probiotic	24
5	4	water (control)	48
6	4	multistrain probiotic	48
7	6	water (control)	72
8	6	multistrain probiotic	72
9	8	water (control)	96
10	8	multistrain probiotic	96

Rats were subjected to 3-hour WIRS by Takagi et al., 1964 [13]. One day prior to the experiment, the rats were not fed, but they had free access to water. For immobilization rats were placed in a perforated metal camera that was putdown vertically into the water for 3 hours so that the water level reaches the jugular fossa of animals. Water temperature was 22-23° C.

After the stress animals of 3-10 groups were treated with water or aqueous solution of MP in a volume of 0.5 ml/200 g orally twice a day. MP containing 14 probiotic strains genera *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Propionibacterium*, was administered at a dose of 140 mg/kg (1.4×10^{10} CFU/kg). Treatment was started in an hour after 3-hour WIRS.

Intact and exposed to stress animals were sacrificed by cervical dislocation after a specified time after the WIRS. Rats blood was collected from the heart into centrifuge tubes without anticoagulant and leaved for 20-30 minutes at room temperature to complete the formation of a clot. Then, blood samples were centrifuged at 1000 g for

15 minutes and the supernatant (serum) were harvested in separate disposable microtubes, frozen at -20° C and used for further studies. Serum ACTH and cortisol content were determined at 1st-3rd days after WIRS, the antiinflammatory cytokines –at 1st-4th days. ACTH and cortisol were determined by ELISA using commercial kits of DRG International Inc. (USA) and SRL LLC "Granum" (Kharkiv, Ukraine)production. Their contents were expressed in pg/ml and nmol/l accordingly. The content of IL-1β was measured by ELISA using commercial kits of GE Healthcare production (Amersham, IL-1β Rat Biotrak ELISA System) and expressed as pg/ml. Content of IL-4, 10, 12B p40 was measured by ELISA using specific polyclonal antibodies (Sigma). Their expression are expressed in units of optical density. All samples were analyzed in two repetitions.

Statistical analysis of data was carried out by the "Statistica 8.0" software package. For the analysis of the data distribution type Shapiro-Wilk`sW criterion was used. Since the obtained results were normally distributed, t-Student test for independent samples was used for the comparison of data. Mean of value (M) and standard error of the mean (m) were calculated. Significant difference was considered at $p \le 0,05$.

Results. It was found that the concentration of ACTH in the serum of intact rats was 23 ± 9,7 pg/ml, and the concentration of cortisol – 27 ± 8,3 nmol/l. As a result of stress cortisol levels increased by 2.2 times ($p<0.001$), while the concentration of ACTH decreased by 7.9 times ($p<0.001$), that confirmed the negative feedback between the level of cortisol in the blood and the level of secretion of ACTH by pituitary gland (Fig. 1a, b) [14]. After 24 hours from the WIRS concentration of cortisol in the blood serum of rats decreased by 5.7 times($p<0.001$) compared with intact controls, which may indicate adrenal depletion under the influence of stress factors and the enter to the third phase of the general adaptation syndrome – the stage of depletion (fig. 1a, b). ACTH concentration after 24 hours was reduced by 3.2-fold ($p<0.01$) compared with intact rats, which may indicate depletion of the pituitary gland. The concentration of ACTH was 2.5 times higher ($p<0.05$) compared with measured immediately after WIRS (Fig. 1a, b). For the next 2 days after WIRS it was established a gradual recovery of the level of ACTH and cortisol to the intact control values. So, after48 hours from the WIRS concentration of ACTH was 1.9 times lower ($p<0.05$), and cortisol – 3.4 times lower ($p<0.001$) compared with intact controls. Within 72 hours after the WIRS ACTH concentration in serum of rats treated with water did not differ significantly from that of intact rats, but cortisol concentration was lower by 1.6-fold the level of the intact control ($p<0.05$) (Fig. 1a, b). So, for 3 days after the stress concentration ACTH and cortisol plasma levels were reduced compared with intact rats, indicatingthe depletion of the endocrine glands, and gradually restored to normal levels.

MP significantly accelerated recovery of functioning of the hypothalamic-pituitary-adrenal system under stress conditions, which was confirmed by a more rapid return of ACTH and cortisol concentrations to values of intact rats (Fig. 1a, b). The level of ACTH under the treatment of MP did not differ from the intact control on the 1st day after the WIRS and the cortisol concentration was restored to the level of intact animals in 3 days after stress exposure. Thus, the effect of MP on the content of stress hormones is one of the mechanisms of its gastroprotective effect. Indeed, we have found significant erosive and ulcerative lesions of the GM on the 1st-3rd days after WIRS despite ofsmall level of ACTH and cortisol in the blood of rats. And the ulcer area in 3 days after WIRS sighnificantly exceeded the registered immediately after stress. Therapy with MP facilitated the restoration of basal levels of ACTH and corticosteroids, which correlated with acceleration ofthe stress-induced lesions healing in the GM.

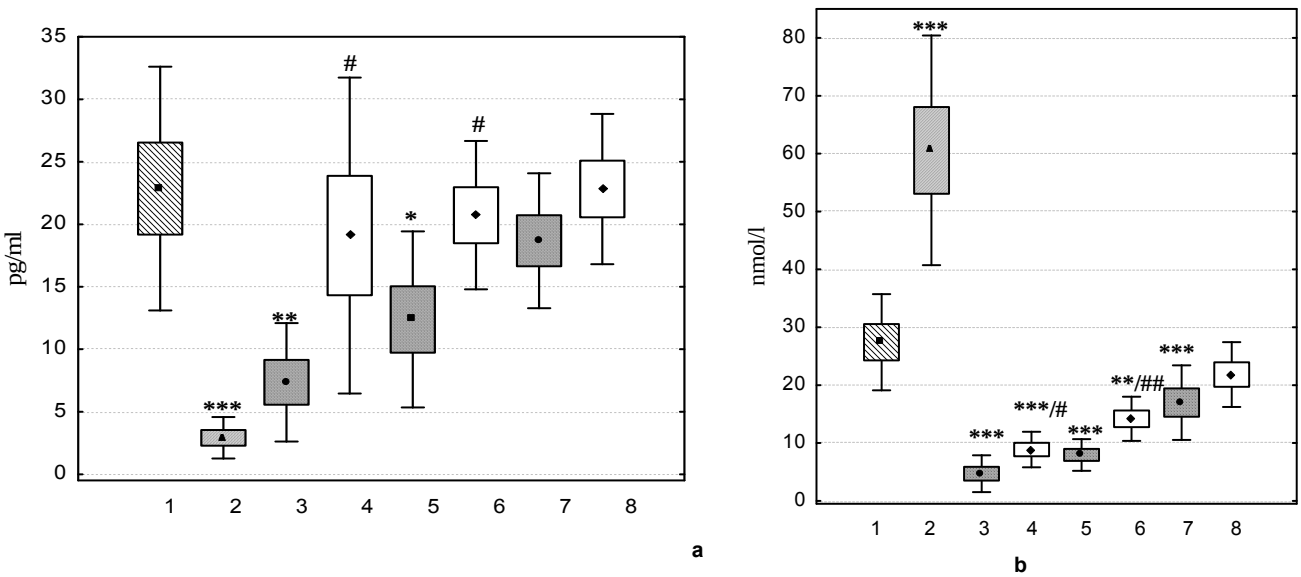

Figure 1. Content of adrenocorticotropic hormone (a) and cortisol (B) in serum of rats after water immersion restraint stress and under the conditions of therapeutic administration of multistrain probiotic (n = 7 per group) (M; box: SD; whiskers: m)
1 – intact control; 2 – immediately after stress; 3, 5, 7 – 1, 2, 3 days after stress in rats who were treated with water;
4, 6, 8, 1, 2, 3 days after stress in rats which were treated with probiotic

*, **, *** – $p<0.05$, $p<0.01$, $p<0.001$ compared with intact control, #, ## – $p<0.05$, $p<0.01$ compared with corresponding groups of rats treated with water.

Study of immunomodulatory properties of MP under conditions of stress-induced lesions of the GM showed that after the application of stress in serum of rats which were injected with water the concentration of proinflammatory cytokines IL-1β and IL-12B p40 significantly increased and remained higher compared to intact animals within 4 days

after WIRS (Table 2). The concentration of antiinflammatory IL-4 was also higher compared to control at all the days of observation after the WIRS, indicating a compensatory function of the immune system in terms of the stress. At the same time, the content of IL-10 in serum of rats after stress did not change.

***Table* 2. The content of proinflammatory and antiinflammatory cytokines in rats serum under water immersion restraint stress and therapeutic administration of multistrain probiotic (M ± m, n = 7 per group)**

index / Groups of rats	Interleukin 1β	Interleukin 12Bp40	Interleukin 4	Interleukin 10
1 (Intact rats)	314±71	0,105±0,015	0,076±0,009	0,199±0,018
2 (immediately after stress)	403±68*	0,268±0,025***	0,076±0,007	0,222±0,015
3 (Control, 1 day after WIRS)	449±66*	0,394±0,056***	0,172±0,040*	0,235±0,008
4 (Multistrain probiotic, 1 day after WIRS)	414±57*	0,311±0,034***	0,270±0,054***/#	0,374±0,063*/#
5 (Control, 2 days after WIRS)	563±51***	0,233±0,035**	0,141±0,023*	0,145±0,035
6 (Multistrain probiotic, 2 days after WIRS)	409±61***/#	0,179±0,032*/#	0,084±0,020#	0,171±0,013
7 (Control, 3 days after WIRS)	511±66**	0,293±0,016***	0,116±0,021	0,211±0,025
8 (Multistrain probiotic, 3 days after WIRS)	319±53##	0,258±0,030***	0,087±0,023	0,190±0,016
9 (Control, 4 days after WIRS)	471±54*	0,137±0,023	0,122±0,017*	0,214±0,020
10 (Multistrain probiotic, 4 days after WIRS)	334±41#	0,082±0,006#	0,072±0,007##	0,174±0,012

*, **, *** – p <0.05, p <0.01, p <0.001 compared with intact controls, # # # – p <0.05, p <0.01 compared with corresponding groups of rats treated with water.

Treatment with MP significantly reduced the concentration of proinflammatory IL-1β and IL-12B p40 after stress. For example, under the MP administration content of IL-1β and IL-12B p40 after WIRS did not differ from the level of intact controls at 3rd and4th day accordingly. It was found a strong effect of MP on the concentration of antiinflammatory cytokines. MP elevatedthe IL-4 concentration by 57% (p<0.05) and IL-10 by 59% (p<0.05) compared with the group of rats treated with water at 1st day after WIRS. In the following days, the concentration of antiinflammatory cytokines IL-4 and IL-10 in the group of rats administered with MP did not differ from that of intact animals. The results indicate an anti-inflammatory effectof MP under conditions of stress-induced lesions of the GM.

These results are consistent with other studies that have shown that probiotic strains reduce the concentration of proinflammatory cytokines under various pathologies of the digestive system [15-17]. Thus, Lin-Lin Chen et al. (2009) found that probiotics can reduce the content of IL-1β levels in experimental colitis [15]. Rodes et al. (2013) revealed that *Bifidobacterium longumsubsp. infantis* reduces the concentration of tumor necrosis factor-α and increases the concentration of anti-inflammatory IL-4 in a model of human intestinal microbiota[16]. Bermudez-Brito et al. (2013) demonstrated a reduction in proinflammatory cytokines level produced by human dendritic cells infected with *Salmonella typhi*, under the influence of *Bifidobacterium breve* CNCM I-4035 [17].

Summing up the results, we can conclude that the probiotic strains reduce the immune response and eliminate stress hyperactivation under stress creating favorable conditions for stress-induced lesions healing in the GM.

Conclusions.

1. MP restored basal levelof ACTH and corticosteroids in conditions of WIRS.

2. MP possessed the antiinflammatory effect under stress action, which was confirmed by a decrease of proinflammatory and increase of antiinflammatory cytokines in the serum of rats.

3. Effect of MP on the system stress and the immune system is one of the mechanisms of thestress-induced lesions healing in GM.

References

1. Veissier I. Stress and welfare: two complementary concepts that are intrinsically related to the animal's point of view / I. Veissier, A. Boissy // Physiol Behav. – 2007. – Vol.92, № 3. – P.429-433.

2. Compensatory gastroprotective role of glucocorticoid hormones / L. P. Filaretova [et al.]// Ross Fiziol Zh Im I M Sechenova. – 2007. – Vol.93, № 11. – P.1217-1228.

3. Involvement of interleukin-1 in immobilization stress-induced increase in plasma adrenocorticotropic hormone and in release of hypothalamic monoamines in the rat / F. Shintani [et al.] // J Neurosci. – 1995. – Vol.15, № 3 Pt 1. – P.1961-1970.

4. Campisi J. Stress-induced extracellular Hsp72 is a functionally significant danger signal to the immune system / J. Campisi, T. H. Leem, M. Fleshner // Cell Stress Chaperones. – 2003. – Vol.8, № 3. – P.272-286.

5. Acute stress modulates the histamine content of mast cells in the gastrointestinal tract through interleukin-1 and corticotropin-releasing factor release in rats / H. Eutamene [et al.] // J Physiol. – 2003. – Vol.553, № Pt 3. – P.959-966.

6. Corticotropin-releasing factor-producing neurons in the rat activated by interleukin-1 / F. Berkenbosch [et al.] // Science. – 1987. – Vol.238, № 4826. – P.524-526.

7. Release of multiple hormones by a direct action of interleukin-1 on pituitary cells / E. W. Bernton [et al.] // Science. – 1987. – Vol.238, № 4826. – P.519-521.

8. Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor / R. Sapolsky [et al.] // Science. – 1987. – Vol.238, № 4826. – P.522-524.

9. From Gastroprotective to Ulcerogenic Effects of Glucocorticoids: Role of Long-Term Glucocorticoid Action / L. Filaretova [et al.] // Curr Pharm Des. – 2013. – [Epub ahead of print].

10. Accelerated Ulcer Healing and Resistance to Ulcer Recurrence with Gastroprotectants in Rat Model of Acetic Acid-induced Gastric Ulcer / Oh T. Young [et al.] // J Clin Biochem Nutr. – 2008. – Vol.42, № 3. – P.204-214.

11. The microbiome: stress, health and disease / Moloney R. D. [et al.] // Mamm Genome. – 2013. – [Epub ahead of print].

12. Вірченко О. В. Дослідження лікувально-профілактичного впливу мультипробіотика "Симбітер" при розвитку гострих уражень в слизовій оболонці шлунка щурів / О. В. Вірченко, Т. В. Берегова, О. І. Цирюк // Вісник проблем біології і медицини. – 2010. – № 1. – P.41-46.

13. Takagi K. Studies on the Drugs for Peptic Ulcer. A Reliable Method for Producing Stress Ulcer in Rats / K. Takagi, Y. Kasuya, K. Watanabe // Chem Pharm Bull (Tokyo). – 1964. – Vol.12, № – P.465-472.

14. Relations of plasma ACTH and cortisol levels with the distribution and function of peripheral blood cells in response to a behavioral challenge in breast cancer: an empirical exploration by means of statistical modeling / G. van der Pompe [et al.] // Int J Behav Med. – 1997. – Vol.4, № 2. – P.145-169.

15. Therapeutic effects of four strains of probiotics on experimental colitis in mice / L. L. Chen [et al.] // World J Gastroenterol. – 2009. – Vol.15, № 3. – P.321-327.

16. Effect of probiotics Lactobacillus and Bifidobacterium on gut-derived lipopolysaccharides and inflammatory cytokines: an in vitro study using a human colonic microbiota model / L. Rodes [et al.] // J Microbiol Biotechnol. – 2013. – Vol.23, № 4. – P.518-526.

17. Cell-free culture supernatant of Bifidobacterium breve CNCM I-4035 decreases pro-inflammatory cytokines in human dendritic cells challenged with Salmonella typhi through TLR activation / M. Bermudez-Brito [et al.] // PLoS One. – 2013. – Vol.8, № 3. – P.e59370.

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ВПЛИВ ТЕРАПЕВТИЧНОГО ВВЕДЕННЯ МУЛЬТИПРОБІОТИКА НА ГІПОТАЛАМО-ГІПОФІЗАРНО-НАДНИРКОВУ СИСТЕМУ І ЦИТОКІНОВИЙ ПРОФІЛЬ В УМОВАХ СТРЕСУ

Вивчали вплив мультиштамного пробіотика (МП) на вміст гормонів стресу (адренокортикотропний гормон (АКТГ) та кортизол) та вміст прозапальних (інтерлейкін (ІЛ) 1β та 12В р40) та антизапальних (ІЛ-4 та ІЛ-10) цитокінів за умов ерозивно-виразкових уражень, викликаних водно-іммобілізаційним стресом (ВІС). Встановлено, що МП суттєво прискорював відновлення функціонування гіпоталамо-гіпофізарно-надниркової системи за умов дії стресу, що відображалося у більш швидкому поверненні концентрації АКТГ та кортизолу до значень інтактних щурів. МП зменшував вміст прозапальних (ІЛ-1β та ІЛ-12В р40) та підвищував вміст антизапальних (ІЛ-4 та ІЛ-10) цитокінів в сироватці крові щурів після ВІС. Отримані дані свідчать, що одним з механізмів лікувального ефекту МП на ураження в слизовій оболонці шлунка, викликані стресом, є вплив на систему стресу та цитокіновий профіль.

Ключові слова: адренокортикотропний гормон, кортизол, цитокіни, стрес, мультиштамний пробіотик.

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ВЛИЯНИЕ ТЕРАПЕВТИЧЕСКОГО ВВЕДЕНИЯ МУЛЬТИПРОБИОТИКА НА ГИПОТАЛАМО-ГИПОФИЗАРНО-НАДПОЧЕЧНИКОВУЮ СИСТЕМУ И ЦИТОКИНОВЫЙ ПРОФИЛЬ В УСЛОВИЯХ СТРЕССА

Изучали влияние мультиштамного пробиотика (МП) на содержание гормонов стресса (адренокортикотропный гормон (АКТГ) и кортизол) и содержание провоспалительных (интерлейкин (ИЛ) 1β и 12В р40) и антивоспалительных (ИЛ-4 и ИЛ-10) цитокинов в условиях эрозивно-язвенных поражений, вызванных водно-иммобилизационном стрессом (ВИС). Установлено, что МП существенно ускорял восстановление функционирования гипоталамо-гипофизарно-надпочечниковой системы в условиях действия стресса, что отражалось в более быстром возврате концентрации АКТГ и кортизола до значений интактных крыс. МП сни жал содержание провоспалительных (ИЛ-1β и ИЛ-12В р40) и повышал содержание антивоспалительных (ИЛ-4 и ИЛ-10) цитокинов в сыворотке крови крыс после ВИС. Полученные данные свидетельствуют, что одним из механизмов лечебного эффекта МП на поражения в слизистой оболочке желудка, вызванные стрессом, является воздействие на систему стресса и цитокиновый профиль.

Ключевые слова: адренокортикотропный гормон, кортизол, цитокины, стресс, мультиштаммный пробиотик.

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THE EFFECT OF ACETIC ZINC ON SERUM ZINC LEVEL AND INTERLEUKIN 1β AND 6 LEVEL IN RATS EXPOSED TO ALCOHOL FOR 21 DAYS

The aim of this study was to examine the effect of acetic zinc supplementation on dynamics of zinc level and inflammatory cytokines (IL-1β and IL-6) production in serum of rats subjected to ethanol exposure for 21 days.The zinc level in serum was determined by flame atomic-absorption spectrophotometry. The level of IL-1β and IL-6 in serum was measured by enzyme-linked immunosorbent assay (ELISA) kits ("Sigma", США). A significant gradual decrease of serum zinc level and elevation of the levels of circulating IL-1β and IL-6 were seen in the ethanol-fed animals been maximal on 16^{th} and 21^{th}. The changes of zinc level and IL-1 β and IL-6 production in ethanol-intoxicated rats were completely corrected after acetic zinc supplementation that was more evident at prolonged ethanol exposure. Zinc level in such animals has been demonstrated to increase and exceed the control by 4.2 and 4.9 times on 16^{th} and 21^{st} day of alcoholization. The IL-1β and IL-6 level diminished and normalized also at these stages of study. Our results suggest that acetic zinc supplementation recovers zinc pool in blood and normalizes inflammatory cytokine production that may be due to reduction of zinc deficiency and attenuating of oxidative stress thus leading to inhibition of inflammation.

Key words. Ethanol, chronic alcohol intoxication, zinc deficiency, inflammatory cytokines, interleukin 1β, interleukin 6, acetic zinc.

Introducton

Ethanol has a variety of detrimental effects on immune system including effects on cell mediated and humoral immune response. It decreases neutrophil infiltration and phagocytic capability, inhibits lymphocyte activation following antigen stimulation, and alteres cytokine production by T cells and macrophages [1]. Prolonged ethanol exposure can directly and indirectly lead to the suppression of immunity and increased susceptibility to infections. The alcohol effects are dose-dependent, long-term ethanol consumption has been associated with inflammation [2]. Alcoholic liver disease is a result of a pro-inflammatory effect of chronic ethanol exposure [3].

Ethanol affects the production of cytokines that involved in inflammatory responses in plasma and a variety of tissues including lung, liver, and very importantly brain [4]. Cytokines are regulatory proteins playing a key role in immune and inflammatory response to infection by pathogens and oncogenesis. Excessive alcohol abusers have increased circulating levels of the inflammatory cytokines such as TNF-α (tumor necrosis factor-α), IL-1β (interleukin 1) and IL-6 (interleukin 6) [5]. A significantly increased production of IL-1β, IL-6, IL 12, and TNF-α by unstimulated peripheral blood monocytes was demonstrated in patients with alcoholic liver disease [6].

Severe zinc deficiency has been observed to develop in patients who chronically abuse alcohol, it's is one of the most consistent biochemical observation in alcoholic liver disease (ALD) [7]. Zinc affects the function of immune system, because it is one of the most highly proliferative organs. Zinc is crucial for normal growth and function of T and B cells, macrophages, neutrophils, and NK cells [8]. Zinc directly influences on blood mononuclear cell, diminishing the production of cytokines (IL-1, IL-6, TNF-α and IFN-γ) [9]. Various immune disorders are associated with zinc deficiency [10]. Decreased serum zinc level is observed in chronic inflammatory or infectious diseases [9, 11].

Zinc has been successfully used to restore impaired immune functions in diseases accompanied by diminished plasma zinc levels (rheumatoid arthritis, acrodermatitis enteropathica, hemodialysis patients, elderly individuals) [10]. A dietary zinc supplement has been proposed as possibly being an efficient method to palliate zinc deficiency in alcoholism [12]. Studies using animal models have demonstrated that Zn treatment prevents alcohol-induced liver injury under both acute and chronic alcohol exposure conditions [13, 14]. Zn has a high potential to be used in the prevention and treatment of ALD [12]. It may be a completely new therapeutic tool for the selective suppression of lymphocyte functions [10] and inhibition of inflammation. In